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INDEX

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Long-term mechanical assisted circulation devices

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Heart failure syndrome is the final presentation of a series of cardiac diseases. According to the Department of Informatics of the Brazilian National Health System (DATASUS), cardiovascular diseases are the third largest cause of hospitalizations in Brazil and heart failure is the main cause of cardiovascular hospital admissions in this country.

Despite all the advances in treatments for heart failure, a significant proportion of such patients evolve to states of refractoriness to clinical treatment. In these situations, it becomes necessary to use advanced procedures such as heart transplantation and long-term mechanical assisted circulation devices.

Heart transplantation is still the gold-standard treatment for refractory heart failure and the mean survival after this procedure is 11 years.[1] However, a large proportion of such patients cannot benefit from this procedure either because of contraindications or because of lack of availability of organs.

In the light of this scenario, there has been great pressure to develop therapies that provide an alternative to transplantation. This has culminated in the introduction of long-term mechanical assisted circulation devices.[2]

The beginnings of the development of mechanical assisted circulation devices date back to 1950, when an extracorporeal circulation machine was first used. In 1964, the National Heart and Lung Institute of the United States created an artificial heart program. Twenty years later, in the 1980s, tests on the HeartMate XVE implant began.

HeartMate XVE was the first generation of long-term mechanical assisted circulation devices. It propelled the blood flow by means of a pulsatile pump that attempted to mimic the flow in the left ventricle. This device was tested in the Randomized Evaluation of Mechanical Assistance for the Treatment of Congestive Heart Failure (REMATCH) trial[3] and was shown to provide a reduction of 48% in the risk of death over a one-year period. However, use of this device presented a high rate of complications such as thromboembolic events and hemolysis.

In 2007, a study comparing HeartMate XVE and HeartMate II, a second-generation device, was published. This new device had a different mechanism, consisting of continuous axial flow. Its technology brought in the advantage of being a smaller device that could be used by individuals of lesser build, with the expectation that it would be possible to use it for a long period, given that it only had a single moving part, its rotor.[4]

Subsequently, axial flow devices dominated in the field of long-term mechanical assisted circulation, for a long period. Centrifugal flow devices then emerged, which brought in the advantages of miniaturization, intrapericardial placement and a bearing-free rotor, achieved through the technologies of electromagnetic or hydrodynamic levitation. These devices reduced hemolysis and adverse events relating to hemocompatibility.[5-7]

The latest report from the Interagency Registry for Mechanically Assisted Circulatory Support (INTERMACS) showed that 77.7% of the long-term devices used in 2019 made use of magnetic levitation, thus showing the growth in this technology over recent years.[8]

Implantation of long-term mechanical assisted circulation devices can be indicated through the following strategies: 1 – As a bridge to candidacy: in situations of clinical conditions that prohibit heart transplantation but which if modifiable would allow the patient to become a candidate for transplantation (for example: pulmonary hypertension and neoplasias that are potentially

curable); 2 – As a bridge to transplantation: in situations in which the device might provide hemodynamic support and clinical stability until the heart transplantation is performed, within a context of progressively increasing severity of the patient's condition and unavailability of an organ for transplantation within the short term; 3 – As destination therapy: in situations in which the device might provide hemodynamic support and clinical stability for a patient with refractory heart failure who presents contraindications for heart transplantation, thus enabling greater survival and better quality of life, in comparison with clinical treatment using medications.[9]

In Brazil, experience with these devices remains sparse,[10] given their high cost and the potential growth in the number of heart transplantations that has occurred over recent decades.[11] These factors have left Brazil well behind with regard current treatments for advanced heart failure.

Within the worldwide scenario, what we see is that improvements in the technology of long-term mechanical assisted circulation devices and in the expertise of medical teams have consequentially increased the survival of such patients. This has given rise to changes in the range of options for treating refractory heart failure. Long-term mechanical assisted circulation devices are increasing gaining space: not only at the transplantation point but also, especially, as destination therapy, which accounted for more than 70% of the indications in 2019.[8]

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Brazilians' level of knowledge, attitudes and practices towards COVID-19: a cross-sectional study

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ABSTRACT

BACKGROUND: Brazil is facing increasing cycles of numbers of infected people and deaths resulting from coronavirus disease 2019 (COVID-19). This situation involves a series of factors, including the behavior of the population, that can be decisive for controlling the disease.

OBJECTIVE: To determine the knowledge, attitudes and practices of the Brazilian population regarding COVID-19.

DESIGN AND SETTING: Cross-sectional survey-type study, conducted using a population sample from different Brazilian states.

METHODS: A quantitative, descriptive and analytical approach was used. Sampling was done according to convenience and via snowballing. The data collection instrument was a knowledge, attitudes and practices system.

RESULTS: 1,655 people from all over Brazil participated in the survey; 80% were living in the southern region and 70.15% were female. More than 90% had knowledge and good attitudes relating to the means of transmission, preventive care and symptoms associated with COVID-19, although their knowledge and attitudes were not fully reflected in daily practices, for which there was lower adherence (80%). Greater knowledge was correlated with older participants, larger number of children, female sex and marital status; better attitude, with female sex and complete higher education; and better practices, with greater age, larger number of children and female sex.

CONCLUSION: A large part of the population has general knowledge about COVID-19, but not all knowledge was applied in practice. Older people, females and university graduates stood out as the best informed and most committed to controlling the disease.

INTRODUCTION

Through the etiological agent severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), coronavirus disease 2019 (COVID-19) has become responsible for causing respiratory[1,2] and systemic[3] disorders among many thousands of people around the world, including severe expressions. These conditions are related to individuals' intense and disordered multisystemic inflammatory response to the disease.[1,2]

COVID-19 has already infected more than 178,927,817 people and has led to the death of at least 3,875,915 people worldwide. In South America, about 500,000 people have lost their lives to this disease in Brazil alone. In the first half of 2021, Brazil was experiencing its worst time regarding the numbers of cases/deaths since the beginning of the pandemic.[4]

Therefore, despite the advances in vaccines against SARS-CoV-2, the classic methods of non-pharmacological prevention and control, such as proper hand hygiene with alcohol gel and use of masks, need to be continued and encouraged,[5] along with social distancing, especially for high-risk groups.[6] Studies have confirmed that the speed with which new cases of COVID-19 occur reduces as social isolation increases.[7]

It is likely that adherence to non-pharmacological prevention measures and the success or failure of such actions are closely influenced by the population's awareness of the subject,[8] which

is endorsed by the theory of knowledge, attitudes and practices. This theory, which was first put forward in the 1960s, assumes that suitable health-related behavior is divided into three continual processes, "the acquisition of knowledge, the generation of attitudes and the change in behavior itself".[9] From this perspective, knowledge is the determining factor for a change in behavior, while beliefs and attitudes are the "driving force" of this change.[9,10] This theory builds on the foundations of another concept that was created in 1950, "the health belief model", which argues that belief is essential for people to adopt healthy behavior, based on preventive pillars.[11]

Through the media and official health agencies, information about COVID-19 has been intensively disseminated. However, given the continuing debate and scientific advances relating to preventive care, diagnostic criteria and treatment, this knowledge does not remain static.[12] Nonetheless, it needs to be asked whether there is any process of acquisition of knowledge and adherence to attitudes/practices regarding preventive measures against COVID-19 among people in Brazil.

Understanding these attitudes, or lack of them, during the pandemic can show up any bottlenecks that may exist and the reasons for failure, in situations relating to the great present challenge of ensuring the safety and effective protection of the population.[13]

OBJECTIVE

Thus, the aim of this study was to assess the level of knowledge, attitudes and practices of the Brazilian population towards COVID-19, in relation to its sociodemographic characteristics, through using a method for ascertaining knowledge, attitudes and practices.

METHODS

The present study received prior approval from the National Research Ethics Committee (Comissão Nacional de Ética em Pesquisa, CONEP) on April 3, 2020, under the number 3.982.636, as provided for in Resolution 466/2012 of the National Health Council. The research that was developed was of survey type, with a quantitative descriptive-analytical approach. The sampling used was obtained according to convenience and through snowballing, given the possible ignorance of the study participants. This method enabled identification and integration of the sample through third-party references.[14]

We spread word of this study among our existing contacts through the instant messaging application WhatsApp. Participants thus recruited were asked to send invitations randomly to their telephone contacts, to try to also enroll them as participants in this research. We aimed to reach the largest number of people and different audiences possible, and to enable participation and responses from all Brazilian states.

The messages sent out provided a link to access an electronic form that we developed on Google Forms. This containing the informed consent statement, guidelines for resolution, and study questions to be answered by people aged 18 years and over. For people who agreed to participate in the study, but who because of limitations indicated that they were unable to provide responses through the form (this was especially the case among elderly participants), the form was applied via telephone, in accordance with the ethical guidance of Resolution No. 510/2016 of the National Health Council.[15]

Data collection took place from July to December 2020. The sample size was calculated using a scale based on the items/subject ratio and at least five participants per item, as proposed by Pasquali.[16] In this study, approximately 71 participants were used per item, with the highest possible number of members. This was a decisive factor with regard to the internal consistency of the scale.[16]

Individuals from 23 Brazilian states took part in the study. The only states from which there were no responses were Roraima, Amapá and Amazonas. Participants answered a questionnaire containing 23 questions with three choices: a) true; b) false; and c) I don't know. These questions sought to recognize clinical and epidemiological knowledge;[15] attitudes and practices in relation to COVID-19;[17] and sociodemographic characteristics (age, sex, marital status, education, state of origin, profession and children).[18] This was adapted from a previously published knowledge, attitudes and practices system.[9]

The questions in this instrument addressed information and concepts set forth by the World Health Organization,[18] regarding etiological agents, transmission routes, protection routes, signs and symptoms, the most vulnerable populations, care and related beliefs. The instrument was built for use in a general population in which people might be unfamiliar with the subject. Therefore, easily understood questions were recommended: these were evaluated from the semantic, conceptual and cultural points of view by three consultants, who were specialists in the field of infectious diseases and in the methodology of this study.

The responses to all the questions were organized to enable association analysis and were counted in a scoring system. Each item in the knowledge, attitudes and practices system consisted of a statement with three alternatives: a) true; b) false; and c) I don't know. The alternatives "false" and "I don't know" were subsequently condensed into a single "false" alternative[19] because of the small number of statements of the type "I don't know".

The assertions were graded as correct or incorrect, and scores were calculated from the sum of these responses. Continuous/numerical variables were then subjected to descriptive analysis (mean, median and standard deviation); and categorical/qualitative variables were subjected to absolute and relative frequency analysis.

Regarding the scores per domain, the maximum score for knowledge was 13, while for practices and attitudes it was five points. Initially, the data were subjected to a normality test (Kolmogorov-Smirnov). Because the data were found not to adhere to normal distribution, nonparametric tests were used for quantitative numerical correlations

(age and number of children) and Spearman's correlation coefficient was calculated. For comparative analysis on nominal qualitative variables, the Mann-Whitney test was used for sex and the Kruskal-Wallis test with Tukey's multiple comparisons was used for marital status and educational level. Variables for which the correlations were significant ($P < 0.05$) were integrated with the multiple regression analysis for each score, which was performed by using the stepwise method, in the SPSS for Windows software, version 19.0.0 (SPSS, São Paulo, Brazil).

RESULTS

One thousand six hundred and fifty-five people participated in the survey, of whom 1,161 (70.15%) were women, and 494 (29.84%) were men. The participants were aged between 18 and 92, with an average age of 35 (SD 14.55). Regarding marital status, 804 (48.55%) of the participants reported being married or in a stable relationship, and 754 (45.53%) were single. Approximately 50% had children. The predominant level of education among the participants was complete higher education (54.65%), followed by incomplete higher education (28.44%). The majority had some form of occupation at the time when they answered the questionnaire. Among the participants, 650 (39.27%) said that they had a job in companies, 226 (13.65%) worked in education and 189 (11.41%) were students. The other 590 individuals (35.67%) were distributed in other less frequent functions (**Table 1**).

Table 1. Sociodemographic characteristics of the study participants

	Variables	Frequency (n)	%
Sex	Female	1161	70.15
	Male	494	29.85
Age (years)	18-37	947	57.22
	38-57	553	33.41
	58-77	148	8.94
	78-92	7	0.42
Marital status	Single	754	45.53
	Married/stable union	804	48.55
	Widowed	16	0.97
	Divorced	81	4.89
Children	Yes	808	48.79
	No	847	51.15
Schooling	Incomplete elementary school	22	1.33
	Complete elementary school	18	1.09
	Incomplete high school	15	0.91
	Complete high school	224	13.53
	Incomplete higher education	471	28.44
	Complete higher education	905	54.65
Home state	Acre	2	0.12
	Alagoas	1	0.06
	Bahia	15	0.90
	Ceará	4	0.24
	Distrito Federal	2	0.12
	Espírito Santo	5	0.30
	Goiás	3	0.18
	Maranhão	5	0.30
	Mato Grosso	23	1.38
	Mato Grosso do Sul	16	0.96
	Minas Gerais	48	2.90
	Pará	4	0.24
	Paraíba	1	0.06
	Paraná	1041	62.90
	Pernambuco	7	0.42
	Piauí	4	0.24
	Rio de Janeiro	44	2.65
	Rio Grande do Norte	5	0.30
	Rio Grande do Sul	124	7.49
	Rondônia	9	0.54
	Santa Catarina	169	10.20
	São Paulo	121	7.31
	Sergipe	1	0.06
	Did not answer	1	0.06

Continue...

Table 1. Continuation

	Variables	Frequency (n)	%
	Company employee	650	39.27
	Educational institution employee	226	13.65
	Distribution company employee	91	5.49
	Financial services company employee	35	2.11
	Student	189	11.41
	Retiree	74	4.47
	Unemployed	22	1.32
	Domestic employee	43	2.59
Profession/Occupation	Communication service employee	34	2.05
	Construction company employee	17	1.02
	Environmental services company employee	4	0.24
	Healthcare institution employee	45	2.71
	Tourist services employee	12	0.72
	Entertainment company employee	18	1.08
	Transportation company employee	6	0.36
	Other types of employment	59	3.56
	Did not answer	130	7.85

Among the interviewees, 1,334 (80%) were from the southern region, 218 (13.18%) from the southeast, 44 (2.66%) from the center-west, 43 (2.59%) from the north and 15 (0.90%) from the northeast. The participation of 1,041 individuals (62.90%) living in the state of Paraná can be highlighted: this was the state in which the distribution of the questionnaire began (**Table 1**).

In the system for evaluating the knowledge, attitudes and practices of the participants in relation to COVID-19, the highest score (percentage of correct answers) was in the knowledge domain (94.84%), followed by the score in the attitudes domain (92.20%). The lowest performance, i.e. the lowest number of correct responses from the participants (80.00%), was in relation to the questions in the practical domain (**Table 2**).

In the knowledge domain, about 98% of the participants showed that they knew that "COVID-19 has droplet transmission", that "the flu vaccine does not prevent COVID-19", that "they should avoid crowded places" and that "social isolation is effective for prevention of COVID-19." Moreover, about 88% recognized that "younger people, elderly people and immunosuppressed children form a group that is more vulnerable to the disease". When asked about preventive care, such as "rubbing one's hands together for 20 seconds" during "hand washing with soap and water, and use of alcohol gel", the average percentage of correct responses (knowledge among the participants) also decreased to 96.60% and 90.40%, respectively (**Table 2).**

It was found that 98.50% of the participants were aware that they "should seek the healthcare service in the event of fever and respiratory distress", but on the contrary, almost 10% of them did not recognize that "COVID-19 can cause respiratory problems", and another 15% did not recognize that "fever and cough are common in COVID-19". In addition, almost half of the participants (46%) were unaware that symptoms such as "runny nose and sneezing are less common" among individuals infected with this disease (**Table 2**).

Regarding attitudes, approximately 6% of the study participants did not "believe in the effectiveness of the World Health Organization and Ministry of Health recommendations" and did not "follow them". For some of them (3.02%), this was because they considered that "social isolation does not decrease contamination"; while for others (6.65%), this was because they did not believe in "the severity of the disease." However, a higher percentage of participants (20%) considered that "the pandemic is non-transitory", and 3.08% showed concern about the disease, through the belief that, directly or indirectly, "it is reflected in damage to health and employment" (**Table 2**).

Regarding the domain of the participants' practices, almost 50% reported "having normally frequented public places", even though 15.17% of them were "living with people in the high-risk group". Although a large part of the population continued to attend public places, the majority (96.68%) claimed to carry out "hand washing" and "social distancing" in public places and had started to "greet people with gestures" (93.66%). However, in their routine, "sanitizing your belongings and objects" was not a practice for 26.40% of the participants.

Correlations between sociodemographic variables and the knowledge, attitudes and practices system showed differences with regard to knowledge, attitudes and practices (**Tables 3**, **4** and **5**).

The variables of age, number of children, sex, marital status and education were correlated with the knowledge domain ($P = 0.001$). Greater age, larger number of children and longer education correlated with greater knowledge. Females and married people had higher scores for this domain than males and single people (**Table 3**).

Table 2. Frequency of correct responses, overall mean and percentage mean (%) of statements relating to the knowledge, attitudes and practices of the study participants towards coronavirus disease 2019 (COVID-19)

Statements relating to the knowledge domain	**Frequency of correct responses (n = 1,655)**
01. Does COVID-19 cause respiratory problems?	1,507 (91.06%)
02. Are fever and coughing common?	1,400 (84.59%)
03. Are a runny nose and sneezing less common?	894 (54.02%)
04. Is it true that not everyone infected will progress to serious complications?	1,586 (95.83%)
05. Are younger people, elderly people and immunosuppressed children more vulnerable?	1,447 (87.43%)
06. Does transmission occur by means of droplets?	1,618 (97.76%)
07. Is social isolation effective for disease prevention?	1,625 (98.19%)
08. Should I avoid crowded places?	1,630 (98.49%)
09. Doesn't the flu vaccine prevent COVID 19?	1,608 (97.16%)
10. Should people with flu symptoms undergo isolation?	1,513 (91.42%)
11. Should I seek a healthcare service if I have fever and respiratory distress?	1,631 (98.55%)
12. Can I use soap and water instead of alcohol gel (community level)?	1,497 (90.45%)
13. Should I wash my hands with soap and water for 20 seconds (rubbing my hands together)?	1,583 (96.65%)
Overall mean number of correct responses	11.81 ± 1.18 (94.84%)
Statements relating to the attitudes domain	**Frequency of correct responses (n = 1,655)**
01. WHO and Ministry of Health recommendations are effective.	1,555 (93.96%)
02. Social isolation reduces contamination.	1,605 (96.98%)
03. I don't follow the guidelines because I believe the disease is not that serious.	1,545 (93.35%)
04. I am concerned about the damage caused by the disease (health, unemployment and other matters).	1,604 (96.92%)
05. I believe the pandemic is temporary, despite the difficulties.	1,318 (79.64%)
Overall mean number of correct responses	4.61 ± 0.67 (92.20%)
Statements relating to the practices domain	**Frequency of correct responses (n = 1,655)**
01. I've been in public places in the last few days.	800 (48.34%)
02. I go to public places even when living with a high-risk group.	251 (15.17%)
03. I carry out hand washing and social distancing.	1,600 (96.68%)
04. I carry out cleaning of belongings and objects.	1,218 (73.60%)
05. I greet people with gestures.	1,550 (93.66%)
Overall mean number of correct responses	4.00 ± 0.97 (80.00%)

Table 3. Average knowledge score versus demographic variables (age, number of children, sex, marital status and educational level of the participants)

Variables	**Knowledge score**		
Age	Correlation coefficient	(P)	Total (n)
	0.173	< 0.001	1,655
Number of children	Correlation coefficient	(P)	Total
	0.156	< 0.001	1,655
Sex	Median (mean ± SD)	(P)	Total (n)
Male	12.00 (11.59 ± 1.25)		494
Female	12.00 (11.90 ± 1.14)	< 0.001	1,161
Marital status	Median (mean ± SD)	(P)	Total (n)
Single	12.00 (11.62 ± 1.18)		754
Married	12.00 (11.98 ± 1.13)	< 0.001	670
Widowed	12.00 (12.31 ± 0.60)		16
Divorced	12.00 (11.80 ± 1.54)		81
Stable union	12.00 (11.91 ± 1.04)		134
Educational level	Median (mean ± SD)	(P)	Total (n)
Incomplete elementary school (1)	11.00 (12.00 ± 2.45)	1 < 6	22
Complete elementary school (2)	11.67 (12.00 ± 1.24)		18
Incomplete high school (3)	11.53 (12.00 ± 1.64)		15
Complete high school (4)	11.66 (12.00 ± 1.40)	4 < 6	224
Incomplete higher education (5)	11.63 (12.00 ± 1.16)	5 < 6	471
Complete higher education (6)	11.96 (12.00 ± 1.05)	< 0.001	905

Note: To correlate knowledge, sex and marital status, the Mann-Whitney and Kruskal-Wallis tests were used, respectively. SD = standard deviation.

Table 4. Average score for attitudes versus demographic variables (sex and educational level of the participants)

Variables	Attitude score		
Sex	Median (mean ± SD)	(P)	Total (n)
Male	5.00 (4.54 ± 0.75)		494
Female	5.00 (4.64 ± 0.63)	0.034	1,161
Educational level	Median (mean ± SD)	(P)	Total (n)
Incomplete elementary school	4.00 (4.00 ± 1.23)		22
Complete elementary school	5.00 (4.44 ± 0.70)		18
Incomplete high school	4.00 (4.07 ± 0.70)		15
Complete high school	5.00 (4.56 ± 0.73)		224
Incomplete higher education	5.00 (4.57 ± 0.70)		471
Complete higher education	5.00 (4.67 ± 0.61)	0.038	905

Note: For multiple comparison of attitudes versus the educational level variable, Tukey's multiple comparison test was used. SD = standard deviation.

Table 5. Average score for practices versus demographic variables (age, number of children and sex of the participants)

Variable	Practices score		
Age	Correlation coefficient	(P)	Total (n)
	0.061	0.014	1,655
Number of children	Correlation coefficient	(P)	Total (n)
	0.054	0.028	1,655
Sex	Median (mean ± SD)	(P)	Total (n)
Male	4.00 (3.77 ± 1.01)		494
Female	4.00 (4.09 ± 0.93)	< 0.001	1,161

Note: To correlate practices with the variable of sex, the Mann-Whitney test was used.
SD = standard deviation.

Regarding the attitudes domain, it was noted that females had higher scores (P = 0.034). There was also an association between education and the attitudes of the participants. The categories of complete high school and incomplete higher education did not differ from each other; however, they presented a lower score than complete higher education (**Table 4**).

Regarding practices, there was a correlation between age and the number of children of the participants. Older age and a larger number of children increased adherence to correct practices (P = 0.014 for age; and P = 0.028 for the number of children). As in the other domains, females also had a high mean score than males (P = 0.001) (**Table 5**).

Univariate analyses in the multiple regression model also showed differences within the knowledge, attitudes and practices system in relation to demographic variables (**Table 6**). Females and individuals with higher education had a better relationship with the knowledge domain (Beta = 0.302 for sex; and Beta = 0.233 for education) and also with the attitudes domain (Beta = 0.100 for sex; and Beta = 0.128 for education). Furthermore, female sex also had a greater association with practice scores (Beta = 0.134).

DISCUSSION

The overall results from this study revealed issues relating to knowledge, attitudes and practices regarding COVID-19 in Brazil. These have been undergoing extensive discussion and continual updating through the media, government bodies and the scientific community since the beginning of the pandemic.

Importantly, the method for evaluating knowledge, attitudes and practices has been adapted, built and used in other studies in

Table 6. Multiple regression analysis: knowledge, attitudes and practices in the study population

Knowledge, attitudes and practices system		Non-standardized coefficients		Standardized coefficients		
		Beta	Standard error	Beta	t	Sig*
Knowledge	Constant		0.074		156.673	0.000
	Complete higher education	0.233	0.062	0.098	3.784	0.000
	Female	0.302	0.062	0.117	4.868	0.000
	Single	-0.256	0.061	-0.108	-4.183	0.000
	Incomplete elementary school	-0.731	0.250	-0.071	-2.922	0.004
Attitudes	Constant	4.539	0.024		185.822	0.000
	Complete higher education	0.128	0.033	0.095	3.864	0.000
	Constant	4.468	0.035		127.459	0.000
	Complete higher education	0.128	0.033	0.095	3.878	0.000
	Female	0.100	0.036	0.068	2.792	0.005
Practices	Constant	3.385	0.030		113.540	0.000
	Single	-0.242	0.044	-0.133	-5.476	0.000
	Constant	3.347	0.033		102.262	0.000
	Single	-0.246	0.044	-0.136	-5.580	0.000
	Female	0.134	0.048	0.068	2.787	0.005

*Sig = significant.

different countries, depending on the cultural context and local reality.[8,20,21] As shown in **Table 1**, most of the population assessed had completed higher education (54.65%), similar to what was shown in other studies.[20,21,22]

The high scores observed for the knowledge domain generally demonstrated that the Brazilian population has knowledge about COVID-19, especially regarding the means of transmission of SARS-CoV-2 and the care required for avoiding this (96%).

Knowledge about COVID-19 in different parts of the world is quite high, but it varies depending on the region.[23] Surveys from two countries on different continents illustrate the differences in subjects' levels of knowledge: the levels ranged from 62% in Paraguay[20] to 90% in China.[21] The latter percentage was similar to what was found in the present study (94.84%) (**Table 2**).

The level of knowledge among participants in the present survey showed a relationship with their level of education. Individuals who had completed higher education had higher knowledge scores than those with incomplete elementary school, complete high school and incomplete higher education (**Table 3**).

On the other hand, in Nepal, for example, where 45.50% of the people had not had higher education, the average knowledge score was 60.00%.[24]

Regarding preventive measures, which were evaluated in relation to part of the population (10.00%), this knowledge was found to be limited. For example, there is misinformation about the use of soap and water as an efficient alternative to use of alcohol gel for hand hygiene, or in the case of absence of alcohol gel. Similarly, in India, the majority of "educated" people and healthcare professionals were aware of how infection occurs and what the preventive measures are. Even so, about 57.00% of the people did not recognize the disease as highly contagious, and almost 10.00% did not realize the importance of hand hygiene and social isolation.[25]

Along the same lines, a study carried out in the United States showed that the participants had good knowledge about the forms of transmission and symptoms. However, these individuals also showed some misconceptions: for example only 37.80% believed that use of an ordinary surgical mask was highly effective for preventing from COVID-19, and 25.60% thought that it was wise to avoid Chinese restaurants.[26] In Pakistan, 54.70% of the subjects reported not knowing that physical contact was the main means for spreading the infection.[27] Contrary to this, in the present study, 97.76% of the participants knew that droplets are the main means of infection transmission.

Appropriation of knowledge relating to preventive care and its updates among the entire population has become essential for controlling COVID-19. Understanding of simple measures that can be applied to indoor environments in situations of limited resources, which is the reality for many Brazilian households, can be highlighted.

Regarding symptoms, 45.98% of the participants in the present study mistakenly said that a runny nose and sneezing were common in COVID-19 (**Table 3**). However, these do not correspond to the classic signs and symptoms of the disease,[28] which are mainly fever (around 90%), dry cough (67.7%-86%), fatigue (38.1%) and dyspnea (18.6%-80%).[18] In the Philippines, a considerable proportion (89.5%) of the sample was able to point out coughing and sneezing as transmission routes.[23]

In Brazil, 15% of the participants did not correlate some of these symptoms with COVID-19. However, recognition of typical signs and symptoms of the disease and the degree of concern that these represent, given the clinical condition manifested, directs possibly infected people to seek medical and/or hospital care at the proper time.

Regarding attitudes, the mean score identified in this study was high, thus confirming the findings from recent studies conducted elsewhere.[23] In this regard, although 20% of the participants noticed that the COVID-19 pandemic was not a temporary phenomenon, 7% still refused to accept and/or follow instructions from official bodies such as the World Health Organization and the Ministry of Health (mask use, hand-cleaning with 70% alcohol or soap and water, avoidance of personal contact and avoidance of situations of crowding, among others).[17]

We observed that attitudes were not necessarily reflected in practice, considering that 26% of the participants reported that they did not clean their belongings and objects, 50% continued to go to public places and 15% were living with high-risk people. Thus, although people may believe the recommendations of official bodies for controlling the COVID-19 pandemic, in practice these recommendations are not put into effect. A large part of the population continues to have contact with many people in their daily routine, perhaps because of work needs or the mistaken feeling that only people with comorbidities (whether respiratory, cardiac or multifactorial) are likely to have a worsened prognosis when affected by COVID-19.[29] However, the real reason why people were going to public places was not investigated, which thus generates a limitation to our study. Nonetheless, it can be assumed that people were doing this in relation to work needs, as shown in a study carried out in Pakistan, where 59.3% of the subjects continued to attend mosques to pray, amid the pandemic.[27]

From correlations and multiple regression analyses, females stood out as having the highest scores in all domains, like in other recent studies that observed higher mean scores for knowledge among women.[20,22,30] Different studies on infectious diseases have also show that females had greater knowledge of the subject than males.[31,32]

With increasing age and larger numbers of children, higher knowledge scores were observed among our Brazilian population. This was put into practice by this population, in combating the COVID-19 pandemic. These results corroborate the findings

of most previous studies that investigated these socioeconomic characteristics.[20,22,23,33,34] On the other hand, in a study carried out in central Nepal, younger people showed more substantial knowledge, according to the authors.[24]

People with higher education or who were undergoing training tended to have scattered knowledge, as demonstrated by the present study. There were correlations with the level of education, both for knowledge (**Table 3**) and for attitudes (**Table 4**). This was confirmed through regression analyses (**Table 6**), such that people with higher education had more adequate knowledge and attitudes towards COVID-19. Lower levels of education can be considered to be a risk factor for the spread of viral infectious diseases and for disease progression to death.[35]

It is important to highlight that the average score for practices was lower (80.00%) than the scores for knowledge and attitudes (94.84 and 92.20%). In the light of this difference, it can be inferred that knowledge alone does not guarantee good practices, or their maintenance. As the pandemic advances, non-compliance with non-pharmacological practices to protect against SARS-CoV-2 infection may become more common.

It is worth remembering that the data in this study were collected in 2020, at a time when respondents were mostly aligned with preventive practices. However, it can be seen in everyday life that many of the measures that were adopted in the middle of the pandemic are not implemented with the same rigor today. Thus, within the Brazilian scenario of restrictions, psychological disturbances (stress and depression)[1,36] and narrative wars between the various levels of government, the politicization of information may have affected not only knowledge, but also especially the beliefs of people in this country. These beliefs are reflected in their attitudes and practices, as described in the published knowledge, attitudes and practices system.[9,10] In addition, progressive weakening of preventive practices may already be a reality in 2021. This is very worrying and is one of the factors responsible for the successive waves of COVID-19.

Furthermore, limitations of the study relating to sample selection may have generated some sampling bias, given that the sample was concentrated in the southern region of the country and among people with higher education levels. Thus, based on this initiative, future applications of the knowledge, attitudes and practices system are suggested, in order to understand the population, considering the changes that are occurring with regard to the COVID-19 pandemic.

CONCLUSION

Through using this knowledge, attitudes and practices system, it was found in this Brazilian population that there was a high level of knowledge about COVID-19. However, there was less commitment to practical application of this knowledge.

Furthermore, some well-informed and active social groups, like older women with children and individuals with higher education levels were noted. These groups showed greater implementation of actions to combat COVID-19. This is an important finding that should be directed towards COVID-19 coping actions for less active groups.

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A 120-second stretch improves postural control and plantar pressure: quasi-experimental study

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ABSTRACT

BACKGROUND: There are no studies on long-term bilateral calf stretching in relation to balance and plantar pressure.

OBJECTIVES: To demonstrate that there is better control of posture and pressures after continuous stretching of the posterior calf muscles.

DESIGN AND SETTING: Pre and post-intervention study conducted in a private clinic**.**

METHODS: We measured static footprints and stabilometry before and after continuous passive plantar flexor stretching of duration 120 seconds, among 24 healthy subjects.

RESULTS: We found differences in Y displacement with eyes closed (P = 0.010), but not among other variables with eyes closed: X displacement (P = 0.263); surface (P = 0.940); laterolateral speed displacement (P = 0.279); and anteroposterior speed displacement (P = 0.914). There were also no differences in eyes-open variables: X displacement (P = 0.341); Y displacement (P = 0.491); surface (P = 0.167); laterolateral speed displacement (P = 0.852); and anteroposterior speed displacement (P = 0.079). The plantar pressures in the heel (maximum pressure, P = 0.048; mean pressure, P = 0.001) and in the midfoot (maximum pressure, P = 0.004; mean pressure, P = 0.004) were reduced, but not in the forefoot (maximum pressure, P = 0.391; mean pressure, P = 0.225). The surface became larger in the forefoot (P = 0.000) and midfoot (P = 0.021).

CONCLUSIONS: Continuous static stretching of plantar flexors for 120 seconds improved stance balance and reduced plantar pressures (maximum and mean) in the rearfoot and midfoot. It also increased the surface in the midfoot and forefoot.

TRIAL REGISTRATION: at clinicaltrials.gov, under the number NTC03743168.

INTRODUCTION

Maintaining balance while standing requires constant regulation because the human body is unstable in a static standing position. Balance is regulated through constant adjustment of the ankle torque. This adjustment results from a combination of peripheral reflexes, intrinsic properties of the ankle tissues and regulation from the peripheral and central nervous systems.[1]

It has been recognized that physical performance and the risk of injury can be conditioned through exercises done prior to physical work.[1] Among these exercises, stretching should be done 15 minutes before the start of the activity in order to benefit from its effects; in fact, just 15 minutes before is when the greatest benefit occurs.[1] Stretching of the ankle flexor muscles can help to improve muscle functionality in different pathological conditions of the foot and ankle.[2-7]

In healthy people, continuous stretching for 30, 60 and 120 seconds was found to improve the range of motion immediately afterwards.[8] It also lowered gastrocnemius muscle oxygenation levels,[9] and decreased sports performance when the duration of stretching was 120 seconds or more,[8] because post-stretch torque loss is associated with decreased central drive.[10]

Lower-extremity muscle strength has proven to be a determining factor in maintaining optimal balance. Low levels of muscle strength have been linked to loss of balance among elderly people.[8] Continuous prolonged stretching over time produces a decrease in strength after 60 seconds of duration.[10]

Measurement of the movement of the center of pressure (COP) while the subject is standing is one of the usual measurements that have been used in different studies to check postural balance, as it has proven to be reliable.[12] Within neuromuscular management of the pressure center to regulate balance, the plantar flexor muscles are of decisive importance.[13] They are capable of

adjusting the anteroposterior movement of the COP, which combined with the action of the invertor and evertor muscles (which control the lateral deviation of the COP) are responsible for adjustment of the COP through movement of the ankle.[14]

A few studies in the literature have examined the effects of stretching exercises on balance capacity.[15-19] Behm et al.[15] found that static stretching exercises had a negative effect on balance. Lima et al.[15] studied the acute effects of unilateral static stretching of the ankle plantar flexor on the COP during a single-leg balance task and found that it had a negative effect.[15] Costa et al.[17] examined the effects of static stretching exercises on dynamic balance capacity. They determined that a static stretching exercise for 15 seconds could have positive effects on balance capacity.

We did not find any correlation between balance while standing and bilateral continuous calf stretching, from reviewing the literature. Therefore, our hypothesis is that there is better stability when short continuous static stretching is performed, due to reduction of muscle stiffness.

OBJECTIVE

The aim of our study was to demonstrate that there is better control of posture after continuous stretching of the posterior calf muscles for more than 120 seconds because there is less torque and stiffness but more range of motion.

METHODS

Subjects

We recruited 24 healthy subjects, comprising 21 females and three males. The sociodemographic characteristics of the sample population are shown in **Table 1**. The sample size was calculated by means of the G*Power software (Düsseldorf University, Düsseldorf, Germany).

We aimed to test for differences in the center of pressure, in the same was as done in a previous study in which the acute effects of unilateral static stretching of the ankle plantar flexor on the COP during a single-leg balance task were investigated. In that study, it was found that the COP area in the anteroposterior direction improved in the stretched limb from before stretching to immediately after stretching, from 1.06 ± 0.24 to 0.87 ± 0.16 (P = 0.015).[15]

To achieve this with statistical 95% confidence, an 80% statistical power analysis ($\alpha = 0.05$; $\beta = 20\%$) and two-tailed tests, a total of 18 participants was found to be required. The subjects were recruited for a month, from October 1, 2018, to October 31, 2018. No subjects were lost over the study period.

The eligibility criteria for the subjects were that they needed to be healthy untrained individuals[15] who had not engaged in flexibility training for at least six months before the study and refrained from such training during the data collection period. The subjects needed to refrain from vigorous exercise and alcohol consumption for 24 hours and from stimulant use (e.g. caffeine) for six hours before testing.[10] They also needed to have not had any previous surgery on the lower extremities; have no history of injury with residual symptoms in the lower extremities within the last year; have no evidence of a leg-length discrepancy (difference in distance from the anterior superior iliac spine to the superior surface of the most prominent aspect of the medial malleolus) of more than 1 cm; have at least 15 degrees of ankle dorsiflexion;[20] and have no evidence of balance deficits, as determined through oral questioning regarding falls[15] and through using the Balance Evaluation Systems Test (BESTest).[21]

The demographic data of the study participants were as follows: 32.2 ± 8.0 years old; 166.20 ± 8.43 cm height; and 62.77 ± 9.52 kg weight. All the demographic data are shown in **Table 1**.

All of the subjects were voluntary participants.[22] The Ethics Committee of A Coruña University approved the study (protocol number: CEIC 28/2016; date: November 28, 2016), and all subjects gave their written informed consent before participating in this research. The ethical standards for human experimentation were in conformity with the Helsinki Declaration. This study was registered at clinicaltrials.gov, under the number NCT03743168.

All measurements were performed at the same hour of the day, between 9 and 11 AM.[15] First, a clinician confirmed the inclusion and exclusion criteria of each subject and performed a baseline balance evaluation.[21]

The protocol consisted of the following:

- A pre-stretching evaluation.
- An ankle plantar flexor static-stretching protocol. The stretching position consisted of a weight-bearing static stretch: the subject stepped up onto a raised platform and placed the forefoot of both feet on the edge of the platform, dropped both heels off the platform almost to the ground without making contact with it, and held that position. There was one set of continuous stretch,[10] consisting of 120 seconds of static passive stretching of calf plantar flexors, to the point of discomfort,[15-16] which was maintained throughout the stretching.[23] The intensity required was 70%-90% of the point of discomfort

Table 1. Sociodemographic characteristics of the sample population (n = 24)

Variable	Mean ± SD	95% CI
Age (years)	32.20 ± 8.08	(28.97-35.44)
Weight (kg)	62.77 ± 9.52	(58.96-66.57)
Height (cm)	166.20 ± 8.43	(162.83-169.58)
BMI (kg/m^2)	22.71 ± 2.90	(21.55-23.87)
Shoe size*	38.81 ± 2.26	(37.90-39.72)

BMI = body mass index; SD = standard deviation; 95% CI = 95% confidence interval; *European sizes.

(POD),[15] where 0 = "no stretch discomfort at all" and 100% = "the maximum imaginable stretch discomfort".[24] All subjects were asked to assess their POD intensity during all stretches, by a clinician.[15]

- An immediate post-stretching evaluation,[15] on the same day.

The pre and post-stretching evaluations were performed in a private clinic, which the subjects attended for the purposes of physiotherapy review treatment. During these evaluations, the subjects were instructed to stand barefoot on a force platform.[22] In preparation for this: the subjects were asked to take up a double-limb stance with placement of their feet on the platform at equal distances from the midline,[25] and set at 30 degrees to the midline.[26] The upper limbs were kept loosely alongside the body during all examinations.[27] The subjects were instructed to stand as still as possible, with their eyes open, while concentrating on a point about two meters away, at eye level.[15] The subjects stood on the same surfaces with eyes open (EO) or eyes closed (EC).[22]

Two tests (EO and EC) were performed, each of 30 seconds in duration,[15,22,27] and the order of the EO and EC conditions was randomized across the subjects.[22] This randomization of the order of the tests was implemented using a bag from which the subject extracted a piece of paper that stated which test was to be performed. Foot plantar pressure was measured during this bipodal standing, with placement of the patient's feet on the platform, before and after stretching. We did two trials on each condition (EO and EC) and the foot area was divided into 3 bilateral areas: bilateral rearfoot, bilateral midfoot, bilateral forefoot.

Variables

The stabilometry measurements consisted of the displacement of the centers of pressure in X and Y with open and closed eyes,[22] the COP area and the COP speed in the anteroposterior (a-p) and mediolateral directions.[22]

Ground reaction forces and moments were recorded and digitized using the Podoprint system (Medicapteurs; Balma, France) with 2,304 sensors in an area of 400 mm x 400 mm, and an acquisition frequency of 200 Hz, with an auto-calibrated system for any use.

Statistical analysis

All data were explored for normality using the Shapiro-Wilks test because the sample size was less than 30 subjects. From this, the data were considered to be normally distributed if $P > 0.05$. A descriptive statistical analysis was performed using means ± standard deviations (SD) and 95% confidence intervals. For each intrasession trial, the intraclass correlation coefficient (ICC) was used to evaluate the reliability of each parameter. To interpret ICC values, we used benchmarks as proposed by Landis and Koch:[28] 0.20 or less, slight agreement; 0.21 to 0.40, fair; 0.41 to 0.60, moderate; 0.61 to 0.80, substantial; and 0.81 or greater, almost perfect.

Standard errors of the mean (SEM) were calculated to measure the range of error of each parameter. The SEM was calculated between sessions, from the ICCs and SDs. $SEM = s\_x.\sqrt{(1- r\_xx)}$; where s_x is the standard deviation of the observed set of test scores, and r_xx is the reliability coefficient for these data, which in this case was considered using the ICC.

Also, the mean value from two measurements on each variable was used. The Wilcoxon signed rank test was performed to test for any differences in nonparametric variables and paired t tests were used for parametric variables.

Lastly, values of normality (VN) were defined for the sample, for all variables. These were obtained from the formula VN = mean +/-1.96 * SD. From the result from each variable, VN was used to calculate the 95% confidence interval. P-values < 0.05 with a 95% confidence interval were considered statistically significant for all tests. The SPSS for Windows software, version 20.0, was used (SPSS Inc., Chicago, Illinois, United States).

The analysis on the intrasession reliability of the variables studied and the values of normality for the total population are presented in **Table 2**.

RESULTS

All the variables showed non-normal distribution ($P < 0.05$).

Stabilometry and static footprint variables before and after bilateral stretching are presented in **Table 3**. Most of the mean values were similar before and after stretching. The exception was the mean Y displacement variable with eyes closed, which was statistically significantly lower after stretching.

After stretching, the plantar pressures (maximum and mean) in the heel, midfoot and forefoot were lower. The surface in the midfoot and forefoot became larger. A static footprint for a representative subject before and after stretching is shown in **Figure 1**.

DISCUSSION

The aim of this intervention study was to analyze changes to balance and footprint variables immediately after 120 seconds of continuous stretching of plantar flexors. After stretching, the plantar pressures (maximum and mean) in the rearfoot and midfoot were reduced. The forefoot pressures did not change. The surface variable in the midfoot and forefoot became larger. We believe that the increase in the surface of the midfoot and forefoot contributed to the decrease in the pressures in the rearfoot and midfoot.

Limitation to dorsiflexion of the ankle in cases of plantar forefoot ulceration has been shown to be beneficial after lengthening of the Achilles tendon, with regard to ulcer recurrence, due to the reduction of plantar pressures.[29] Interestingly, with 120 seconds of

continuous plantar flexor stretching, we observed reduced pressures in the forefoot, in feet without an equinus condition. Thus, healthy people may avoid having foot pathological conditions in which the symptoms consist of increased pressure, such as metatarsalgia or hallux abductor valgus.[30] Additional studies are needed in order to ascertain the duration of the effect of the pressure reduction.

The balance variables evaluated with eyes open before and after stretching did not show any significant differences. On the other hand, with eyes closed, the Y displacement became significantly lower after stretching. This finding was concordant data obtained in other studies on stance balance. The ankle and gastrocnemius muscle are activated through the anteroposterior movement of the center of pressure during bipodal standing. For mediolateral movement, a separate hip load/unload strategy implemented by the hip abductors/adductors forms a dominant defense for standing with feet side-by-side.[31] Logically, our intervention on the gastrocnemius affected only the posterior displacement.

Comparing our results with previous research, the types and protocols of stretching differed in all studies, and the results too. Behm et al.[16] found that static stretching exercises had a negative effect on balance, using an intermittent stretching protocol. Morrin and Redding[18] compared the effects of three different stretching

Table 2. Analysis on intrasession reliability of the variables studied and values of normality in the total population (n = 24)

Variable	Pretest (n = 24)			Posttest (n = 24)		
	ICC (95% CI)	SEM	Values of normality 95% CI	ICC (95% CI)	SEM	Values of normality 95% CI
Rearfoot maximum pressure (kPa)	0.822 (0.549-0.929)	10.24	59.97-155.17	0.990 (0.976-0.996)	1.96	62.13-139.13
Rearfoot mean pressure (kPa)	0.988 (0.970-0.995)	0.62	29.81-52.31	0.981 (0.952-0.992)	0.79	26.16-48.66
Rearfoot surface (cm^2)	0.992 (0.982-0.997)	0.94	925.69-884.14	0.995 (0.989-0.998)	0.90	1097.99-1047.69
Midfoot maximum pressure (kPa)	0.996 (0.990-0.998)	0.75	-10.82-35.90	0.812 (0.526-0.926)	6.58	-12.45-47.05
Midfoot mean pressure (kPa)	0.994 (0.984-0.997)	0.44	-5.13-17.59	0.910 (0.771-0.964)	1.90	-4.18-20.66
Midfoot surface (cm^2)	0.991 (0.979-0.996)	1.87	383.13-305.51	0.995 (0.989-0.998)	1.33	413.97-340.04
Forefoot maximum pressure (kPa)	0.972 (0.930-0.989)	0.82	49.16-90.94	0.743 (0.349-0.898)	8.16	41.15-103.93
Forefoot mean pressure (kPa)	0.969 (0.922-0.988)	0.48	20.01-30.75	0.496 (-0.274-0.800)	5.08	12.82-40.88
Forefoot surface (cm^2)	0.987 (0.969-0.994)	1.55	1269.83-1216.52	0.986 (0.967-0.994)	1.94	1379.85-1309.96
X displacement with eyes open (mm)	0.990 (0.974- 0.996)	0.43	0.21-17.41	0.986 (0.965-0.995)	0.59	-2.15-17.63
Y displacement with eyes open (mm)	0.945 (0.860- 0.978)	1.98	4.07-37.19	0.985 (0.961-0.994)	1.33	-2- 40.76
Surface with eyes open (mm^2)	0.986 (0.964-0.994)	0.79	5.17-29.13	0.986 (0.965- 0.995)	1.03	5.17-29.13
Mean speed of laterolateral displacement with eyes open (mm/s)	0.865 (0.659-0.947)	0.10	0.65-1.73	0.920 (-1.294-.641)	0.20	-0.09-2.69
Mean speed of anteroposterior displacement with eyes open (mm/s)	0.785 (0.457- 0.915)	0.09	0.49-1.47	0.919 (0.794-0.968)	0.11	0.09-1.73
X displacement with eyes closed (mm)	0.950 (0.874-0.980)	1.04	1.31-17.03	0.985 (0.963-0.994)	0.52	-1.73-15.07
Y displacement with eyes closed (mm)	0.972 (0.928-0.989)	1.63	4.68-40.66	0.982 (0.955-0.993)	1.28	-0.91-36.71
Surface with eyes closed (mm^2)	0.985 (0.963-0.994)	1.33	2.30-21.28	0.966 (0.915-0.987)	2.58	-3.93-50.95
Mean speed of laterolateral displacement with eyes closed (mm/s)	0.768 (0.415-0.908)	0.11	0.87-1.77	0.851 (0.623-0.941)	0.18	0.34-2.22
Mean speed of anteroposterior displacement with eyes closed (mm/s)	0.853 (0.630-0.942)	0.12	0.62-1.90	0.092 (-1.294-0.641)	0.94	-0.49 -3.39

SD = standard deviation; 95% CI = 95% confidence interval.

protocols. The results showed that combination stretching gave rise to significantly enhanced balance and vertical jump height scores than those from static stretching. Their stretching protocol was performed on the hamstring muscles.

It needs to be considered that, depending on the muscle studied, different acute effects from static stretching on muscle-tendon mechanics can be observed.[31] Chatzopoulos et al.[19] found that static stretching had a negative effect on dynamic balance,[32] in a protocol consisting of three minutes of jogging followed by seven minutes of static stretching. Lima et al.[15] tested a protocol for unilateral static discontinuous stretching of ankle plantar flexors and analyzed the open-eye condition in relation to unilateral balance.[15] In contrast, we analyzed a bipodal stance, which requires low-intensity contraction of the triceps surae, compared with unipodal balance. Lim et al.[33] indicated that static stretching exercises had no effect on static balance.

There is a consensus regarding the dose-effect relationship of the duration of stretching.[34-35] Young et al.[34] studied the effects of the volume and intensity of warm-up static stretching on explosive force production and range of motion (ROM) of the plantar flexors. Two minutes of stretching at 90% intensity had no significant influence on muscle function in these variables.

We believe that the negative effects on static balance that were seen in previous studies differed from ours due to the type of

Table 3. Stabilometry and static footprint variables before and after bilateral stretching

Variable	Pretest (n = 24) Mean ± SD (95% CI)	Posttest (n = 24) Mean ± SD (95% CI)	P-value*
Rearfoot maximum pressure (kPa)	107.57 ± 24.29 (96.20-118.94)	100.66 ± 19.63 (91.47-109.85)	0.048
Rearfoot mean pressure (kPa)	41.06 ± 5.74 (38.37-43.75)	37.41 ± 5.74 (34.72-40.10)	0.001
Rearfoot surface (cm^2)	85.37 ± 10.60 (80.89-89.85)	83.62 ± 12.83 (78.20-89.04)	0.196
Midfoot maximum pressure (kPa)	12.54 ± 11.92 (6.95-18.12)	17.30 ± 15.18 (1019-24.40)	0.004
Midfoot mean pressure (kPa)	6.23 ± 5.80 (3.51-8.94)	8.24 ± 6.34 (5.27-11.21)	0.004
Midfoot surface (cm^2)	17.39 ± 19.80 (9.03-25.76)	19.99 ± 18.86 (12.03-27.96)	0.021
Forefoot maximum pressure (kPa)	70.05 ± 10.66 (65.06-75.04)	72.54 ± 16.02 (65.04-80.04)	0.391
Forefoot mean pressure (kPa)	25.38 ± 2.74 (24.09-26.66)	26.85 ± 7.16 (23.49-30.20)	0.225
Forefoot surface (cm^2)	91.41 ± 13.60 (85.67-97.16)	99.50 ± 16.43) (92.56-106.43)	0.000
X displacement with eyes open (mm)	8.81 ± 4.39 (6.75- 10.86)	7.74 ± 5.05 (5.38-10.11)	0.341
Y displacement with eyes open (mm)	20.63 ± 8.45 (16.67-24.59)	19.38 ± 10.91 (14.27-24.49)	0.490
Surface with eyes open (mm^2)	10.02 ± 6.72 (6.88-13.17)	11.98 ± 8.75 (7.88-16.07)	0.167
Mean speed of laterolateral displacement with eyes open (mm/s)	1.19 ± 0.28 (1.05-1.32)	1.30 ± 0.71 (0.96-1.63)	0.852
Mean speed of anteroposterior displacement with eyes open (mm/s)	0.98 ± 0.25 (0.86-1.10)	0.91 ± 0.42 (0.71-1.11)	0.079
X displacement with eyes closed (mm)	7.86 ± 4.68 (5.67-10.05)	6.67 ± 4.29 (4.66-8.68)	0.263
Y displacement with eyes closed (mm)	22.67 ± 9.18 (18.37-26.97)	17.90 ± 9.60 (13.41-24.03)	0.010
Surface with eyes closed (mm^2)	23.58 ± 10.86 (18.50-28.66)	23.51 ± 14.00 (16.95-30.06)	0.940
Mean speed of laterolateral displacement with eyes closed (mm/s)	1.32 ± 0.23 (1.21-1.43)	1.28 ± 0.48 (1.05-1.51)	0.279
Mean speed of anteroposterior displacement with eyes closed (mm/s)	1.26 ± 0.33 (1.10-1.42)	1.45 ± 0.99 (0.99-1.91)	0.914

SD = standard deviation; 95% CI = 95% confidence interval; *Wilcoxon test: P-value < 0.05 taken to be significant.

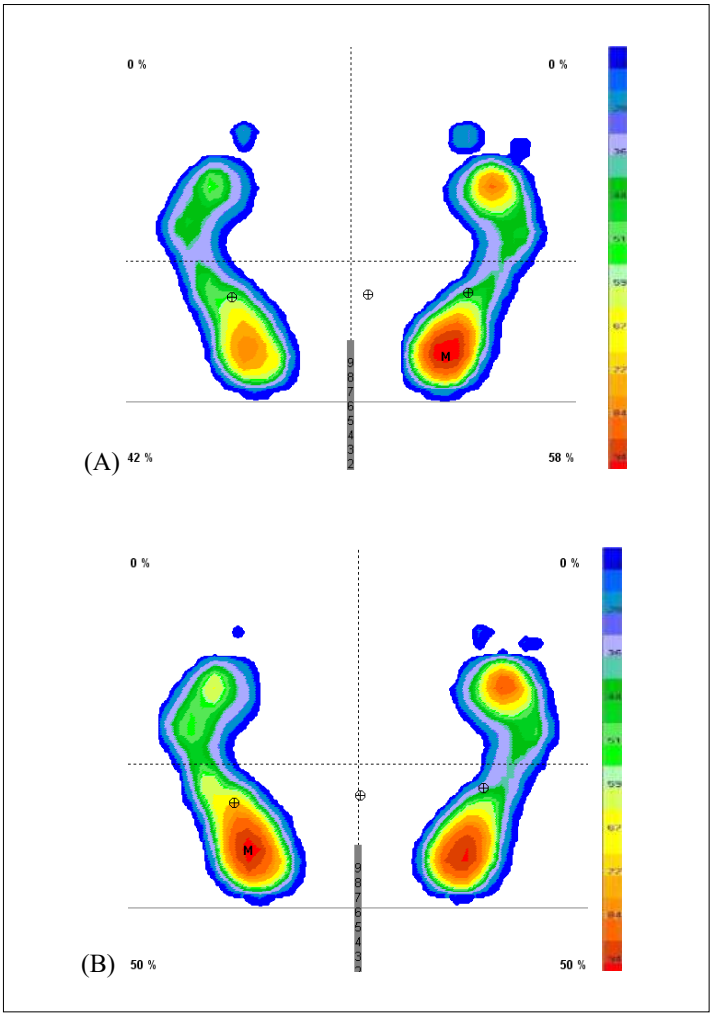

Figure 1. Distribution of pressure in static footprint for a representative subject before stretching (A); and distribution of pressure in static footprint for a representative subject after stretching (B). The scale at the right indicates pressure (g/cm^2).

stretching and its duration, and also regarding the measurement with eyes closed. In this regard, Costa et al.[17] determined that static stretching exercises with durations of 15 and 45 seconds could have positive effects on dynamic balance capacity.

The physiological changes that can occur in short-duration medium-intensity static stretching on the osteotendinous reflex and Golgi reflex are probably different from the changes seen through longer-duration high-intensity stretching. Therefore, the input to the central nervous system from long and high-intensity stretching, and the subsequent reorganization of the central nervous system, ought to be different from the input from short and medium-intensity stretching.[17]

We consider that medium-intensity stretching with a duration of 120 seconds has an effect consisting of proprioceptive improvement and believe that this was the cause of the results from our study. Any stimulus that increases proprioception shows its importance when a proprioceptive route as important as the visual route is then removed. However, additional studies are needed in order to acquire evidence regarding the physiological causes of the findings and their possible applications in sports and rehabilitation.

One limitation of this study was that the sample of subjects was not equitable in gender terms. Nonetheless, it is true that most studies on stretching have not reflected equity between men and women since this factor is not considered to affect the result.[8,10]

The present study showed better stance balance and lower pressures in the rearfoot and midfoot. This was probably due to the increased surface area in the forefoot. This implies that there is a need for stretching among subjects with previous histories of lesions due to high midfoot and rearfoot pressures, especially in barefoot exercises and in sports that imply a need for greater static balance against force, such as archery.

CONCLUSIONS

This study demonstrated that continuous static stretching of plantar flexor muscles for 120 seconds improved stance balance and reduced the plantar pressures (maximum and mean) in the rearfoot and midfoot. Stretching increased the surface variable in the midfoot and forefoot.

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Relationship between awareness of cervical cancer and HPV infection and attitudes towards HPV vaccine among women aged 15-49 years: a cross-sectional study

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ABSTRACT

BACKGROUND: Cervical cancer is a type of cancer caused by human papillomavirus (HPV).

OBJECTIVE: To determine the relationship between awareness of cervical cancer and HPV infection and attitudes towards HPV vaccine among women aged 15-49 years.

DESIGN AND SETTING: Cross-sectional study conducted at Karabük Training and Research Hospital, Turkey.

METHODS: 500 women who visited the gynecology outpatient clinic of a public hospital between July 15 and December 31, 2019, were selected through random sampling. Data were collected using a socio-demographic questionnaire comprising nine questions (created by the researchers), the HPV and Cervical Cancer Awareness Questionnaire and the Carolina HPV Immunization Attitudes and Beliefs Scale.

RESULTS: The relationship between the awareness questionnaire and the beliefs scale was explained through simple effect modeling of a structural equation. The women's knowledge score regarding cervical cancer and HPV infection was 4.69 ± 4.02 out of 15. Women were afraid of being diagnosed with cervical cancer and HPV infection, but they did not have sufficient information. They had poor information about the HPV vaccine, did not know how to obtain the vaccine and did not have enough information about its benefits and harmful effects. Women who were afraid of getting cervical cancer, and who thought that they were at risk, had more information about the HPV vaccine.

CONCLUSION: Women need information about cervical cancer, HPV infection and the HPV vaccine. Midwives, nurses and physicians who provide healthcare services in gynecological follow-ups should provide information to women about the HPV vaccine and cervical cancer.

INTRODUCTION

Although the incidence of cervical cancer has decreased in developed countries over recent decades through effective screening programs, it continues to be an important health problem, especially in developing countries. With 569,847 new cases and 311,365 deaths worldwide in 2018, cervical cancer is expected to be the fourth most common type of cancer and the fourth leading cause of cancer death among women. Out of these deaths, 90% occur in underdeveloped or developing countries. In 2018, 2,356 new cases and 1,280 deaths related to cervical cancer were seen in Turkey.[1]

Human papillomavirus (HPV) is the most common sexually transmitted infection.[2] It has been estimated that the lifetime probability of acquiring HPV exceeds 80% among women and 90% among men.[3] Different groups of HPVs exist, with different epithelial tropisms (cutaneous and mucosal) and life-cycle strategies. Many HPVs are classified as low risk (lrHPV) because they are very rarely associated with neoplasia or cancer in the general population. These lrHPVs typically cause indeterminate/undetectable infections or benign papillomas that can last for months or years but are eventually cleared by the host's immune system. High-risk HPV (hrHPV) types are the cause of many major human cancers, including almost all cases of cervical cancer, a large proportion of other anogenital cancers and an increasing number of head and neck tumors.[4] HPV infections can be temporary or permanent. Most cervical HPV infections (around 90%) are cleared by cell-mediated immunity within one to two years of exposure. In lrHPV infections, clearance occurs within a shorter period than in hrHPV infections. Among all HPV infections, 5%-10% cause persistent disease.[5]

Cervical cancer has a long preinvasive period due to lesions associated with persistent hrHPV infection. Early diagnosis of these preinvasive lesions using screening methods (HPV DNA tests,

cervical cytological tests, etc.), effective treatment of these lesions and administration of HPV vaccines can prevent this disease. Most cases of cervical cancer occur in women who have never been screened or were screened poorly.[4,5]

HPV vaccination has the potential to greatly reduce the morbidity and mortality associated with genital HPV infections and is recommended by the American Society of Obstetrics and Gynecology (ACOG) for all women and men aged 9-26 years.[6] As cervical cancer has a long preinvasive period, it can be diagnosed and treated early by means of the screening programs that have been developed. For this reason, evaluating society's attitudes and beliefs about cervical cancer and HPV vaccine and increasing the level of knowledge are important in terms of preventive medical practices.

Greater awareness among sexually active women aged 15-49 years regarding cervical cancer will decrease the rate of occurrence of this disease and increase the levels of knowledge about HPV vaccines and the vaccination rate.[6–10]

OBJECTIVE

The aim of this study was to determine the relationships between knowledge of cervical cancer, awareness of HPV infection and attitudes towards HPV vaccines among women aged 15-49 years.

METHODS

Research type and sampling

This cross-sectional study was planned with the aim of determining the levels of knowledge about HPV and cervical cancer, levels of knowledge regarding preventive measures, health-related beliefs and awareness about HPV vaccines among women aged 15-49 years. The study population comprised women (28,356 women) within this age group who were living in the province of Karabük, Turkey.[11] However, the sample used in this study comprised 500 women, as calculated through G-power analysis with a 95% confidence interval and 5% margin of error, assuming 75.2% prevalence. Data were collected at the gynecology and diseases outpatient clinic of a public hospital between July 15 and December 31, 2019.

Data collection tools

To collect data, a questionnaire on sociodemographic characteristics comprising nine questions was prepared in line with the literature by the researchers. In addition, the HPV and Cervical Cancer Awareness Questionnaire and the Carolina HPV Immunization Attitudes and Beliefs Scale (CHIAS) were used. (12,13) Permission for this study was obtained from the Bülent Ecevit University Human Research Ethics Committee (dated June 27, 2019; approval no. 600) and from the institute at which this research was conducted. After obtaining permission from the women who agreed to participate in the study, data were collected through face-to-face interviews. The study was conducted in accordance with the Declaration of Helsinki.

Evaluation of data

The statistical analysis for this study was done with the aid of the SPSS 20 computer software (SPSS, Chicago, United States). Given that the skewness and kurtosis values of the data remained within the +2.0/-2.0 range limit, the data were considered to follow normal distribution.[14] Computer-assisted data analysis was used for the basic evaluation (correlations and frequencies) on the study data. We found that relationships between pairs of scales were explained through first-order factor analysis. A computer-assisted analysis program was used for factor analysis. Pearson's correlation analysis was used to determine the relationships between scales and subdimensions.[15] The data obtained were evaluated with a 95% confidence interval and a significance level of $P < 0.05$.

RESULTS

The Cronbach's alpha coefficient of the awareness scale for cervical cancer and HPV infection was determined as 0.91 by Ingledue.[12] In the validity-reliability study for use of this awareness scale in Turkish, conducted by Özdemir and Kısa, Cronbach's alpha coefficient was 0.71.[16] In our study, the Cronbach's alpha coefficient of the awareness scale for cervical cancer and HPV infection was 0.81. The Cronbach's alpha coefficient of the CHIAS was determined by McRee et al.[17] Accordingly, the alpha values for "harm", "obstacles", "effects" and "uncertainty" were 0.69, 0.69, 0.61 and 0.66, respectively.[17] Cronbach's alpha was 0.62 in the validity-reliability study for use of the Carolina HPV Immunization Attitudes and Beliefs Scale in Turkish, conducted by Sunar and Süt.[18] In our study, the Cronbach's alpha of the CHIAS was 0.80; the alpha values for the subdimensions "harm", "obstacles", "effects" and "uncertainty" were 0.73, 0.75, 0.74 and 0.70, respectively.

The sociodemographic characteristics of the women participating in the study (n = 500) are shown in **Table 1**. The mean age of the participants was 23.52 ± 5.656 years, and the mean number of sexual partners was 0.22 ± 0.529. Among these women, 78.6% were high school graduates (n = 393), 79% were single (n = 395) and 50.6% (n = 253) had a monthly income of between 0 and 500 Turkish lira (approximately 0 to 70.32 United States dollars). In addition, 63.6% (n = 318) had health insurance from the Turkish Social Insurance Institution (SGK) and 95% (n = 485) had not reached the menopause (**Table 1**).

Levels of knowledge regarding HPV and cervical cancer

The mean knowledge score from the first 15 questions on the awareness scale for cervical cancer and HPV infection was 4.69 ±

4.02. More than half of the women provided incorrect answers to the information questions about cervical cancer and HPV infection in the first section. At the same time, the mean knowledge scores were also low.

The perceived threat due to cervical cancer

Regarding the perceived threat due to cervical cancer, perceived sensitivity and severity scores, which are subdimensions of health beliefs, were evaluated. The subdimension score for perceived sensitivity was determined to be a maximum of 45.[12,16]

Perceived sensitivity

The mean perceived sensitivity score in our study was 25.32 ± 6.38. Among the participants, 50% were worried about getting cervical cancer and HPV. Conversely, 50% of the women stated that they did not have any information about prevention of cervical cancer and the measures to be taken against HPV infection.

Table 1. Sociodemographic characteristics of the women participating in the study (n = 500)

	n (%)
Age in years	23.52 (5.656)*
Education level	
Illiterate	4 (0.8)
Literate	15 (3)
Primary school graduate	21 (4.2)
Secondary school graduate	18 (3.6)
High school graduate	49 (9.8)
University/college level	393 (78.6)
Marital status	
Single	395 (79)
Married	91 (18.2)
Widow	5 (1)
Significant partnerships	8 (1.6)
In-home or out-of-home partnerships	1 (0.2)
Monthly income	
0-500 TL	253 (50.6)
501-1000 TL	111 (22.2)
1001-1500 TL	22 (4.4)
1501-2000 TL	26 (5.2)
2001-2500 TL	30 (6)
2501-3000 TL	21 (4.2)
Over 3000 TL	37 (7.4)
Health insurance	
Social insurance institution	318 (63.6)
Pension fund	60 (12)
Pension fund for the self-employed (Bağ-kur)	65 (13)
Social security institution	43 (8.6)
Optional insurance	9 (1.8)
Unemployment insurance	5 (1)
Menopausal status	
Yes	6 (1.2)
No	485 (97)
Not sure	9 (1.8)

*Mean (standard deviation); TL = Turkish lira.

Perceived severity

Similarly, the mean perceived severity score was 17.74 ± 4.03. The perceived severity scores of the participants ranged from 6 to 30 points. Among the women, 41.2% saw HPV infection as a life-threatening disease, whereas 38.2% saw cervical cancer as a curable disease.

For each item derived from the previous versions of the CHIAS, the expressions were changed to reflect the perspective of a young adult rather than a parental perspective. In this process, the sentence format used by Dempsey et al. was taken as an example.[19] The subdimension scores of the CHIAS were as follows: harm = 13.8 ± 3.37; obstacles = 8.53 ± 1.97; effects = 4.61 ± 1.30; and uncertainty = 4.42 ± 21.22.

In our study, the relationships between the subdimensions of the HPV and cervical cancer awareness questionnaire and those of the CHIAS were explained through simple effect modeling from a structural equation model (SEM). The adaptation values were as follows: minimum discrepancy (CMIN) = 34.911; degrees of freedom (df) = 13; minimum discrepancy/degrees of freedom ratio (CMIN/df) = 2.685; root mean square error of approximation (RMSEA) = 0.058; comparative fix index (CFI) = 0.938; and goodness-of-fit index (GFI) = 0.981. Because CMIN/df was not within the required limits, correction indices were examined. The "effects" subdimension of CHIAS provided a correction in accordance with the modification index, with the item of levels of knowledge of cervical cancer and HPV: the F1 and F2 dimensions.

The analysis was repeated by removing the "effects" subdimension item from the model. Then, the adaptation values were as follows: CMIN = 8.617; df = 8; CMIN/df = 1.077; RMSEA = 0.012; CFI = 0.998; and GFI = 0.994. All of the adaptation criteria were thus met within the desired limits.

The nonstandard path coefficient of F2 was 0.152, and this was statistically significant (P < 0.001). The standard path coefficient for this item was 0.249. The nonstandard path coefficient of the perceived severity subdimension was 1, and this was statistically significant (p < 0.001). The standard path coefficient for this item was 0.851. The nonstandard path coefficient of the perceived sensitivity subdimension was 1.366, and this was statistically significant (P < 0.001). The standard path coefficient for this item was 0.736.

The nonstandard path coefficient of the "harm" subdimension was 1, and this was statistically significant (P < 0.001). The standard path coefficient for this item was 0.62. The nonstandard path coefficient of the "obstacles" subdimension was 0.336, and this was statistically significant (P < 0.001). The standard path coefficient for this item was 0.357. The nonstandard path coefficient of

the "uncertainty" subdimension was 0.223, and this was statistically significant (P < 0.001). The standard path coefficient for this item was 0.382. The nonstandard path coefficient for the levels of knowledge of cervical cancer and HPV was 0.174, and this was statistically significant (P < 0.001). The standard path coefficient for this item was 0.149 (**Figure 1**; **Table 2**).

Pearson's correlation was used because it showed parametric distribution between the awareness scale for cervical cancer and HPV infection and the subdimensions of the CHIAS (**Table 3**). There were no relationships between the subdimension of levels of knowledge of cervical cancer and HPV of the awareness scale for cervical cancer and HPV infection and the "harm", "obstacles" and "uncertainty" subdimensions of the CHIAS. On the other hand, a positive and highly significant relationship was found with the "effects" subdimension. Very high positive correlations were found between the perceived sensitivity subdimension of the awareness scale for cervical cancer and HPV infection and the "harm", "effects" and "uncertainty" subdimensions of the CHIAS. Moreover, very high significant positive correlations were found between the perceived severity subdimension of the awareness scale for cervical cancer and HPV infection and the "harm", "effects" and "uncertainty" subdimensions (**Table 3**).

DISCUSSION

Cervical cancer is a type of cancer that is monitored regularly through screening programs around the world, including in Turkey, and it can be treated quickly when detected.[20,21] Through use of these screening programs for cervical cancer, mortality

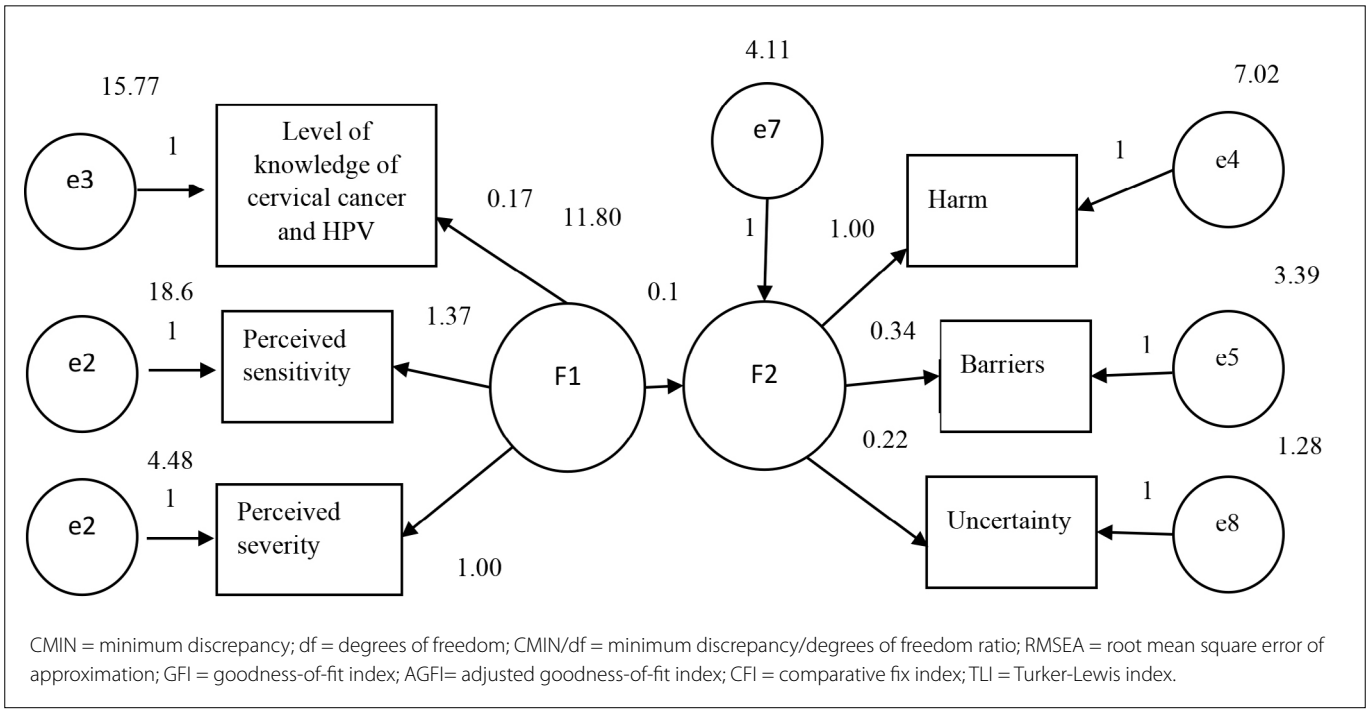

Figure 1. Nonstandard path coefficients between the HPV and cervical cancer awareness questionnaire and the Carolina HPV Immunization Attitudes and Beliefs Scale (CHIAS).

Table 2. Structural equation model (SEM) analysis between the HPV and cervical cancer awareness questionnaire and the Carolina HPV Immunization Attitudes and Beliefs Scale (CHIAS)

Scale subdimensions and factor two	Impact direction	Factors	β^0	β^1	Std error	Statistical test	P	R^2
F2	<---	F1	0.249	0.152	0.051	2.981	0.003	0.062
Perceived severity	<---	F1	0.851	1				0.725
Perceived sensitivity	<---	F1	0.736	1.366	0.3	4.559	< 0.001	0.542
Harm	<---	F2	0.62	1				0.384
Barriers	<---	F2	0.357	0.336	0.099	3.401	< 0.001	0.128
Uncertainty	<---	F2	0.382	0.223	0.065	3.421	< 0.001	0.146
Level of knowledge about cervical cancer and HPV	<---	F1	0.149	0.174	0.064	2.729	0.006	0.022

β^0 = standardized coefficient; β^1 = non-standardized coefficient; Std error = standard error; R^2 = coefficient of determination.

and morbidity due to this disease are gradually decreasing.[21] The important factors in treating this disease are early diagnosis and women's awareness. The degree of cancer or the size of the lesions that can be treated are decisive in the treatment process.[20,22] Although cervical cancer is preventable, reports in the literature have demonstrated that, despite knowing about cervical cancer, women are not aware of the factors involved in its development.[20,23,24]

In our study, the women's knowledge score about cervical cancer and HPV infection was low (4.69 ± 4.02), contrary to the findings in studies in the literature. Although 50% of the women were afraid of getting cervical cancer and HPV infection, they did not have any information about preventing this infection. In a study conducted by Montgomery et al. to determine the level of knowledge of cervical cancer and HPV, the knowledge score was 7.39 among 149 women.[23] In a study by Ozan et al., 336 women who visited a gynecology outpatient clinic were assessed regarding their level of knowledge of cervical cancer and HPV, and it was observed that although 86.6% of them knew about cervical cancer, only 33.6% knew about HPV infection.[24] Pehlivanoğlu et al. found that 26.8% out of 295 women who visited a family medicine outpatient clinic had never heard of the Pap smear test and 43.4% did not know about HPV infection.[20]

Although the women in our study had heard about HPV vaccines, their attitudes and knowledge regarding HPV vaccination was inadequate. This situation might have originated from the women's low awareness of cervical cancer and inadequate knowledge of HPV infection. The level of knowledge of HPV vaccines that we found in our study was consistent with data in the literature.[9,13,25]

Pelullo et al. conducted a study among 556 nursing students, with the aim of examining their knowledge and attitudes regarding HPV vaccines. They found that although almost all the students had heard about the vaccine, only 36.5% were aware of its risk factors.[13]

In a study by Yılmaz et al., in which 624 nursing students were examined in terms of their knowledge, behavior and attitudes in relation to HPV vaccines, their levels of knowledge regarding the vaccine were lower than their levels of knowledge regarding HPV infection.[9] Although 87.7% of those students knew that an HPV vaccine for women exists, only 52.4% were aware of the existence of an HPV vaccine for men.[9]

In our study, the subdimension scores for "obstacles" "harm" and "uncertainty" in relation to getting the HPV vaccine were low. This might have been due to lack of knowledge about the vaccine, lack of vaccine availability, the women's lack of awareness about the vaccine effects and unavailability of the vaccinees. This result was consistent with data in the literature.[9,13,26]

In our study, significant relationships were found between the responses to the questionnaire regarding levels of knowledge of cervical cancer and HPV infection and the subdimensions of the CHIAS. Women who were worried about cervical cancer had higher levels of knowledge about HPV. Meanwhile, a significant relationship was found between women who thought they had a high probability of getting cervical cancer or HPV infection and the total knowledge score and the "harm" subdimension score. There were significant relationships between women's fear of being infected with HPV virus and the "effects" and "obstacles" subdimension scores of the CHIAS. This might have been due to inability to cover the cost of the vaccine, lack of a vaccination program across the country and lack of information about where women can obtain the vaccine. This result was consistent with data in the literature.[9,13]

Knowledge about HPV vaccines increased with the perceived sensitivity, and there was a positive relationship between them. Conversely, a negative relationship was observed between the level of knowledge of cervical cancer and HPV infection and the "harm" subdimension. As the level of knowledge increased, the number of women thinking that the vaccine was harmful decreased. These results were consistent with data in the literature.[9,10,13,27]

Pelullo et al. reported that there was a positive relationship between the levels of knowledge of HPV vaccines among the students in their study and these students' awareness of the risk factors.[13]

Giuseppe et al. explored HPV awareness among 1,348 adolescent girls and young women and reported that those who saw themselves at risk of cervical cancer and HPV infection had higher levels of knowledge about HPV vaccination.[10]

In a cross-sectional study, in which Napolitoni et al. examined women's knowledge and attitudes regarding HPV infection and vaccines, a positive significant relationship was found between women carrying and/or knowing about HPV risk factors and their levels of knowledge and attitudes in relation to HPV vaccines.[27]

Table 3. Correlation between the awareness scale for cervical cancer and HPV infection and the subdimensions of the Carolina HPV Immunization Attitudes and Beliefs Scale (CHIAS)

n = 500	CHIAS subdimensions							
	Harm		Barriers		Effects		Uncertainty	
	r	P	r*	P	r*	P	r*	P
Level of knowledge of cervical cancer and HPV	-0.83	0.65	-0.06	0.88	0.20**	0.00	0.04	0.31
Perceived sensitivity	0.12**	0.00	0.06	0.12	0.15**	0.00	0.10*	0.02
Perceived severity	0.12**	0.00	0.06	0.12	0.12**	0.00	0.09*	0.04

*Correlation is significant at the 0.05 level; **Correlation is significant at the 0.01 level.

CONCLUSION

In our study, women's knowledge and attitudes towards cervical cancer and HPV infection were found to be inadequate. This inadequacy had an effect on their levels of knowledge regarding HPV vaccines. The highest score on the scale of knowledge about HPV vaccines was 56, but the mean score of these women was 33.37 ± 5.05. Through this result, it was seen that women did not have enough information about HPV vaccines and that HPV vaccine-related education was needed. It was also found that women were not getting vaccinated because of their lack of knowledge about vaccine access, its effects and its cost. Considering the efforts made towards ensuring widespread use of cervical cancer screening programs, similar strategies and programs need to be developed for HPV vaccine programs, in order to provide greater immunity against HPV infection. Further qualitative and quantitative studies are needed in order to determine HPV vaccine awareness in Turkey.

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Relationships between self-reported dyspnea, health conditions and frailty among Brazilian community-dwelling older adults: a cross-sectional study

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ABSTRACT

CONTEXT: Dyspnea is a symptom present in several chronic diseases commonly seen among older adults. Since individuals with dyspnea tend to stay at rest, with consequently reduced levels of physical activity, they are likely to be at greater risk of developing frailty, especially at older ages.

DESIGN AND SETTING: Cross-sectional study at community level, Brazil.

OBJECTIVE: To analyze the relationships between self-reported dyspnea, health conditions and frailty status in a sample of community-dwelling older adults.

METHOD: Secondary data from the follow-up of the Frailty in Brazilian Elderly (FIBRA) study, involving 415 community-dwelling older adults (mean age: 80.3 ± 4.68 years), were used. The variables analyzed were sociodemographic characteristics, reported dyspnea, clinical data and frailty phenotype. Associations between dyspnea and other variables (age, sex, education and body mass index) were verified through the crude (c) and adjusted (a) odds ratios.

RESULTS: The prevalence of dyspnea in the entire sample was 21.0%. Dyspnea was more present in individuals with pulmonary diseases, heart disease, cancer and depression. Older adults with multimorbidities (adjusted odds ratio, ORa = 2.91; 95% confidence interval, CI = 1.41-5.99) and polypharmacy (ORa = 2.02; 95% CI = 1.15-3.54) were more likely to have dyspnea. Those who reported dyspnea were 2.54 times more likely to be frail (ORa = 2.54; 95% CI = 1.08-5.97), and fatigue was their most prevalent phenotype component.

CONCLUSION: Dyspnea was associated with different diseases, multimorbidities, polypharmacy and frailty. Recognizing the factors associated with dyspnea may contribute to its early identification and prevention of its negative outcomes among older adults.

INTRODUCTION

Dyspnea is a symptom that is present in different chronic diseases, with high prevalence among older adults.[1,2] The American Thoracic Society defines dyspnea as "a subjective experience of breathing discomfort that consists of qualitatively distinct sensations that vary in intensity".[3] Since dyspnea is a frequent symptom among community-dwelling older adults,[2,4] it is important that healthcare professionals should routinely evaluate and document the presence of dyspnea, just like they do for pain.[3,5]

In clinical practice, dyspnea is initially assessed by a single question (e.g. "Are you short of breath? Yes or No).[6] Single-item questionnaires have clinical importance, as they are simple and quick to use, but they fail to cover all aspects of dyspnea.[7] A large number of scales for measuring dyspnea have been described in the literature. Scales based on reports of breathlessness during activities of daily living (e.g. the modified Medical Research Council Dyspnea Scale and the Baseline Dyspnea Index) and on the perception of breathlessness while making exertions (e.g. the modified Borg scale and visual analogue scale) are the ones most frequently used.[8] Because multidimensional tools such as the Multidimensional Dyspnea Profile have the ability to assess different aspects of dyspnea, they are promising tools for use in both laboratory and clinical settings.[9] Nonetheless, most breathlessness scales were developed and validated for patients with respiratory diseases and, consequently, they need to be properly tested before being applied to other populations.[7]

A systematic review showed that the estimated prevalence of dyspnea among community-dwelling older adults was 36%, and that dyspnea can have an impact on different activities of daily living.[2] In Brazil, a cross-sectional study among community-dwelling older adults observed that 30.9% of the participants had dyspnea symptoms. Moreover, most of the older adults who reported dyspnea had poor physical performance, were women, had multimorbidity and were frail.[4] In addition to presenting an association between dyspnea and cardiorespiratory diseases, older adults with moderate to severe dyspnea are more likely to be unable to perform a single chair stand, have depressive symptoms, use psychoactive drugs and be underweight and/or obese, than those with no or mild dyspnea.[1,10] Because presence of dyspnea is associated with several chronic diseases and health issues, some authors have argued that dyspnea in older adults should be considered to be a multifactorial geriatric condition.[10,11]

Common adaptive responses to the presence of dyspnea include avoidance of physical effort and, consequently, adoption of a sedentary lifestyle.[3] In patients with chronic obstructive pulmonary disease (COPD), this can precipitate a downward spiral of dyspnea-induced inactivity, thus resulting in physical deconditioning, progression of disease and disability.[12] Thus, some studies have shown that COPD patients were more likely to be frail, and that this was associated with airflow limitations, reduced physical function, dyspnea, disability, anxiety and depression.[13]

Frailty is a medical syndrome with multiple causes and contributors that confers vulnerability to stressors and increases the risk of different negative outcomes at older age, such as dependency and/or death.[14] Regarding physical frailty, physical inactivity plays an important role in the progression of this syndrome, as it directly influences some determinant components for development of frailty.[15] Although the overlap between dyspnea-induced inactivity and vicious cycles of frailty seems obvious, the relationships between dyspnea, health conditions and frailty among community-dwelling older adults are not well established.[16] Vaz Fragoso et al.,[16] for example, found that older adults with respiratory impairment had higher chances of becoming frail, and vice versa. In another study,[17] the same authors observed that poor physical-functional performance and frailty status were associated with moderate to severe exertional dyspnea in community-dwelling older adults.

Considering that the older population is growing and that dyspnea is a highly prevalent symptom in this phase of life, it would be useful to identify the factors associated with this important symptom, especially with a view to adequate healthcare management and/or prevention of negative outcomes among older adults. In addition, in the presence of dyspnea, the person tends to stay at rest, which can cause a series of negative consequences, such as physical inactivity, deconditioning and disability, which are commonly associated with the onset and progression of frailty syndrome.

OBJECTIVE

In order to collaborate in healthcare practices for older adults, the aim of the present study was to analyze the relationships between self-reported dyspnea, health conditions and the frailty phenotype in a sample of community-dwelling older adults.

METHODS

Study design and participants

This was a cross-sectional study that used the records of community-dwelling older adults who participated in the second wave of the Frailty in Brazilian Elderly (FIBRA) study, conducted in the city of Campinas and in the Ermelino Matarazzo district of the city of São Paulo, state of São Paulo, Brazil, between 2016 and 2017. The first wave of data collection occurred between 2008 and 2009 and its database contained a record of 1284 older adults. Details about the sample size calculation, recruitment, data collection and main findings of the first wave can be found elsewhere.[18]

The second wave of the FIBRA study was submitted to and approved by the local ethics committees on November 23, 2015 (approval number, CAAE: 49987615.3.0000.5404) and on September 17, 2018 (CAAE: 92684517.5.0000.5404). The present study was also approved by the ethics committee board on October 31, 2019 (CAAE: 22196619.7.0000.5390).

Procedures

The participants in the first wave of the FIBRA study were contacted and invited to participate in the follow-up study. At the beginning of the single session of data collection, which was conducted at the participants' homes, these older adults were informed about study procedures and voluntarily signed an informed consent statement, after agreeing to participate.

Older adults who fulfilled the inclusion and exclusion criteria completed the interview. The inclusion criteria were as follows: the participants needed to have participated in the first wave and to have remained as permanent residents in the geographical location. Older adults with cognitive impairment suggestive of dementia, severe disability (wheelchair-bound or bedridden), motor and/or cognitive sequelae from stroke, moderate/severe Parkinson's disease, severe visual and/or hearing impairments, or decompensated diseases or terminal illness, were excluded. Considering that the number of chronic diseases (cardiovascular, respiratory, cancerous, etc.) increases with age,[19] older adults with these diseases were not excluded from the study. In addition, given that information on comorbidities was accessed through self-reporting (detailed below) and access to medical files was not feasible, it was not possible to classify the diseases reported with exactness (e.g. the type of respiratory disease reported). Current smokers were also

included in the study since their prevalence in the total sample was low (i.e. 3.0%) (**Table 1**).

From the original cohort, 549 older adults (≥ 65 years of age) agreed to participate in the second wave. Another 192 had died, and 543 could not located or refused to participate. Among those who completed the initial part of the interview (sociodemographic information, anthropometric measurements, physical frailty assessment and Mini-Mental State Examination, MMSE), 419 had MMSE scores above the cutoff point, adjusted for years of schooling, and participated in the second block of measurements. Out of these, 415 participants answered the question about the presence of dyspnea (i.e. the dependent variable) and thus were included in the present study.

It is important to note that a sample size ≥ 329 participants was found to be adequate for the present study, based on an estimated prevalence of dyspnea of 30.9%,[4] a 95% confidence level and an error of 0.5%.

The following variables/scales were extracted from the FIBRA study database:

- Self-reported dyspnea: The participants were asked about the presence of dyspnea through a simple question: "Do you have breathlessness, yes or no?".
- Sociodemographic variables: Sex (men, women); age ranges (72-79 years and ≥ 80 years); and schooling (illiterate, 1-4 years and ≥ 5 years).
- Anthropometric data: Weight was measured in kilograms, using the G-Tech brand scale; and height in centimeters, using a scale (200 cm), graduated in centimeters and millimeters. Body mass index (BMI) was obtained by dividing the weight (kg) by the height squared (m^2). BMI was classified in accordance with the cutoff values established by the Pan-American Health Organization (PAHO). In this way, older adults are categorized as follows: underweight with BMI ≤ 23 kg/m^2; normal weight with BMI between > 23 kg/m^2 and < 28 kg/m^2; overweight with BMI between > 28 kg/m^2 and < 30 kg/m^2; and obese ≥ 30 kg/m^2.[20]
- Smoking habit: Participants were considered to be current smokers if they answered "yes" to the following question: "Do you currently smoke?".
- Self-reported chronic diseases: Information was obtained from dichotomous items (yes or no) in which it was investigated whether a doctor had diagnosed heart diseases, hypertension, diabetes mellitus, depression, pulmonary diseases, osteoporosis, stroke, cancer and/or osteoarthritis at any time during the 12 months prior to the interview. Multimorbidity was considered to be the coexistence of two or more of these diseases.
- Frailty: This was assessed based on the phenotype criteria proposed by Fried et al.[15] Older adults were qualified as frail (three or more criteria), pre-frail (one or two criteria) or non-frail (no criteria), considering the following five criteria:
  - Self-reported unintentional weight loss: this was considered to be present if, over the last year, it was greater than or equal to 4.5 kg or 5% of body weight.
  - Fatigue: this was considered to be present if the older adults answered "occasionally" or "most of the time" for either of the following two self-report items regarding how they felt during the last week: "I felt that everything I did was an effort" and "I could not get going". These items were obtained from the Center for Epidemiologic Studies Depression Scale (CESD).[21,22]
  - Low grip strength of the dominant hand: this was considered to be present, as measured using a Jamar dynamometer (Lafayette Instruments, Lafayette, Indiana, United States), if the average measurement from three grip attempts with the arm flexed at 90° at the forearm was among the 20% lowest distribution values, after adjusting for sex and BMI.
  - Gait slowness: this was considered to be present, as assessed from the time in seconds spent to walk a distance of 4.0 meters at the usual speed, if the average of three attempts was among the 20% highest distribution values (in seconds) for the entire sample, after adjusting for sex and height.[23]
  - Inactive level of physical activity: The level of physical activity corresponded to the weekly frequency and daily duration of physical exercise, sports and household chores, based on responses to the items of the Minnesota Leisure Time Activity Questionnaire.[24] To calculate the weekly caloric expenditure on leisure activities and on household chores, we considered the number of items to which the older adult replied affirmatively, multiplied by the number of days in the week and the number of minutes per day. Then we calculated the quintiles of the distribution of this variable, for men and women separately. We considered participants to be inactive if they scored among the 20% lowest distribution values for weekly caloric expenditure, corrected according to sex.[25]

Statistical analysis

The sample was characterized by calculating the absolute and relative frequencies and 95% confidence interval (95% CI) of the variables considered. Associations between dyspnea and the other variables were verified using Pearson's chi-square test or Fisher's exact test. After that, simple and multiple logistic regression analyses were also used to estimate crude and adjusted odds ratios. The adjusted analysis was performed independently according to age, sex, education and BMI classifications for each variable. For all analyses, the significance level was set at $P < 0.05$.

RESULTS

Four hundred and fifteen older people (mean age 80.3 ± 4.68 years old) answered the question about shortness of breath. The

majority of the participants were very old (56.4% were aged 80 years and over), were females (69.8%), had had one to four years of education (59.7%), had normal weight (i.e. 43.8% had BMI between > 23 kg/m² and < 28 kg/m²) and were not currently smokers (97.0%). In addition, these older adults mostly had two or more self-reported diseases (69.3%), used less than five medications (62.6%) and were classified as pre-frail (64.3%) (**Table 1**).

The prevalence of dyspnea in the entire sample was 21% (95% CI = 17.3 to 25.2). The frequencies and associations between dyspnea and sociodemographic variables, anthropometric data, health conditions and frailty are presented in **Table 1**. No significant difference between the sexes was found with regard to the prevalence of dyspnea, but the prevalence was greater (P = 0.014) in the younger age group (26.7% for 72-79 years of age) than in the older group (16.7% for 80 years and over). However, presence of dyspnea was not associated with BMI (P = 0.075) or with the smoking habit (P = 0.985).

In relation to health conditions, dyspnea was also more prevalent among participants with polypharmacy (29.8% versus 16.0%; P = 0.002) and multimorbidities (24.0% versus 10.2%; P = 0.002). **Figure 1** shows the prevalence of dyspnea according to the self-reported diseases evaluated in the present study. Dyspnea was more frequent among older adults with heart diseases (31.5% versus 17.0%; P = 0.002), cancer (41.2% versus 18.1%; P = 0.001), pulmonary diseases (35.5% versus 17.6%; P = 0.004) and depression (31.3% versus 17.8%; P = 0.012). In addition, the prevalence of dyspnea was higher (P = 0.022) in older adults who were frail (35.2%) than in those classified as pre-frail (19.6%) or non-frail (17.2%) (**Table 1**). The analysis on frailty components showed that dyspnea was more present in older adults who presented fatigue (34.7% versus 15.4%; P = 0.000) and gait slowness (32.8% versus 18.8%; P = 0.012) (**Figure 2**).

After adjusting for age, sex, schooling and BMI classification, the logistic regression showed that the participants with pulmonary diseases (adjusted odds ratio, ORa = 2.25; 95% CI = 1.08 to 4.67), heart diseases (ORa = 2.06; 95% CI= 1.17 to 3.62), cancer (ORa = 3.25; 95% CI = 1.48 to 7.18) and depression (ORa = 1.94; 95% CI = 1.04 to 3.59) were more likely to have dyspnea. Other health conditions also associated with the presence of dyspnea were multimorbidities (ORa = 2.91; 95% CI = 1.41 to 5.99) and polypharmacy (ORa = 2.02; 95% CI = 1.15 to 3.54). Moreover, older adults who reported dyspnea had 2.54 times (95% CI = 1.08 to 5.97) more chances of being classified as frail than as pre-frail or non-frail (**Table 2**).

Among the frailty phenotype components, fatigue (crude odds ratio, ORc = 2.90; 95% CI = 1.77 to 4.75) and gait slowness (ORc = 2.10; 95% CI = 1.16 to 3.79) showed associations with dyspnea in the crude analysis. After controlling for confounding variables (i.e. age, sex, schooling and BMI classification), only fatigue remained significantly associated with dyspnea (ORa = 2.75; 95% CI = 1.62 to 4.66).

Figure 3 shows the overlaps between the presences of dyspnea, multimorbidity and frailty/pre-frailty. The main overlapping detected was between multimorbidity and frailty (44.8%), followed by dyspnea versus multimorbidity versus frailty/pre-frailty (14.5%). The rates of overlapping of dyspnea with multimorbidity and frailty/pre-frailty were 3.1% and 2.5%, respectively. In addition, dyspnea only appeared separately from multimorbidity and frailty in fewer than 1% of the participants. It is important to note that 7.3% of the participants did not have any of these conditions, either alone or in combination.

DISCUSSION

In the present study, the prevalence of dyspnea among community-dwelling older adults was 21%, which was within the range reported by previous studies, even those differing in assessment method used (i.e. single question versus the Medical Research Council Dyspnea Scale).[2,4] Older adults who reported having

Table 1. Percentage distribution of the sample and the proportion of dyspnea according to sociodemographic variables, anthropometric data, health conditions, and frailty. FIBRA study, Campinas and Ermelino Matarazzo district in São Paulo, São Paulo, Brazil, 2016/2017

Variables	n (%)	Dyspnea		P-value
		No n (%)	Yes n (%)	
Sex				
Male	125 (30.2)	105 (84.0)	20 (16.0)	0.100
Female	289 (69.8)	222 (76.8)	67 (23.2)	
Age groups (years)				
72-79	180 (43.6)	132 (73.3)	48 (26.7)	**0.014**
≥ 80	233 (56.4)	194 (83.3)	39 (16.7)	
Schooling level				
Illiterate	52 (13.3)	38 (73.1)	14 (26.9)	
1-4 years	234 (59.7)	188 (80.3)	46 (19.7)	0.398
≥ 5 years	106 (27.0)	87 (82.1)	19 (17.9)	
Body mass index classification				
Underweight	72 (17.3)	62 (86.1)	10 (13.9)	
Normal	180 (43.8)	147 (81.7)	33 (18.3)	0.075
Overweight	50 (12.0)	38 (76.0)	12 (24.0)	
Obese	113 (27.2)	81 (71.7)	32 (28.3)	
Current smoker				
Yes	12 (3.0)	9 (75.0)	3 (25.0)	0.985
No	399 (97.0)	306 (79.1)	81 (20.9)	
Polypharmacy				
0-4	219 (62.6)	184 (84.0)	35 (16.0)	**0.002**
≥ 5	131 (37.4)	92 (70.2)	39 (29.8)	
Multimorbidity				
0-1	118 (30.7)	106 (89.8)	12 (10.2)	**0.002**
≥ 2	267 (69.3)	203 (76.0)	64 (24.0)	
Frailty				
Not frail	93 (22.6)	77 (82.8)	16 (17.2)	
Pre-frail	265 (64.3)	213 (80.4)	52 (19.6)	**0.022**
Frail	54 (13.1)	35 (64.8)	19 (35.2)	

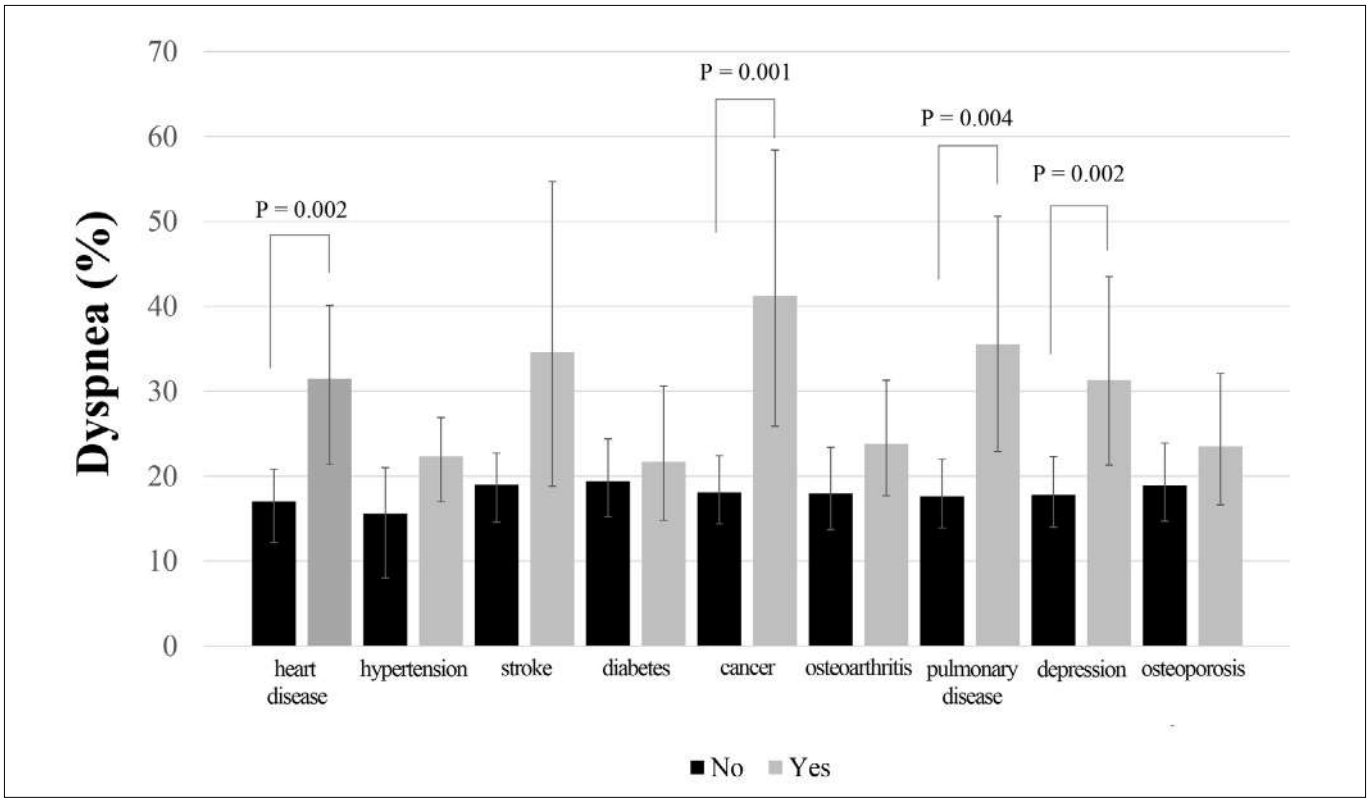

Figure 1. Prevalence of dyspnea according to self-reported diseases.

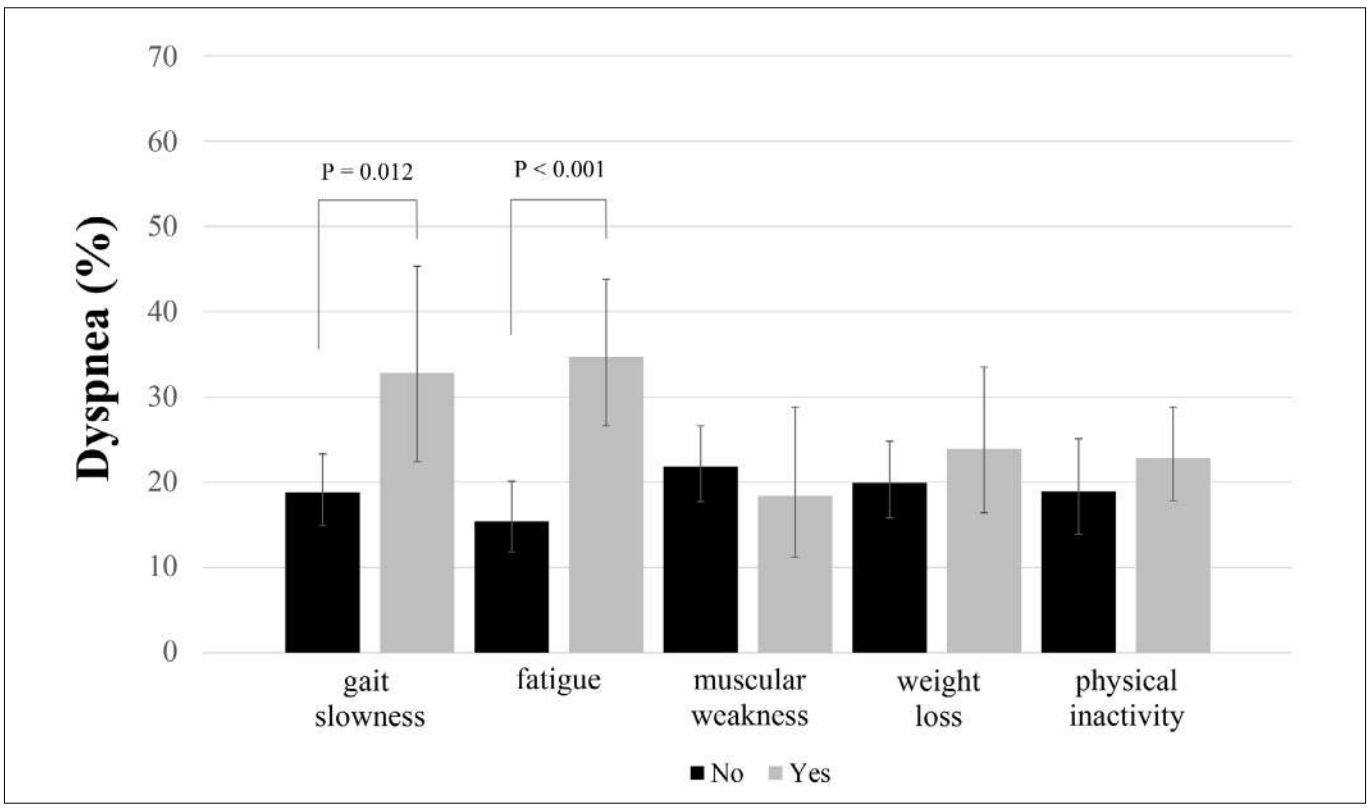

Figure 2. Prevalence of dyspnea according to the presence of frailty phenotype components.

breathlessness were more likely to have pulmonary diseases, heart diseases, cancer, depression, multimorbidity and polypharmacy. In addition, dyspnea occurred more frequently among participants who were classified as frail (i.e. 35.2%), and fatigue was the phenotype component that was most strongly associated with dyspnea.

There is evidence in the literature that the prevalence of dyspnea increases with advancing age.[2,26,27] In a representative sample of North American older adults,[1] it was observed that one in four adults aged 70 and over experienced clinically significant breathlessness, evaluated via a single question (i.e. "How often do you become short of breath while awake?"). Greater prevalence of multimorbidities[10] and polypharmacy,[28] reduced physical fitness[3] and decreased efficiency of the respiratory system[11] make older adults more susceptible to dyspnea, which corroborate the increasing prevalence of dyspnea among older age ranges.

Although our results were in agreement with the literature in terms of the overall prevalence of dyspnea among older adults, it was surprisingly higher among those aged 72 to 79 years than among those aged 80 and over. Nonetheless, a previously published geriatric dyspnea model supports the notion of paradoxical reduction in

Table 2. Crude and adjusted odds ratios for dyspnea, according to sociodemographic and anthropometric characteristics, health and clinical conditions, and frailty status and phenotype components of the FIBRA study. City of Campinas and Ermelino Matarazzo district of the city of São Paulo, São Paulo, Brazil, 2016/2017

Variables	Dyspnea					
	OR_{crude}	95% CI	P-value	$OR_{adjusted}$	95% CI	P-value
Sociodemographic and anthropometric characteristics						
Sex						
Female versus male	1.58	0.91-2.75	0.101	1.37	0.77-2.44	0.439
Age groups (years)						
≥ 80 versus 72-79	0.55	0.34-0.89	**0.015**	0.59	0.35-0.98	**0.041**
Schooling level						
1-4 years versus illiterate	0.66	0.33-1.33	0.247	0.64	0.31-1.32	0.228
≥ 5 years versus illiterate	0.59	0.27-1.30	0.194	0.56	0.26-1.24	0.209
Body mass index classification						
Underweight versus normal	0.72	0.33-1.55	0.398	0.64	0.27-1.49	0.301
Overweight versus normal	1.41	0.66-2.98	0.373	1.39	0.63-3.08	0.412
Obese versus normal	1.76	1.01-3.07	**0.047**	1.54	0.86-2.78	0.148
Health and clinical conditions						
Self-reported diseases						
Heart diseases	2.24	1.32-3.80	**0.003**	2.06	1.17-3.62	**0.012**
Hypertension	1.55	0.88-2.73	0.128	1.53	0.84-2.82	0.167
Stroke	2.25	0.96-5.27	0.061	1.50	0.58-3.86	0.399
Diabetes	1.14	0.66-1.98	0.620	1.10	0.62-1.95	0.746
Cancer	3.17	1.52-6.59	**0.002**	3.25	1.48-7.18	**0.003**
Arthritis	1.42	0.86-2.34	0.164	1.41	0.82-2.40	0.211
Pulmonary diseases	2.57	1.31-5.03	**0.006**	2.25	1.08-4.67	**0.029**
Depression	2.11	1.17-3.80	**0.013**	1.94	1.04-3.59	**0.036**
Osteoporosis	1.31	0.77-2.22	0.308	1.41	0.79-2.50	0.239
Multimorbidity						
≥ 2 versus 0-1	2.78	1.44-5.39	**0.002**	2.91	1.41-5.99	**0.004**
Polypharmacy						
≥ 5 versus 0-4	2.23	1.32-3.75	**0.003**	2.02	1.15-3.54	**0.014**
Frailty status and phenotype components						
Frailty						
Pre-frail versus not frail	1.17	0.63-2.18	0.609	1.14	0.58-2.22	0.705
Frail versus not frail	2.61	1.20-5.67	**0.015**	2.54	1.08-5.97	**0.032**
Frailty components (versus absence of the criterion)						
Weight loss	1.26	0.73-2.18	0.398	1.48	0.81-2.69	0.197
Low physical activity	1.26	0.78-2.04	0.330	1. 20	0.72-2.03	0.482
Low grip strength	0.80	0.42-1.52	0.509	0.81	0.40-1.64	0.563
Gait slowness	2.10	1.16-3.79	**0.013**	1.71	0.89-3.30	0.106
Fatigue	2.90	1.77-4.75	**< 0.001**	2.75	1.62-4.66	**< 0.001**

OR = odds ratio; CI = confidence interval; adjusted: age, sex, schooling and body mass index classification. In bold: statistically significant associations.

the perception and reporting of dyspnea, explained in terms of the influence of physiological, neurological, psychological and social changes with aging.[29] Thus, it is plausible that the self-reported dyspnea rate is reduced in the older age ranges. Additionally, another study with the same cohort showed that a considerable portion of longer-living older adults (i.e. 80 years of age and over) were in good health,[30] which contradicted the results from investigations in other countries.[31-33] One hypothesis is that people with better health and socioeconomic conditions are more likely to reach advanced ages. So, it is possible that the lower prevalence of dyspnea in the oldest-old group can also be partly due to this (i.e. older adults with worse conditions, including those associated with the presence of dyspnea, would die earlier).[34,35] Given that cohort studies involving very old community-dwelling adults are scarce in Brazil, the possibility for discussion of the present results is limited.

Regarding the factors associated with dyspnea in older adults, the present results partly corroborate the findings of Smith et al.[1] Among community-dwelling older adults, those authors found that individuals with respiratory diseases, multimorbidity, heart diseases, obesity and low educational level were more likely to have dyspnea. In addition, dyspnea was associated with depression, anxiety, fatigue, pain, dependence with regard to activities of daily living and high use of healthcare services. Interestingly, Johnson et al.[36] showed that age was inversely related to restrictive breathlessness during the last year of life. They argued that individuals who lived to old age achieved that age because they probably did not have the medical conditions associated with restrictive dyspnea, which thus supports the hypothesis raised above.

In old age, the presence of multimorbidity may be more complex due to overlapping of other conditions such as physical impairment, mental disorders, frailty and polypharmacy.[30,37,38] These conditions impose a considerable burden at an individual level, and on healthcare and social services too.[33] Previous studies demonstrating the complexity of healthcare management for older people showed that multimorbidity and polypharmacy were also associated with dyspnea,[1,28] which was concordant with our findings. Smith et al.,[1] for example, observed that older adults with significant breathlessness were more likely to have multimorbidity than single chronic conditions.

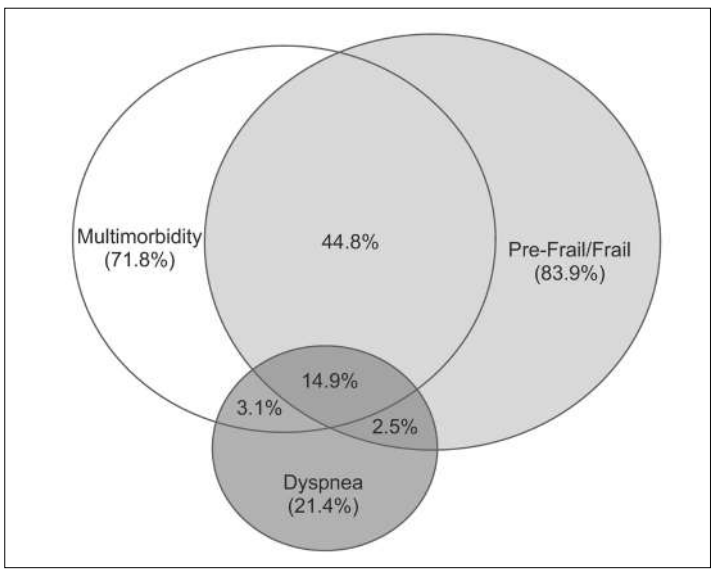

Figure 3. Overlapping between dyspnea, multimorbidity and frailty/pre-frailty status.

A cross-sectional study conducted in Brazil also showed that community-dwelling older adults with dyspnea, assessed using the modified Medical Research Council Dyspnea Scale, had more diseases than those without this symptom.[4] Regarding polypharmacy, Akgün et al.[28] observed that it was strongly related to dyspnea (as measured on a shortness-of-breath scale from 0 to 10 scale within the Edmonton Symptom Assessment System) among older adults with serious life-limiting diseases. After adjusting for different factors (age, sex, diagnosis and statin discontinuation), each additional medication was associated with 8% and 16% increased risk of mild and moderate-to-severe dyspnea, respectively. In addition to associations between dyspnea and some chronic self-reported diseases, multimorbidity and polypharmacy, it was also observed in the present study that dyspnea could occur in combination with multimorbidity and/or frailty/pre-frailty conditions. These, in turn, may bring more complexity to healthcare for community-dwelling older adults.

The relationship between respiratory impairment/diseases and frailty among older adults has been demonstrated in different studies.[13,16,39] In a meta-analysis, Marengoni et al.[39] found that older adults with COPD had twofold increased odds for presenting frailty, compared with those without COPD. Among community-dwelling older adults, Vaz Fragoso et al.[16] showed that respiratory impairment was associated with frailty at the baseline and that there was an increased likelihood of developing frailty features after three years of follow-up. Although studies involving COPD patients have supported the existence of a relationship between dyspnea and frailty,[13,39,40] it is not known whether this is true for community-dwelling older adults.

In the present study, older adults who reported dyspnea were more likely to be classified as frail, even after adjustment for age, sex, schooling and BMI classification. Moreover, although fatigue and gait slowness were the frailty phenotype components that were associated with reports of dyspnea, only fatigue remained associated with dyspnea after controlling for confounding factors. According to Vaz Fragoso et al.,[17] poor performance in the single chair stand was associated with a greater chance of having moderate-severe exertional dyspnea, which became attenuated when controlled for some frailty components singly. Similarly, Silva et al.[4] observed that there was high prevalence of frailty (23.6%) among Brazilian community-dwelling older adults who reported having dyspnea. After

adjusting for different confounders, higher dyspnea score was found to be independently associated with poor physical performance.[4] Among COPD patients, Medina-Mirapeix et al.[40] demonstrated that low grip strength was the most prevalent component among those classified as frail, followed by low physical activity levels and fatigue. However, only fatigue was associated with dyspnea and severity of COPD symptoms in the final multivariate models.

Some conceptual models for explaining the dyspnea-inactivity vicious cycle in COPD are available in the literature. Recently, Ramon et al.[41] proposed a model in which a sequence of events (e.g. expiratory airflow limitation, increased resting lung volumes and dynamic hyperinflation) might lead to a cycle consisting of the following: dyspnea - reduced physical activity - deterioration of exercise capacity - dyspnea. Although not included in Ramon's model, fatigue might be easily fit into the dyspnea-inactivity vicious cycle, independently of a specific diagnosis. In an observational study involving community-dwelling older adults, Egerton et al.[42] showed that fatigue was associated with lower physical activity levels. This finding did not confirm whether fatigue was a cause of low physical activity levels or whether it occurred as a result from inactivity. Therefore, those authors suggested that reduced activity might also lead to decreased physical capacity, which would hence increase inactivity-related fatigue through a reversed causal pathway. Surprisingly, neither low physical activity levels nor low grip strength was associated with dyspnea in our study. Therefore, it is still unclear whether dyspnea-inactivity and frailty cycles overlap or not among community-dwelling older adults and whether a reversed causal pathway might exist in this population. This pathway would consist of the following: frailty - reduced physical activity - deterioration of physical reserves and increased exercise intolerance - dyspnea.

In summary, it was observed that a considerable proportion of the community-dwelling older adults of our study reported shortness of breath. Unlike other age groups, this symptom may be related not only to specific conditions (such as respiratory and cardiovascular diseases) but also to the presence of multimorbidities, polypharmacy and frailty. Thus, self-reported dyspnea should be considered to be an important symptom among older adults and should not be attributed to age *per se*. In addition to single-item questionnaires, it is important that shortness of breath should be investigated in a broader manner in this population, especially taking into account its impact on activities of daily living. However, it needs to be borne in mind that the scales most used for assessing dyspnea were developed before publication of the Consensus of the American Thoracic Society.[3] Thus, these scales cannot cover the entirety of the definition of dyspnea in terms of functional, sensory-perceptual and affective aspects. In this way, multidimensional scales to evaluate dyspnea should be also considered when assessing the geriatric population. It is noteworthy that, in addition to adequate clinical management of chronic diseases and usage of medications, care for older adults with dyspnea should be carried out in an interdisciplinary and integrated manner, as much as possible.

The present study had some limitations that should be addressed. First of all, the cross-sectional nature of the study did not allow us to draw causal associations between dyspnea, health conditions and frailty. Therefore, further longitudinal studies should include objective respiratory measurements that would help clarify such relationships and the effect of respiratory impairment on the progression of frailty, especially among community-dwelling older adults. Second, as the present sample was not representative of the community-dwelling older population and only included individuals aged 72 years and over, the results need to be interpreted with caution. Third, as the presence of dyspnea was evaluated just by a single question, the impact of its severity, commonly assessed using the Medical Research Council Dyspnea Scale, was unknown in our sample. In addition, methodological bias cannot be ruled out, given that different approaches were used to measure dyspnea (a single screening question) and frailty (a well-established tool). However, it is important to note that no reliable and valid dyspnea scales for Brazilian community-dwelling older adults were available at the time of design and application of the research protocol.

CONCLUSIONS

Among community-dwelling older adults, dyspnea was associated with different diseases, multimorbidities, polypharmacy and frailty, even after controlling for age, sex, schooling and body mass index. Among those reporting dyspnea, fatigue and gait slowness were more likely to be present. Recognizing the factors associated with reports of dyspnea may contribute to prevention of negative outcomes and to management of frailty in this population.

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The first appointment with a nephrologist: Brazilian patients' demographic and kidney function characteristics. A retrospective study

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ABSTRACT

BACKGROUND: The number of nephrologists has risen slowly, compared with the prevalence of chronic kidney disease (CKD) in Brazil. Data on patients referred to nephrology outpatient clinics remains scarce.

OBJECTIVE: To determine the demographic and kidney function characteristics of patients at their first appointment with a nephrologist.

DESIGN AND SETTING: Retrospective study conducted at three nephrology outpatient clinics (public and private services), in São Paulo, Brazil.

METHODS: From December 2019 to February 2020, we collected patient data regarding demographics, kidney function parameters and comorbidities. We then analyzed data on 394 patients who met a nephrologist for their first appointment.

RESULTS: The main comorbidities were hypertension (63.7%), diabetes (33.5%) and nephrolithiasis (22.3%). Regarding CKD stages, 24.1%, 9.1%, 13.7%, 15.2%, 15.2% and 2.3% of the patients were in stages 1, 2, 3a, 3b, 4 and 5, respectively. Proteinuria was absent or mild, moderate and high in 17.3%, 15.2% and 11.7%, respectively; and 16.2% had not undergone previous investigation of serum creatinine or proteinuria (55.8%). For 17.5%, referral to a nephrologist occurred late. Patients in public services were older than those in private services (59 years versus 51 years, respectively; P = 0.001), more frequently hypertensive (69.7% versus 57.5%; P = 0.01) and reached a nephrologist later (22.4% versus 12.4%; P = 0.009).

CONCLUSION: Referrals to a nephrologist were not being made using any guidelines for CKD risk and many cases could have been managed within primary care. Late referral to a nephrologist happened in one-fifth of the cases and more frequently in the public service.

INTRODUCTION

The number of nephrologists in Brazil increased by 25%, from 3,500 to 4,400 between 2008 and 2018.[1] Within the same ten years, the number of patients on maintenance dialysis rose by 52%, from 87,044 to 133,000, approximately.[2] Nephrologists are distributed differently across the country. While the northern region has 0.7 nephrologists per 100,000 inhabitants, the southern and southeastern regions have 6.7 nephrologists per 100,000 inhabitants.[1] This disproportion between the numbers of nephrologists and the numbers of patients who need them is seen worldwide, even in developed countries.[3] In the United States, between 1996 and 2012, the number of patients who started dialysis rose from 300,000 to 500,000, while the number of nephrologists decreased from 18 to only 10 per 1,000 patients.[3,4]

According to KDIGO (Kidney Disease Improving Global Outcomes), every person who presents chronic kidney disease (stages 4 and 5) and the ones who have high levels of albuminuria (albumin to creatinine ratio > 300 mg/g) should be referred to a nephrologist.[5] The Brazilian Ministry of Health also recommends that patients who are in stages 4 or 5 of chronic kidney disease (CKD) should be followed up by a specialist.[6]

Delay in reaching a nephrologist is associated with unfavorable outcomes and higher healthcare expenditure.[7-11] In Brazil, late referral to a nephrologist was first demonstrated in 1995.[12] In that study, about 60% of the patients who started dialysis had not been followed up on an outpatient basis by a nephrologist.

On the other hand, some studies have suggested that many patients who are referred to a nephrologist can be easily followed up within primary care.[13,14] Bahiense-Oliveira et al. showed

that 52% of the patients assisted by a nephrologist did not need to be assessed or treated by this specialty at their first appointment.[13] Another study showed that 35.7% of the patients assessed by nephrologists had stages 1 and 2 of CKD and only a few of them (26%) presented higher levels of proteinuria or albuminuria, meaning that many patients could have continued to be cared for within primary care.[14]

OBJECTIVE

Because of the need for accurate medical referral to nephrologists and the lack of these specialists, the aim of this study was to describe the characteristics (sociodemographic and CKD stages) of patients who were assessed by nephrologists at their first appointment, in both public and private services.

METHODS

Study design and participants

This was a multicenter retrospective study based on medical records. We included three outpatient clinics in the metropolitan area of São Paulo: two clinics affiliated with private health insurance services and one public clinic within the Brazilian National Health System (Sistema Único de Saúde, SUS). We analyzed information on first appointments with a nephrologist that took place between December 2019 and February 2020, among patients who were ≥ 18 years old. We excluded those who had undergone kidney transplantation or who were on kidney replacement therapy. In the Brazilian public service, patients can only reach specialists through a medical referral from primary care or from other specialists. In private services, patients can reach specialists either through referrals or through their own initiative.

The protocol for this study was approved by the Ethics Committee of Universidade Federal de São Paulo on June 5, 2020 (CAAE 31053420.9.1001.5505).

Definitions and parameters of interest

Basic characteristics and clinical information relating to diagnoses of hypertension, diabetes mellitus (DM), urinary lithiasis, recurrent urinary tract infection, polycystic kidney disease and glomerulonephritis were obtained from the patients' charts. We defined hypertension as the use of anti-hypertensive drugs or the presence of this diagnosis in the patient's chart. DM was defined from use of oral antidiabetic drugs or insulin therapies or the presence of this diagnosis in the patient's chart.

Laboratory assessments included serum creatinine and proteinuria. We used serum creatinine, age, and gender to determine the estimated glomerular filtration rate (eGFR), in accordance with the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation.[15] The racial factor was not included in the eGFR calculation because of the multiethnic composition of the Brazilian population and because of a previous study that demonstrated that this adjustment did not contribute to greater accuracy in this population.[16] CKD was defined as eGFR < 60 ml/min/1.73 m^2 or the presence of biomarkers for renal dysfunction, such as proteinuria, dysmorphic hematuria or abnormal kidney ultrasound. We classified CKD into five stages in accordance with the current guidelines: stage 1 (eGFR ≥ 90 ml/min/1.73 m^2 and any renal dysfunction biomarker); stage 2 (eGFR of 60-89 ml/min/1.73 m^2 and any renal dysfunction biomarker); stage 3a (eGFR of 45-59 ml/min/1.73 m^2), stage 3b (eGFR of 30-44 ml/min/1.73 m^2); stage 4 (eGFR of 15-29 ml/min/1.73 m^2); and stage 5 (eGFR < 15 ml/min/1.73 m^2). We considered stages 3b, 4 and 5 to be advanced CKD.[5]

We used the following methods to determine the levels of proteinuria: urinalysis (dipstick); random urinary albumin-to-creatinine ratio (ACR); random urinary protein-to-creatine ratio (PCR); 24-hour albuminuria; and 24-hour proteinuria. We stratified the patients into three categories according to their level of proteinuria: absent or mild (urinalysis negative or 1+, ACR < 30 mg/g, PCR < 150 mg/g, 24-hour albuminuria < 30 mg or 24-hour proteinuria < 150 mg); moderate (1+ or 2+ on urinalysis, ACR of 30-300 mg/g, PCR of 150-500 mg/g, 24-hour albuminuria of 30-300 mg and 24-hour proteinuria of 150-1000 mg); and high (3+ on urinalysis, ACR > 300 mg/g, PCR > 500 mg/g, 24-hour albuminuria > 300 mg and 24-hour proteinuria > 1000 mg).[5]

Among the reasons for referring patients to a nephrologist, we considered the following: hypertension, diabetes, nephrolithiasis, recurrent urinary tract infection, hematuria (red blood cells above the laboratory reference levels) and acute kidney injury (serum creatinine > 0.3 mg/dl, in comparison with the baseline serum creatinine, investigated within the preceding three months before data collection).[17] We collected data on the specialties from which patients were referred to a nephrologist (internal medicine, endocrinology, cardiology or urology). Also, we registered whether patients reached a nephrologist by themselves, with no medical referral. Late referral was defined as referral in stages 4 or 5.[7-11]

Patients for whom serum creatinine and proteinuria information was available were classified into CKD risk groups: low risk, moderate risk, high risk and very high risk.[18]

Sampling and statistical analyses

We calculated the sample size based on the following equation:[19] $N = n^*X / (X + n - 1)$; in which $X = Z_{(\alpha/2)}{}^2 * p*(1-p) / error^2$. "$Z_{(\alpha/2)}$" is the critical value for a normal distribution when $\alpha/2$ (confidence interval = 95%, $\alpha = 0.05$ and critical value of 1.96), "p" represents the proportion of the referred patients in the sample, "error" is the estimated margin around "p", and "n" means the size of the population. We estimated that 40% of the patients

were correctly referred to a nephrologist, in conformity with previous research.[12,13] We considered that the size of the population was 100,000 inhabitants. We set error and confidence intervals of 8% and 95%, respectively. In this manner, we determined that a minimum of 144 medical records from public and private services would need to be analyzed.

We used the statistical package SPSS, version 18.0 (SPSS Inc., Chicago, Illinois, United States). We described the frequencies of the categorical variables. Age showed non-normal distribution and so we presented data on its median and interquartile range (IQR). We used χ^2 or Fisher exact tests to compare the frequencies, as appropriate. Also, we used the Mann-Whitney test to compare non-normal continuous variables. We set the significance level for P-values at < 0.05.

RESULTS

The demographic and clinical characteristics (renal data and comorbidities) of our sample are shown in **Table 1**. The median age was 55 years old (IQR, 42-67); 51.5% of the patients were male; and the most frequent comorbidities were hypertension (63.7%), DM (33.5%), nephrolithiasis (22.3%) and recurrent urinary infection (8.6%). The distribution of the patients regarding CKD grading was 24.1% in stage 1, 9.1% in stage 2, 13.7% in stage 3a, 15.2% in stage 3b, 15.2% in stage 4 and 2.3% in stage 5.

Proteinuria was observed to be mild or absent in 17.3% of the patients, while 15.2% presented moderate and 11.7% presented high levels of proteinuria. The percentages of patients referred to a nephrologist with no information on serum creatinine and proteinuria were 16.2% and 55.8%, respectively. Among patients with

Table 1. Characteristics of the patients and comparison between public and private healthcare services

	All (n = 394)	Public (n = 201)	Private (n = 193)	P-value*
Age, years (range)	55 (42-67)	59 (47-69)	51 (38-64)	0.001
Male, n (%)	203 (51.5)	97 (48.3)	106 (54.9)	0.18
Reasons for referring to a nephrologist/comorbidities, n (%)				
Hypertension	251 (63.7)	140 (69.7)	111 (57.5)	0.01
Diabetes mellitus	132 (33.5)	74 (36.8)	58 (30.1)	0.15
Nephrolithiasis	88 (22.3)	41 (20.4)	47 (24.4)	0.35
Recurrent urinary tract infection	34 (8.6)	19 (9.5)	15 (7.8)	0.55
Polycystic kidney disease	15 (3.8)	8 (4.0)	7 (3.6)	0.85
Glomerulonephritis	4 (1.0)	1 (0.5)	3 (1.6)	0.29
Acute kidney injury	14 (3.6)	8 (4.0)	6 (3.1)	0.64
Hematuria	8 (2.0)	4 (2.0)	4 (2.1)	0.95
Other	59 (15.0)	18 (9.0)	41 (21.2)	0.001
CKD stage, n (%)				
1	95 (24.1)	41 (20.4)	54 (28.0)	0.07
2	36 (9.1)	23 (11.4)	13 (6.7)	0.11
3a	54 (13.7)	31 (15.4)	23 (11.9)	0.31
3b	60 (15.2)	39 (19.4)	21 (10.9)	0.02
4	60 (15.2)	43 (21.4)	17 (8.8)	< 0.001
5	9 (2.3)	2 (1.0)	7 (3.6)	0.08
Proteinuria stratification, n (%)				
Absent or mild	68 (17.3)	28 (13.9)	40 (20.7)	0.74
Moderate	60 (15.2)	40 (19.9)	20 (10.4)	0.008
High	46 (11.7)	30 (14.9)	16 (8.3)	0.58
Referral with no serum creatinine result, n (%)	64 (16.2)	22 (10.9)	42 (21.8)	0.004
Referral with no proteinuria result, n (%)	220 (55.8)	103 (51.2)	117 (60.6)	0.06
No CKD, n (%)	16 (4.1)	0 (0.0)	16 (8.3)	< 0.001
Advanced CKD, n (%)**	129 (32.7)	84 (41.8)	45 (23.3)	< 0.001
Late referral, n (%)***	69 (17.5)	45 (22.4)	24 (12.4)	0.009
Referred from, n (%)				
No referral	72 (18.3)	0 (0.0)	72 (37.3)	< 0.001
General practitioner (internist)	228 (57.9)	187 (93.0)	41 (21.2)	< 0.001
Endocrinologist	28 (7.1)	3 (1.5)	25 (13.0)	< 0.001
Urologist	11 (2.8)	0 (0.0)	11 (5.7)	0.002
Other	55 (14.0)	11 (5.4)	44 (22.8)	< 0.001
Discharge from the nephrologist	39 (9.9)	7 (3.5)	32 (16.5)	< 0.001

*Public versus private; **Chronic kidney disease (CKD) stages 3b, 4 and 5; ***CKD stages 4 and 5.

hypertension and without DM, the referral rates for those with results from laboratory tests on serum creatinine and proteinuria were 87% and 48%, respectively. Among those with DM, the corresponding referral rates reached 91% and 56%, respectively.

Late referral (stages 4 and 5) was found in 17.5% of the participants. Most of the patients (57.9%) were referred to a nephrologist by a general practitioner. About 10% of the patients were discharged by the nephrologist after the first appointment.

Compared with the private insurance patients, the individuals seen in public outpatient clinics were older (59 [IQR, 47-69] versus 51 [IQR, 38-64] years old; P = 0.001), more commonly hypertensive (69.7% versus 57.5%; P = 0.01) and were referred later to a nephrologist (22.4% versus 12.4%; P = 0.009). In the public service, patients were more frequently referred to a nephrologist without serum creatinine results (10.9% versus 21.8%, respectively; P = 0.004) and without proteinuria tests (51.2% versus 60.6%; respectively, P = 0.06). The type of doctor who most frequently referred patients to a nephrologist was the general practitioner (93%) in the public service; while in the private service patients reached a nephrologist predominantly without any referral (37.3%) followed by referral from a general practitioner (21.2%).

According to the risk map for CKD,[19] 19.9% of the patients were at low risk, 21% at moderate risk, 24% at high risk and 34.8% at very high risk (**Table 2**). Compared with patients seen at private outpatient clinics, those seen within the public healthcare system presented lower probability of being at low risk of CKD (11.2% versus 32.4%, respectively; P < 0.001) and higher risk of CKD (69.4% versus 44.1%; respectively, P = 0.001) (**Table 3**).

DISCUSSION

The Brazilian guidelines regarding CKD define that the risk stratification should be conducted within primary care through assessing serum creatinine and proteinuria levels.[6] The Brazilian guidelines for hypertension and DM also include serum creatinine and proteinuria tests performed annually, as a minimum.[20]

This study showed that for one patient in six, no information on serum creatinine was available at the time of the first appointment with a nephrologist. Additionally, more than half of the patients were not investigated regarding urinary protein levels. Failure in screening for CKD has also been observed in other regions in which the rates of serum creatinine monitoring (32.5% to 73.5%) and proteinuria assessment (2.5% to 40%) were low.[21-23]

Considering the impact of aging on the decline in renal function,[24] 23 patients (5.9% of the sample) may not necessarily have needed to be referred to a nephrologist (patients aged > 75 years; eGFR < 60; and proteinuria assessment not performed or absent). Nonetheless, most of them were referred without any assaying of proteinuria (20 patients).

The prevalences of hypertension and DM in our sample were 63.7% and 33.5%, respectively. According to a survey by the Brazilian Nephrology Society,[2] the most common causes of CKD stage 5 are hypertension and DM. Indeed, these diseases can be identified and prevented within primary care.[25-28] Proteinuria plays an important role in accelerating the progression rate of CKD, but its assessment was neglected among 52% of hypertensive and 44% of diabetic patients. Although testing of proteinuria levels is important, this was not usually performed.

Compared with the patients seen via the private healthcare service, the public service patients who reached a nephrologist showed higher rates of advanced CKD (stages 3b, 4, and 5), but serum creatinine and proteinuria were more frequently assessed before the referral. Because of the scarcity of nephrology appointments within the public service, those individuals may reach the

Table 2. Patients' distribution* according to the risk map for chronic kidney disease[19]

Chronic kidney disease stages	Estimated glomerular filtration rate** (ml/min/1.73 m²)	Proteinuria stratification		
		Absent or mild n (%)	Moderate n (%)	High n (%)
1	> 90	26 (15.7)[a]	19 (11.4)[b]	9 (5.4)[c]
2	60-89	7 (4.2)[a]	10 (6.0)[b]	9 (5.4)[c]
3a	45-59	6 (3.6)[b]	12 (7.2)[b]	7 (4.2)[d]
3b	30-44	10 (6.0)[c]	11 (6.6)[d]	13 (7.8)[d]
4	15-29	12 (7.2)[d]	8 (4.8)[d]	4 (2.4)[d]
5	< 15	0 (0.0)[d]	0 (0.0)[d]	3 (1.8)[d]

*166 patients for whom data on serum creatinine and proteinuria were available. Risk map according to the Kidney Disease Outcomes Quality Initiative (KDOQI); **CKD-EPI equation; [a]low risk; [b]moderate risk; [c]high risk; [d]very high risk.

Table 3. Comparison between public and private health insurance patients regarding the risk of chronic kidney disease[19]

Risk stratum	Public (n = 98)	Private (n = 68)	P
Low	11 (11.2)	22 (32.4)	< 0.001
Moderate	19 (19.4)	16 (23.4)	0.52
High or very high	68 (69.4)	30 (44.1)	0.001

specialist later in time, and with advanced stages of CKD. This may also happen because patients with hypertension or DM are highly adherent to their treatment within primary care. Public service patients had better chances of undergoing CKD screening, probably due to the higher degree of control and requirements for setting up appointments with specialists. Doctors who work for SUS need to provide written justification in advance, to explaining why the patient should be referred to a specialist.

The main type of physician responsible for referring patients to a nephrologist within the public service was the general practitioner, while in private healthcare services there was a broader range of sources such as self-referral, general practitioners and other specialists, thus suggesting that the private healthcare service is highly compartmentalized.

About 25% of the patients referred to a nephrologist did not belong to the major risk groups for CKD (hypertension, DM, elderly people, polycystic kidney disease and glomerulonephritis). This percentage was greater in the public service than in private services (31.1% versus 19.9%, respectively; P = 0.01). Patients at low risk of CKD were more commonly seen in the private healthcare services. These results suggest that there are higher rates of unnecessary appointments with specialists within private services, which can be explained by the convenience of reaching a specialist when the patient can afford it.

Some limitations of this study should be mentioned. First, this was a retrospective study, and some of the patients could not take their laboratory results to their first appointment with the nephrologist, which may have prevented nephrologists from registering patients' lab results in the charts. Second, the limited number of centers included in this study prevented us from generalizing our results to other public or private Brazilian healthcare services. Third, the data available in relation to diabetes and hypertension (diagnosis and pharmacological treatment) may have provided an underestimate of their prevalences, especially because patients with diabetes and hypertension are oligosymptomatic at the beginning of their natural history. However, the result that we have presented through our sampling may work as a comparison for other, future research, in order to educate both patients and healthcare professionals about the early stages of CKD.

CONCLUSIONS

There are opportunities to improve the stratification of the risk of chronic kidney disease (CKD) in both public and private healthcare services. Proteinuria plays an important role in predicting CKD and seems to have been ignored in many patients who are at high risk of CKD, such as hypertensives and diabetics. Late referral to a nephrologist and unnecessary appointments with this specialist are common in public and private services, respectively. Further research aimed at monitoring healthcare quality in the early stages of CKD may improve the way in which physicians refer their patients to a nephrologist.

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Adverse effects associated with favipiravir in patients with COVID-19 pneumonia: a retrospective study

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ABSTRACT

BACKGROUND: Favipiravir is generally used in treating coronavirus disease 2019 (COVID-19) pneumonia in Turkey.

OBJECTIVE: To determine the side effects of favipiravir and whether it is a good treatment option.

DESIGN AND SETTING: Retrospective study conducted in Atatürk Chest Diseases and Chest Surgery Training and Research Hospital, Ankara, Turkey.

METHODS: 357 patients who completed favipiravir treatment at the recommended dose were included. 37 patients with drug side effects and 320 patients without drug side effects were examined in two groups.

RESULTS: Side effects were observed in 37 (10.36%) out of 357 patients using favipiravir. The most common side effect was liver dysfunction, in 26 (7.28%) of the patients. The following other side effects were also observed: diarrhea (1.4%), nausea (0.84%), abdominal pain (0.28%) and thrombocytopenia (0.28%). One patient (0.28%) presented both increased transaminases and nausea.

CONCLUSION: In this study, it was determined that favipiravir may constitute an alternative for treating COVID-19 pneumonia given that its side effects are generally well tolerated and not serious.

INTRODUCTION

Since the first appearance of coronavirus disease 2019 (COVID-19) in Wuhan, China, in December 2019, this disease has evolved into a global pandemic.[1] It is associated with a wide clinical spectrum of conditions, ranging from asymptomatic disease to fatal pneumonia.[2] Treatment protocols remain limited to guideline recommendations and clinical studies. No specific treatment or prophylaxis has been introduced for use.

Favipiravir is a purine analogue and ribonucleic acid (RNA)-dependent polymerase inhibitor that is used for influenza treatment in Japan. It has shown efficacy against many RNA viruses, including Ebola, neurovirus and *Enterovirus*.[3] It is generally considered safe, with good tolerability, low side-effect potential and a half-life of five hours. The reported side effects include diarrhea, elevated transaminase levels, hyperuricemia and neutropenia.[4]

OBJECTIVE

In this study, we aimed to assess the side effects of favipiravir treatment among patients diagnosed with COVID-19 pneumonia, in order to discuss its role as a therapeutic option.

METHODS:

This retrospective study was conducted in Atatürk Chest Diseases and Chest Surgery Training and Research Hospital, Ankara, Turkey. The study included patients admitted to our hospital and hospitalized due to confirmed COVID-19, between September 1, 2020, and October 1, 2020. COVID-19 was diagnosed using the reverse-transcription polymerase chain reaction (RT-PCR) test. In this retrospective study, data on clinical characteristics, along with laboratory and chest computed tomography (CT) findings, were retrieved from digital databases and patient files. Patients > 18 years of age were deemed eligible if complete laboratory and chest CT data for them were available, along with a COVID-19 diagnosis via real-time RT-PCR; or if they showed highly suspicious disease based on clinical-radiological findings, despite RT-PCR negativity.

In accordance with the treatment guidelines for adult COVID-19 patients endorsed by the Turkish Ministry of Health, these patients received a loading oral favipiravir dose of 1600 mg

twice daily, followed by a maintenance dose of 600 mg twice daily, for a total duration of 5 to 10 days.[5]

Demographic data, underlying conditions, clinical signs and symptoms, laboratory and radiological findings, oxygen support requirement, supportive treatments and side effects arising during favipiravir treatment were recorded. Side effects were defined as elevated transaminases, gastrointestinal symptoms (nausea, diarrhea or abdominal pain), high blood sugar or thrombocytopenia. In addition, a few of the patients also had elevated baseline transaminases, but they were not excluded because transaminase levels could also be raised due to the disease itself.

The local ethics committee approved this study (date: Oct 8, 2020; number: 696).

Statistical analysis

Statistical analysis was performed using SPSS 25.0 (Statistical Package for the Social Sciences for Windows, Inc.; Chicago, Illinois, United States). The compatibility of the data to normal distribution was investigated by means of the Kolmogorov-Smirnov test. Data showing the characteristics of continuous variables were expressed as the mean ± standard deviation or median (minimum-maximum); and categorical data as the number and percentage (%). Independent groups were compared using Student's t test or the Mann-Whitney U test. The relationships between variables were evaluated using Pearson's correlation analysis. Categorical variables were compared using the chi-square test. P values < 0.05 were considered statistically significant.

RESULTS

Overall, 357 patients who completed the recommended favipiravir regimen were included. The patients were divided between those who did and those who did not experience drug side effects (n = 37 and n = 320, respectively). In both groups, males comprised the majority of cases (25/37, 67.6%; and 203/320, 63.4%, respectively). The mean ages in the two groups were comparable (62.88 ± 13.9 and 58.95 ± 13.08 years, respectively). Patients with favipiravir-related side effects had higher body mass index (BMI) (28.73 ± 4.51 versus 30.39 ± 4.76, P = 0.03) (**Table 1**).

The most common comorbidities in both groups were hypertension and diabetes mellitus (DM). The most frequently used medications were oral anti-diabetics, angiotensin-converting enzyme inhibitors (ACEIs), angiotensin-receptor blockers (ARBs) and antiaggregating/anticoagulant agents. Patients without favipiravir-related side effects were more likely to be on ARB treatment (3/77; P = 0.04). **Table 1** compares the demographic data, clinical characteristics and radiological findings in the two groups.

The duration of favipiravir treatment was comparable in the two groups. However, patients with favipiravir-related side effects had a significantly longer hospital stay (7.13 ± 4.2 versus 10.78 ± 5.62 days; P < 0.001) and were twice as likely to require intensive care unit admission (5.9% versus 10.8%; P = 0.279). While there were more patients requiring advanced medical treatment among those with favipiravir-related side effects, the difference was insignificant (21.6% versus 14.7%; P = 0.38) (**Table 1**). Among the 357 patients who received favipiravir, 37 (10.36%) had side effects. Among the 231 patients who received favipiravir alone, 19 (8.2%) had side effects, while among the 126 patients who received hydroxychloroquine with favipiravir, 18 (14.3%) exhibited side effects. The incidence of side effects did not differ significantly between these two groups (P = 0.1).

The most frequent drug-related adverse effect (elevated transaminase levels) occurred in 26 patients (7.28%, 26/357); in all cases, this did not require treatment discontinuation and was resolved through supportive therapy. Other adverse effects comprised diarrhea (1.4%), nausea (0.84%), abdominal pain (0.28%) and thrombocytopenia (0.28%). One patient showed elevated transaminases in conjunction with nausea (**Table 2**; **Figure 1**).

A correlation analysis on determinants of favipiravir-related side effects showed that there were positive correlations with BMI and elevated baseline transaminase and ferritin levels; and negative correlations with elevated creatinine and ARB/ACEI use. However, all of these correlations were weak (r ≤ 0.2) (**Table 3**).

DISCUSSION

One major challenge with COVID-19 relates to the absence of reliable evidence regarding therapeutic options. Furthermore, urgent therapeutic needs have resulted in the use of empirical treatments and other agents that were previously investigated for treatment of other coronaviruses. Several drugs, such as chloroquine, umifenovir, remdesivir and favipiravir, are being used for COVID-19 treatments in many countries, including Iran, Japan and China.[6-9]

Favipiravir, an antiviral agent in the nucleotide-analogue class, has been approved for influenza treatment in Japan. Its activity profile against influenza, Ebola and many other RNA viruses has been established as consisting of prevention of viral replication via inhibition of viral RNA polymerase.[10] It is used as a pro-drug, with 94% bioavailability, 54% protein binding and low volume of distribution. Cmax (maximum concentration) is reached within two hours following a single dose, while both Tmax (maximum concentration time) and half-life increase after multiple doses. It has a half-life of 2.5 to 5 hours and is metabolized via rapid renal elimination after hydroxylation, mainly through the action of aldehyde oxidase and marginally through xanthine oxidase. It exhibits dose-and time-dependent pharmacokinetic effects. While not metabolized by the cytochrome P450 (CYP) system, favipiravir inhibits one of the system's components (CYP 2C8). As such, caution is advised when it is co-administered with drugs metabolized by the CYP system.[11,12]

The dose of favipiravir administered varies based on indication. For COVID-19, higher doses are generally preferred.[13,14] According to the national COVID-19 treatment guidelines issued by the Ministry of Health, a twice-daily dose of 1600 mg is recommended on the first day of treatment, followed by 600 mg, twice daily, for a total duration of 5 to 10 days, in patients with

Table 1. Demographic characteristics of the patients

	No side effects (n = 320)	With side effects (n = 37)	P-value
Age, years, n ± SD	62.88 ± 13.9	58.95 ± 13.08	0.102
Gender, n (%)			
Male	203 (63.4%)	25 (67.6%)	0.753
Female	117 (36.6%)	12 (32.4%)	
Favipiravir alone (N = 231), n (%)	212 (91.8%)	19 (8.2%)	0.1
Favipiravir + hq (N = 126), n (%)	108 (85.7%)	18 (14.3%)	
BMI, kg/m² ± SD	28.73 ± 4.51	30.39 ± 4.76	**0.036**
Comorbidities, n (%)			
Hypertension	141 (44.1%)	8 (21.6%)	0.014
DM	96 (30%)	7 (18.9%)	0.224
CAD	57 (17.8%)	4 (10.8%)	0.401
CHF	19 (6%)	1 (2.7%)	0.707
COPD	49 (15.3%)	2 (5.4%)	0.167
Asthma	22 (6.9%)	3 (8.1%)	0.734
Cancer	32 (10%)	4 (10.8%)	0.778
CRF	13 (4.1%)	-	0.377
ILD	1 (0.3%)	1 (2.7%)	0.198
Rheumatological	9 (2.8%)	1 (2.7%)	ns
Medications administered, n (%)			
OAD	63 (19.7%)	2 (5.4%)	0.057
ACEI/ARB	77 (24.1%)	3 (8.1%)	**0.045**
Betablocker	49 (15.3%)	3 (8.1%)	0.352
Antiaggregant/coagulant	38 (11.9%)	4 (10.8%)	ns
Blood parameters			
WBC (×10⁹/l), mean ± SD	7780.16 ± 4839.21	8188.61 ± 4355.72	0.628
Lymphocytes (×10⁹/l), median (range)	1030 (128-44990)	1350 (300-10920)	**0.016**
Platelets (×10⁹/l), mean ± SD	230534.38 ± 104771.79	250837.84 ± 116872.8	0.271
Neutrophil/lymphocyte ratio, median (range)	4.71 (0.12-79.92)	4.46 (0.08-23.44)	0.237
AST(IU/l), median (range)	34 (8-262)	46 (20-332)	**0.012**
ALT(IU/l), median (range)	26 (7-258)	35 (9-348)	**0.016**
CRP (mg/dl), median (range)	86 (1-493)	84.62 (2.5-395.74)	0.811
D-dimer (µg/l), median (range)	790 (156-80000)	780 (200-12430)	0.347
Ferritin (ml/ng), median (range)	320.5 (15-1650)	536.6 (41-1650)	**0.03**
Creatinine (mg/dl), mean ± SD	1.12 ± 0.52	0.94 ± 0.25	**0.04**
SO_2 (%), mean ± SD	87.52 ± 8.77	88.22 ± 6.36	0.64
CT findings, n (%)			
Normal	8 (3.2%)	1 (2.9%)	ns
Frosted glass	232 (92.1%)	30 (88. %2)	0.505
Consolidation	87 (34.5%)	19 (55.9%)	**0.026**
One sided	93 (29.1%)	15 (40.5%)	0.252
Double-sided	190 (59.4(%)	20 (54.1%)	0.252
Favipiravir usage time (days), n ± SD	6.97 ± 2.38	7.73 ± 2.63	0.072
Days of hospitalization, n ± SD	7.13 ± 4.27	10.78 ± 5.62	**< 0.001**
Intensive care transportation, n (%)	19 (5.9%)	4 (10.8%)	0.279
Need for advanced oxygen therapy, n (%)	37 (11.6%)	4 (10.8%)	ns
Need for advanced medical treatment, n (%)	47 (14.7%)	8 (21.6%)	0.387
Exitus, n (%)	37 (11.6%)	4 (10.8%)	ns

WBC: White blood cell count, BMI = body mass index; DM = diabetes mellitus; CAD = coronary artery disease; CHF = congestive heart failure; COPD = chronic obstructive pulmonary disease; CRF = chronic renal failure; ILD = interstitial lung disease; OAD = oral antidiabetics; ACEI/ARB = angiotensin-converting enzyme inhibitor/angiotensin-receptor blocker; AST = aspartate aminotransferase; ALT = alanine aminotransferase; CRP = C-reactive protein; SO_2 = oxygen saturation; ns = non-significant; SD = standard deviation, hq = hydroxychloroquine.

Table 2. Side effects seen with favipiravir

Patients using favipiravir	357
Patients with drug side effects, n (%)	37 (10.4%)
Side effects, n (%)	
Elevated transaminases	25 (7%)
Diarrhea	5 (1.4%)
Nausea	2 (0.56%)
High blood sugar	2 (0.56%)
Abdominal pain	1 (0.28%)
Thrombocytopenia	1 (0.28%)
Nausea + elevated transaminases	1 (0.28%)

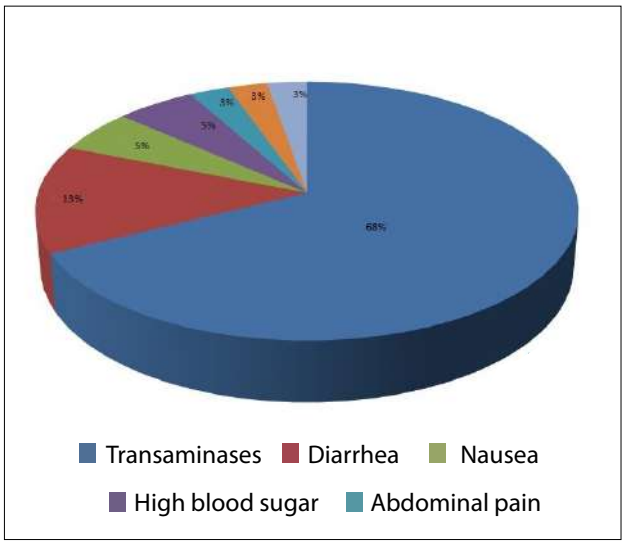

Figure 1. Distribution of side effects.

Table 3. Relationship between favipiravir side effects and clinical-laboratory-radiological features

Clinical-laboratory-radiological features	Favipiravir side effects	
	r (Pearson's correlation coefficient)	P-value
Body mass index	0.111	**0.036**
Lymphocyte count	0.05	0.344
Aspartate aminotransferase	0.212	**< 0.001**
Alanine aminotransferase	0.216	**< 0.001**
Ferritin	0.144	**0.009**
Creatinine	-0.109	**0.04**
Consolidation in thorax tomography	0.143	**0.015**
Angiotensin-converting enzyme inhibitors/angiotensin-receptor blockers	-0.117	**0.027**

pneumonia and probable/definitive disease.[15] All of our patients received this regimen. The most common side effects included elevated hepatic enzymes, gastrointestinal disorders, hyperuricemia and neutropenia.[5]

In a randomized Chinese study comparing favipiravir (at similar doses) with umifenovir (200 mg three times daily, for 10 days), 116 patients received favipiravir and 120 umifenovir. The recovery rates at day seven were comparable (P = 0.139), although the time to resolution of fever and coughing was shorter among favipiravir recipients. Side effects occurred in 31.9% of patients (n = 37) in the favipiravir group versus 23.33% (n = 28) in the umifenovir group (P = 0.14).[16] In our study, 10.36% of the patients had side effects associated with favipiravir use. Transaminase elevation occurred in 8.62% of the patients in the favipiravir group (n = 10) versus 10% (n = 12) among umifenovir patients. Again, in the Chinese study, 13.79% of patients in the favipiravir group had increased serum uric acid levels, while this laboratory parameter was not examined in our study. Gastrointestinal reactions were reported in 13.79% of the patients, while occurring at a lower rate (2.24%) in the current study.

In a Japanese study,[17] side effects were reported in 20% of the patients who received favipiravir in doses lower than those approved for COVID-19. Side effects were generally minor and, like in another study,[5] included hyperuricemia (5%), diarrhea (5%), neutropenia and elevated hepatic enzymes (2%).[17] In a review published in October 2020 that examined 32 studies registered at clinicaltrials.gov, elevated transaminase, eczema and pruritus, abdominal pain, nausea and diarrhea were reported in ≥ 1%, < 0.5%, 0.5%, 0.5 to 1% and ≥ 1% of the patients receiving favipiravir, respectively.[5] In the current study, the most common adverse effect was elevated transaminases (7.28%), with no cases of allergic side effects, while gastrointestinal disorders (abdominal pain, diarrhea and nausea) were found in lower proportions.

Since favipiravir is metabolized and inhibited by aldehyde oxidase, serum concentrations should be monitored and dosing should be adjusted in those with hepatic impairment. Favipiravir, or its metabolites, has been detected in semen and breastmilk.[11,12] Although favipiravir may have pharmacokinetic interactions with oseltamiver, its co-administration with acetaminophen in healthy volunteers resulted in excess exposure to acetaminophen. Meanwhile, as co-administration with theophylline may elevate blood drug concentrations, a concomitant increase in the risk of side effects occurs, thus requiring added caution during use of this combination.

In an interaction chart published by Liverpool Drug Interaction Group in March 2020, favipiravir does not have clinically significant interaction potential with ARBs/ACEIs.[18] Therefore, the presence of more patients with ARB/ACEI use in our group without favipiravir-related side effects may simply have represented a coincidental finding, supportive of the absence of clinical interactions between favipiravir and ARBs/ACEIs.

Clinically significant interactions between favipiravir and hydroxychloroquine are unlikely. Favipiravir is mainly metabolized

by aldehyde oxidase, while hydroxychloroquine is metabolized by the CYPs 2C8, 3A4 and 2Da. It is eliminated via urine, such that 3% of the administered dose is recovered within 24 hours. While favipiravir inhibits CYP 2C8, significant side effects are unlikely, given that hydroxychloroquine is metabolized through multiple pathways.[19] This mechanism may explain the lack of difference between patients who received favipiravir alone and those who received favipiravir with hydroxychloroquine.

Favipiravir appears to be a good therapeutic option for treatment of COVID-19, as it can be administered orally and may also be given to symptomatic patients who do not require hospitalization. Like other antiviral agents, it may be recommendable to initiate favipiravir treatment soon after emergence of symptoms, given its ability to reduce viremia. This may certainly have some epidemiological implications in pandemics, such as in relation to COVID-19. Although side effects are generally well tolerated, laboratory parameters should be closely monitored.

The limitations of the present study were that it was conducted in a single center and had a retrospective design. This study was carried out in accordance with the recorded information only. Thus, it was not easy to reach conclusions regarding risk factors, given that it is possible that not all of them were considered because of the nature of retrospective cohorts.

CONCLUSIONS

Favipiravir is a valuable drug for treatment of mild to moderately severe symptomatic COVID-19 patients. However, further randomized and controlled studies are warranted to provide more reassuring data for physicians regarding its use.

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A new biomarker in severe pneumonia associated with coronavirus disease 2019: hypoalbuminemia. A prospective study

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ABSTRACT

BACKGROUND: Effective triage and early detection are very important for controlling and treating coronavirus disease 2019 (COVID-19). Thus, the relationships between hypoalbuminemia and other acute-phase reactants in such cases need to be evaluated.

OBJECTIVES: To investigate the importance of albumin levels in cases of severe pneumonia due to COVID-19.

DESIGN AND SETTING: Prospective study conducted in Ankara City Hospital (a stage 3 hospital), Turkey.

METHODS: Data from 122 patients diagnosed with pneumonia due to COVID-19 who were admitted to this hospital were analyzed statistically in comparison with date from 60 healthy controls. Three groups were established: healthy controls, intubated patients and non-intubated patients. Lung tomography scans from the patients were examined one-by-one. Real-time polymerase chain reaction (RT-PCR) test results were recorded.

RESULTS: Albumin levels were statistically significantly lower in the intubated and non-intubated groups than in the control group, in comparing the three groups ($P < 0.01$). The other acute-phase reactants, i.e. neutrophil-to-lymphocyte ratio and C-reactive protein levels, were significantly higher in the intubated and non-intubated groups than in the control group ($P < 0.05$). Albumin levels were also significantly lower in the intubated group than in the non-intubated group ($P = 0.02$). No differences were detected with regard to other parameters ($P > 0.05$).

CONCLUSIONS: Hypoalbuminemia may constitute a biomarker indicating the severity of pneumonia due to COVID-19.

INTRODUCTION

Coronavirus disease 2019 (COVID-19) was caused by a novel coronavirus infection called severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).[1] The infection spread rapidly all over the world and was declared to be a pandemic by the World Health Organization. A total of 78,604,532 documented cases had been reported worldwide as of December 26, 2020, and 1,744,235 patients had died.[2] Since there is no current specific treatment and drug against this novel virus, it is very important to determine the risk factors for severe prognosis.

Lung involvement is a serious complication in severe infection and requires hospitalization in an intensive care unit. Usually, the lungs are bilaterally affected. Treatment with mechanical ventilation may be necessary as a result of severe respiratory failure. There is no special drug for treatment of these patients, and supportive treatments are applied. The blood parameters of these patients can be variable. Examination of blood parameters in cases of severe pneumonia due to COVID-19 is providing a guide to prognosis and treatment in cases with comorbidities.

Albumin is a protein synthesized by the liver plays important roles in maintaining nutrition and plasma osmolality.[3] Serum albumin, C-reactive protein (CRP), white blood cell (WBC) and neutrophil-to-lymphocyte (N/L) ratio values are known to be acute-phase reactants. The WBC, CRP and N/L ratio are objective systemic inflammation markers that are usually taken to measure the susceptibility of the patient to mortality.[4-6] In our study, the relationships between these acute-phase reactants were compared in cases of severe pneumonia due to COVID-19.

OBJECTIVES

Serum albumin, C-reactive protein, white blood cell and neutrophil-to-lymphocyte ratio values are known to be acute-phase reactants. Their levels change during acute-period events in metabolism. Changes to in the levels of these biomarkers during the acute period of COVID-19 pneumonia can provide us with information about the severity of the disease and can be used to predict the prognosis.

In our study, the relationships between these acute-phase reactants were compared in cases of severe pneumonia due to COVID-19. Effective triage and early detection are very important for controlling and treating this disease. For this purpose, the relationship between hypoalbuminemia and other acute-phase reactants was compared in cases of severe pneumonia due to COVID-19.

METHODS

Approval was obtained from the Ethics Committee of Ankara City Hospital (date: April 14, 2021; number: 14.04.2021/1527). The blood samples were taken from 122 patients and 60 healthy volunteers and were evaluated using a computer after working in the laboratory.

The subjects were divided into three groups: group 1: healthy controls; group 2: intubated; and group 3: non-intubated. Lung computed tomography (CT) scans from the patients who were diagnosed with COVID-19 were examined one-by-one. Patients who were diagnosed with COVID-19 as a result of lung tomography reports were included in the study. Polymerase chain reaction (PCR) results from the analysis system were reviewed. The tomography findings from 42 PCR-negative patients were compatible with presence of COVID-19. These patients were included in the study.

Albumin, CRP, WBC and N/L ratio values of all the cases included in the study were separately entered into the statistics software. Individuals who were under the age of 18 years, trauma patients, and pregnant women were excluded from the study.

Statistical analysis

Statistical analyses were done by using the IBM SPSS Statistics (version 22) computer software (IBM, Armonk, United States, 2011). The distribution of the variables was examined by using the Kolmogorov-Smirnov and Shapiro-Wilk tests. The quantitative data were expressed as the mean ± standard deviation (SD) or the median and interquartile range (IQR). One-way analysis of variance (ANOVA), with Tukey's post-hoc test, and the independent-group Student t test were applied to the data that showed normal distribution. The Kruskal-Wallis, Mann-Whitney U and Dunn post-hoc tests were applied to the data that did not show normal distribution. Chi-square testing was performed on categorical data. P-values < 0.05 were considered statistically significant.

RESULTS

The demographic data of the pneumonia cases due to COVID-19 and the control group are presented in **Table 1**. No significant differences were detected with regard to age or gender. Among the 122 patients, 80 were not intubated and 42 were intubated and treated with mechanical ventilation. PCR test results were negative in the cases of 42 patients and positive for 80 patients. The tomography scans of all the patients were examined and the findings were entered into the system. The tomography findings from 42 PCR-negative patients were compatible with COVID-19. The tomography results from the two different patient groups with COVID-19 pneumonia are shown in **Figure 1**.

The P-value was calculated for each parameter by making comparisons between the groups in the statistical analyses. From examination of the data of the three groups, it was found that the albumin levels were lower in the intubated and non-intubated groups than in the control group, at statistically significant levels ($P < 0.01$). The distribution of the data is shown in **Figures 2** and **3**. The albumin levels were lower in patients with COVID-19 pneumonia who received negative results from the PCR (**Figure 2**). Also, comparison of the intubated and non-intubated groups showed that the albumin levels were significantly lower in the intubated and non-intubated groups, as seen in **Table 1** ($P = 0.02$). Albumin levels were also found to be significantly lower in the intubated group than in the non-intubated group, as shown in **Figure 3** ($P = 0.02$).

When the other acute-phase reactants, i.e. N/L ratio and CRP level were compared among the three groups, they were found to be significantly higher in the intubated and non-intubated groups ($P < 0.05$). However, no differences were detected between the intubated and non-intubated groups ($P > 0.05$). The WBC values did not show any statistically significant differences among the three groups or between the two groups (intubated and non-intubated) (**Figure 4**).

Comparison of the intubated and non-intubated groups without involving the control group showed that there was only a statistically significant difference in the albumin levels ($P = 0.002$). As demonstrated in **Figure 3**, the albumin levels were found to be even lower in intubated cases than in non-intubated cases. No significant differences were detected with regard to CRP, WBC and N/L ratio ($P > 0.05$).

DISCUSSION

In our study investigating the role of acute-phase reactants in the etiopathogenesis of COVID-19 pneumonia, the low serum albumin level in these cases makes us think that hypoalbuminemia may be a biomarker. The data relating to WBC, CRP and N/L ratio were similar to those reported in the literature. Hypoalbuminemia was seen to be an important prognostic criterion in cases of mortality among individuals with COVID-19

Table 1. Characteristics of routine blood parameters of the three groups: healthy controls, intubated patients and non-intubated patients

Characteristics	Control group (n = 60)	Non-intubated group (n = 80)	Intubated group (n = 42)	P-value*	P-value**
Age in years: median (IQR), range	40.0 (29-72), 18-79	70.0 (57-75), 23-100	80.0 (73-87), 55-100	0.361	0.693
Gender: male/female	34/26	49/31	25/17	0.861	0.876
Laboratory analysis: median (IQR)					
Na, mEq/l	140 (138-142)	138 (135-141)	138 (134-141)	0.236	0.495
K, mEq/l	4.2 (4.0-4.5)	4.2 (3.9-4.7)	4.3 (3.8-4.5)	0.194	0.221
Ca, mg/dl	9.1 (9.0-9.4)	8.6 (8.1-9.3)	8.5 (7.8-9.0)	0.110	0.820
Glucose, mg/dl	98 (85.25-100.2)	115 (95.8-168.5)	118.5 (96.5-165)	**0.003**	0.222
LDH, U/l	198 (172-244.5)	308 (217.5-454.0)	323.5 (204-460)	**0.006**	0.951
WBC $\times 10^9$/l	7.75 (6.7-1.7)	7.3 (5.4-10.8)	7.1 (5.5-9.6)	0.341	0.460
Hemoglobin, g/dl	13.8 (12.7-14.5)	12.0 (10.6-13.6)	11.9 (9.9-12.9)	**< 0.001**	0.795
PLT $\times 10^9$/l	250 (200.8-304.5)	251.5 (160.5-340)	235.5 (148.5-317.3)	0.584	0.570
N/L ratio	2.1 (1.4-5.4)	5.9 (3.4-12.8)	6.9 (2.9-11.7)	**0.002**	0.411
Albumin, g/dl	36.0 (44.0-48.0)	35.0 (32.0-39.0)	31.0 (27.8-35.0)	**< 0.001**	**0.002**
CRP, g/l	0.03 (0.02-0.07)	0.05 (0.01-0.12)	0.08 (0.03-0.12)	**0.041**	0.807
Comorbidities					
Systemic hypertension, n (%)	-	24 (19.7%)		-	-
Diabetes mellitus, n (%)	-	25 (20.5%)		-	-
Ischemic heart disease, n (%)	-	34 (27.9%)		-	-
Chronic renal disease, n (%)	-	5 (4.1%)		-	-
Cancer, n (%)	-	4 (3.3%)		-	-
Cerebrovascular events, n (%)	-	4 (3.3%)		-	-
Chronic obstructive pulmonary disease, n (%)	-	5 (4.1%)		-	-
Neurological disease (Alzheimer, Parkinson etc.)	-	9 (7.4%)		-	-
Other, n (%)	-	12 (9.8%)		-	-

IQR = interquartile range; Na = sodium; K = potassium; Ca = calcium; LDH = lactate dehydrogenase; WBC = white blood cell; PLT = platelet; N/L ratio = neutrophil-to-lymphocyte ratio; CRP = C-reactive protein. P-values less than 0.05 were considered significant and are highlighted in bold. *Comparison of three groups; **Comparison of intubated and non-intubated groups.

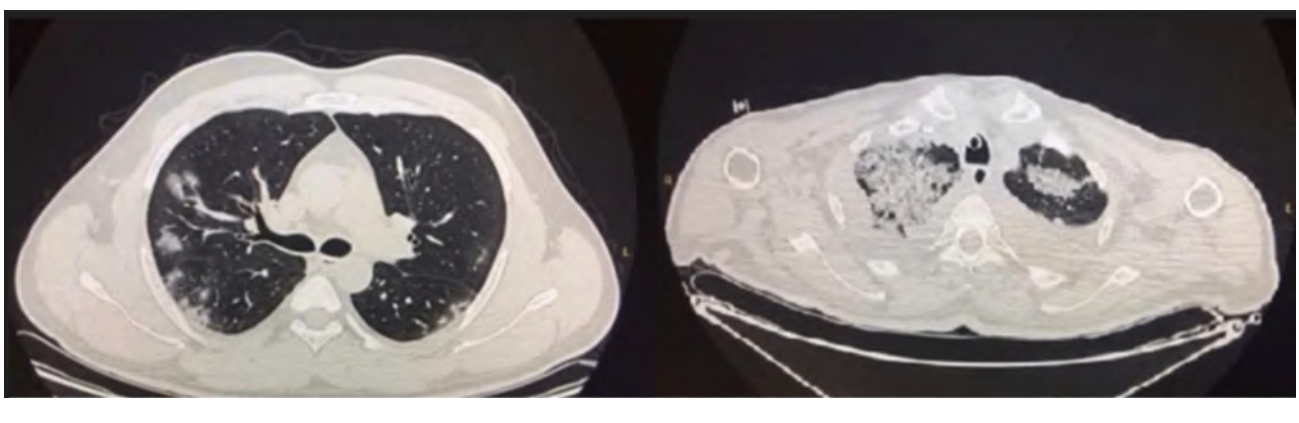

Figure 1. Computed tomography (CT) sample images of patients with COVID-19.

pneumonia. Adding albumin to the treatment may be effective in reducing mortality in cases of hypoalbuminemia, which we think may constitute a biomarker showing the severity of the disease.

The mechanisms of hypoalbuminemia in COVID-19 have not been fully investigated or explained. In some studies, hypoalbuminemia has been detected in severe COVID-19 cases. There is a possibility that the coronavirus may also affect liver functions, and thus may be as the reason for the low serum albumin levels in cases of severe COVID-19 pneumonia. Therefore, hypoalbuminemia may suggest the presence of liver dysfunction.[7]

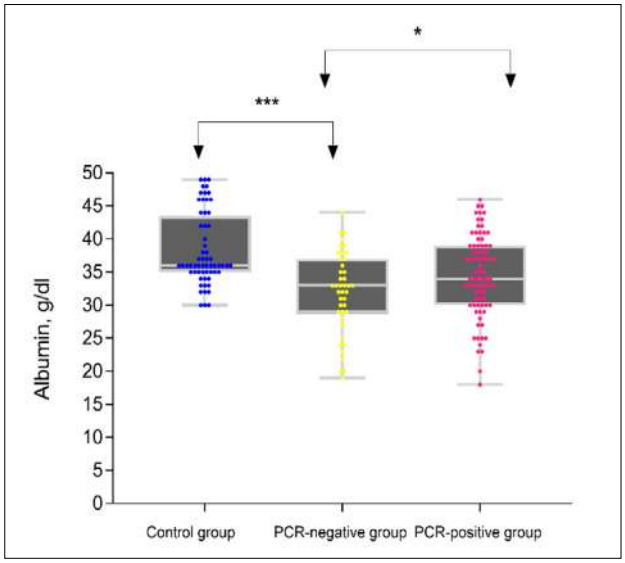

Figure 2. Albumin levels in the three groups (healthy controls, PCR-negative patients and PCR-positive patients) according to polymerase chain reaction (PCR) results.

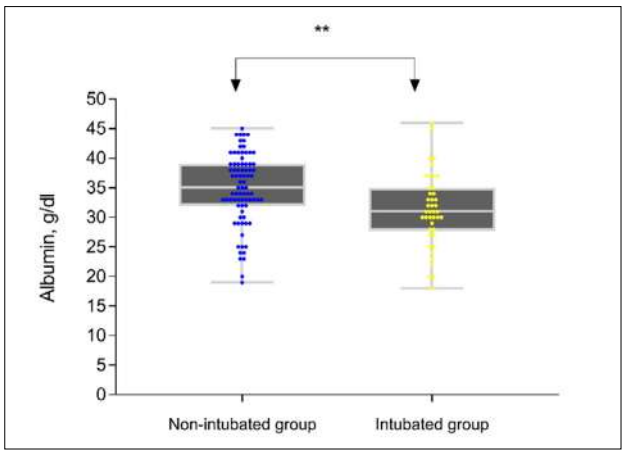

Figure 3. Albumin levels of the patient groups according to intubation.

COVID-19 is a pandemic that first appeared in Wuhan, China, in December 2019, and has spread all over the world. There is still no specific treatment for the disease and vaccine studies are still continuing. Patients with severe bilateral lung involvement are intubated and treated with mechanical ventilation. This is done especially in cases of patients with blood oxygen levels falling below certain levels. Supportive treatments are applied in such cases of severe pneumonia due to COVID-19, which might otherwise lead to death.

Albumin is a protein that is synthesized by the liver and plays important roles in maintaining nutrition and plasma osmolality.[3] Li et al. argued that low albumin levels are indicative of poor nutritional status and also reduce the immunity of the body. Furthermore, they reported that the immune response of the host to ribonucleic acid (RNA) virus infection is often weakened because of the nutritional insufficiency, which is not always taken into consideration in making the clinical diagnosis and implementing the treatment.[7] Comorbid conditions may be the cause of low albumin levels in patients diagnosed with COVID-19 and hypoalbuminemia.

Hypoalbuminemia is related to inflammation. Inflammation causes expansion of the interstitial gap and the volume of albumin distributed increases through increased capillary permeability, with escape of serum albumin. It has been shown that the half-life of albumin is shortened and that the total albumin mass is reduced. These two factors cause hypoalbuminemia, despite increased fractional synthesis rates in the plasma. For this reason, hypoalbuminemia stems from inflammatory conditions that prevent adequate responses to events, such as surgery or chemotherapy, and is associated with poor quality of life and decreased survival. Decreased serum albumin levels are indicative of clinical deterioration. Albumin acts as a main extracellular cleaner, antioxidant and supplier of amino acids for cell synthesis. Management of hypoalbuminemia needs to be based more on correcting the causes of the continuing inflammation than on albumin infusion.[8-10]

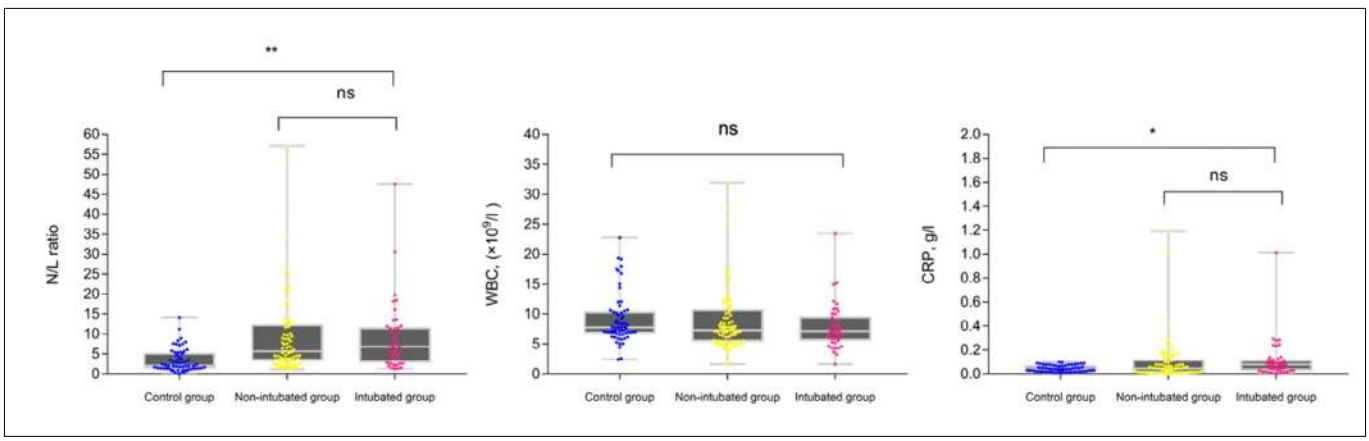

Figure 4. C-reactive protein (CRP), neutrophil-to-lymphocyte (N/L) ratio and white blood cell (WBC) levels of the three groups (healthy controls, non-intubated patients and intubated patients).

The inflammation in cases of pneumonia that develop due to COVID-19 can cause hypoalbuminemia. Improvement of states of hypoalbuminemia through COVID-19 treatment can contribute to reducing the inflammation.

In the present study, the albumin levels were low in the analyses made in the two groups diagnosed with COVID-19 pneumonia (intubated and non-intubated), without involving the control group. The lack of significant differences in other parameters in the comparisons between these two groups suggests to us that hypoalbuminemia is more important for showing the severity of the disease. The significantly lower albumin levels in the intubated group than in the non-intubated group suggests to us that these lower levels show the greater severity of the disease. Addition of albumin to the treatment, in cases of detection of hypoalbuminemia in intubated patients, may contribute to the recovery. Wider studies are needed on this issue.

States of comorbidity that can lower serum albumin levels may contribute to hypoalbuminemia in the COVID-19 pandemic. These conditions are encountered in intensive care patients. The presence of comorbidities may adversely affect the treatment process. In some published papers, it was reported that serum albumin levels were negatively affected in situations of hypervolemia.[8-10] Cases of COVID-19 pneumonia with hypervolemia may be more affected. Avoiding hypervolemia can contribute to recovery. Conditions such as hypervolemia, proteinuria or liver failure, which may decrease serum albumin levels in COVID-19 pneumonia, should be treated.

It was reported in some previous studies that hypoalbuminemia, lymphopenia, decreased lymphocyte and neutrophil percentages, high C-reactive protein levels and high lactate dehydrogenase (LDH) levels were common laboratory abnormalities.[11-14] It was also found that serum albumin values, lymphocyte cell counts and percentages, neutrophil percentages and LDH and CRP levels were highly correlated with acute lung damage.[8,9] These conditions can play critical roles in cases of severe pneumonia that develop due to COVID-19. In our study, it was found that albumin values were low, compared with those of the control group. Also, the CRP levels and N/L ratios were high and the WBC values were not significant. Moreover, our findings were in line with those in the literature.

We believe that an idea of the severity of the disease of this pandemic, which has affected the entire world, can be obtained in terms of lower albumin levels in cases of greater severity of lung involvement. Treatments for hypoalbuminemia can contribute to improving the condition of patients with pneumonia.

Limitations

Our study population was small. Serum albumin levels are also affected by conditions such as hypervolemia. It needs to be asked whether serum albumin levels might also cause worsening of COVID-19 pneumonia accompanied by hypervolemia, and whether these levels might have a role in etiopathogenesis. More extensive studies may be required.

CONCLUSION

Hypoalbuminemia may constitute a biomarker indicating the severity of cases of pneumonia due to COVID-19.

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Echocardiographic epicardial fat thickness and immature granulocyte are novel inflammatory predictors of acute ischemic stroke: a prospective study

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ABSTRACT

BACKGROUND: Acute ischemic stroke (AIS) is the most common type of stroke. Inflammation is the primary factor in the pathogenesis of atherosclerosis. Use of immature granulocytes (IGs) has been recommended as a new indicator of systemic inflammation. However, data on the association between echocardiographic epicardial fat tissue thickness (EFT) and IGs in patients with AIS are limited.

OBJECTIVE: To evaluate the association between the presences of IGs, epicardial fat tissue and AIS.

DESIGN AND SETTING: Prospective study in a tertiary-care university hospital in Antalya, Turkey.

METHODS: Our study included 53 AIS patients and 41 healthy controls with age and gender compatibility. Blood samples and transthoracic echocardiography of all participants were compared.

RESULTS: IG levels were significantly higher in patients with AIS than in controls (0.62 ± 0.36 versus 0.28 ± 0.02, P < 0.001). The mean EFT was 3.74 ± 0.61 mm in the control group and 6.33 ± 1.47 mm in the AIS patient group. EFT was significantly greater in AIS patients than in controls (P < 0.001). For the optimum cut-off value for IG (0.95), the area under the curve (AUC) was determined to be 0.840; sensitivity was determined to be 81.1% and specificity, 92.5%. For the optimum cut-off value for EFT (4.95 mm), the AUC was determined to be 0.953; sensitivity was determined to be 90.6% and specificity, 90%.

CONCLUSIONS: IG and echocardiographic EFT are clinical markers that can be used to predict AIS risk.

INTRODUCTION

Stroke is a sudden localized and focal neurological syndrome. It is a major medical and economic problem that can result in severe disability and mortality. Acute ischemic stroke (AIS) is the most common type of stroke.[1] Inflammation is the primary factor in the pathogenesis of atherosclerosis, and it is well known that inflammation indicators change before stroke and atherosclerotic events.[2] In addition, many studies have shown that there are correlations between some inflammatory indicators such as high-sensitivity C-reactive protein (hs-CRP), neutrophil/lymphocyte ratio, cardiovascular diseases and AIS.[3,4] Over recent years, use of immature granulocytes (IGs) has been recommended as a new indicator of systemic inflammation. The prognostic and predictive role of IGs has been shown in relation to many diseases.[5,6] However, the role of IGs in patients with AIS has not been clearly revealed until now.

Epicardial fat tissue is located between the myocardium and the visceral sheets of the pericardium and it may play an adverse role in relation to the heart through production and secretion of proinflammatory and proatherogenic mediators. Its presence has been correlated with presence of atherosclerotic diseases such as coronary artery disease and aortic stenosis.[7-9] Although many imaging methods are used for measuring epicardial fat tissue thickness (EFT), echocardiographic measurement is the method most preferred because of its inexpensiveness, repeatability and easy applicability. Although there are studies showing that EFT is associated with many diseases such as metabolic syndrome, diabetes mellitus and coronary artery disease, there are very few studies in the literature examining its association with AIS.[8-10] Moreover, data on the association between echocardiographic EFT and IG in patients with AIS are limited.

OBJECTIVES

The purpose of this study was to evaluate the association between the presences of IG, epicardial fat tissue and AIS.

METHODS

Study design and participants

Our study was designed as a single-center prospective case-control study. Its subjects consisted of patients who were admitted to our tertiary-care emergency department (ED) and were hospitalized with a diagnosis of stroke between December 2019 and December 2020. Our study was conducted in accordance with the Helsinki Declaration, upon approval from the local ethics committee (decision date: December 26, 2019; and number: 27/20). All participants were informed about the study before entering it, and a written consent statement was obtained from each of them.

Data collection

Our study included 53 AIS patients and 41 healthy controls with age and gender compatibility. The individual medical history and clinical characteristics of all participants were recorded, and body mass index (BMI) was calculated as weight (kg)/height^2 (m^2). Patients were diagnosed with AIS through clinical and physical examination, radiology imaging and neurology consultation. All patients underwent brain tomography (CT) and/or diffusion-weighted magnetic resonance (MRI) imaging. Some patients were diagnosed with AIS through imaging with an appearance consistent with acute infarction, in repeated MRI imaging after hospitalization.

Patients with the following characteristics at the time of hospital admission were not included in the study: patients < 18 years old; obese patients (BMI > 30 kg/m^2); pregnant women; patients with myeloproliferative disease; those with chronic inflammatory, chronic liver or kidney disease; those with malignancy; those with granulocyte colony stimulating factor, immunosuppurative agent or steroid use; those with uncontrolled hypertension or diabetes; and those presenting infection.

Blood samples were taken from peripheral venous blood within one hour at the latest, at the time of admission to the emergency department. All hemogram parameters, including IG, were measured using an automated blood analyzer (Coulter LH 780 Hematologic Analyzer, Beckman Coulter Inc., Brea, United States).

Echocardiogram and epicardial fat measurement

Transthoracic echocardiography on all participants was performed in the left lateral recumbent position using a 2.5-3.5 MHz ultrasound probe (Mindray M5 device; Mindray DS USA Inc., Mahwah, New Jersey, United States). All sonographic examinations were performed at the bedside in the ED and were recorded to include three cardiac cycles. EFT was measured by an experienced emergency physician, and the guidelines of the American Echocardiography Society for standard echocardiographic measurements were followed.[11] Measurements were made from the parasternal long axis.

In EFT measurements, the practitioner detected epicardial fat tissue as an area of relatively low echogenicity located between the right ventricle and the inner sheet of the pericardium. The greatest EFT in this area was measured in the end-systolic phase of the cardiac cycle, parallel to the aortic valve[12] (**Figure 1**). Three consecutive measurements were made; the average was calculated and was noted on the patient's follow-up paper as the EFT measurement. The investigator who made the measurements was blind to the laboratory values, final results and study group of the patients.

Data analysis

Statistical analyses in our study were performed using the SPSS 21.0 software package (SPSS Inc., Chicago, Illinois, United States). Continuous variables were expressed as the mean ± standard deviation, and categorical variables as the number (%), for patients with AIS and the control group. The data were evaluated with regard to normal distribution, and the independent t test or Mann-Whitney U test was used, according to suitability. In addition, categorical data were evaluated using the chi-square test. Spearman's correlation was calculated and examined to ascertain any correlations between the variables. The optimum cutoff value for IG and EFT, with regard to predicting AIS, was evaluated through receiver operating characteristic (ROC) analysis. Variables that might affect AIS were evaluated by means of logistic regression analysis. Statistical significance was defined as $P < 0.05$.

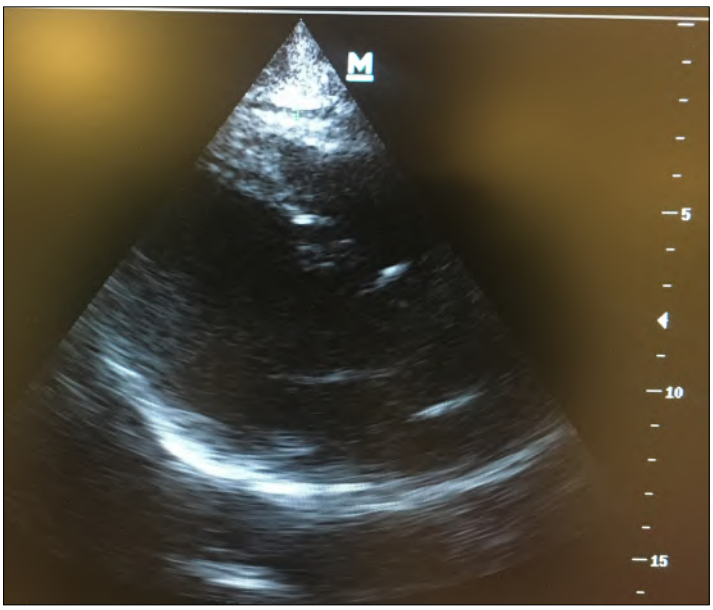

Figure 1. Measurement of epicardial fat tissue thickness by means of echocardiography.

RESULTS

Among the 53 patients with AIS included in our study, 28 of them were male and 25 were female; their mean age was 71.15 ± 12.32 years. Among the 41 healthy participants, 19 of them were male and 22 were female; their mean age was 69.78 ± 10.31 years. There was no significant difference between the patient and control groups in terms of risk factors except for age, gender, BMI and hypertension (HT). The healthy control group had significantly lower systolic blood pressure, diastolic blood pressure, heart rate, leukocyte count and glucose level. IG levels were significantly higher in the patients with AIS than in the controls (0.62 ± 0.36 versus 0.28 ± 0.02; P < 0.001) (**Figure 2**). The mean EFT was 3.74 ± 0.61 mm in the control group and 6.33 ± 1.47 mm in the AIS patient group. EFT was significantly greater in the AIS patients than in the controls (P < 0.001) (**Figure 3**). The main characteristics of the patients are shown in **Table 1**.

The efficacy of IG and EFT for determining AIS was calculated by plotting ROC curves (**Figure 4**). For the optimum cutoff value of IG, which was 0.95, the area under the curve (AUC) was determined to be 0.840; sensitivity was determined to be 81.1% and specificity, 92.5% (**Table 2**). For the optimum cutoff value of EFT, which was 4.95 mm, the AUC was determined to be 0.953; sensitivity was determined to be 90.6% and specificity, 90% (**Table 2**). We also found a significant positive correlation between EFT and IG (r = 4.974; P < 0.001). We found that HT (odds ratio, OR: 2.990; 95% confidence interval, CI: 1.281 to 6.79; P = 0.011), high glucose (OR: 10.450; 95% CI: 3.935 to 27.749; P < 0.001), high IG (OR: 1.782; 95% CI: 1.624 to 1.937; P < 0.001), atrial fibrillation (OR: 1.612; 95% CI: 1.112 to 1.887; P = 0.008); and high EFT (OR: 2.733; 95% CI: 2.559 to 2.907; P < 0.001) were independent risk factors in multivariant logistics regression analysis (**Table 3**).

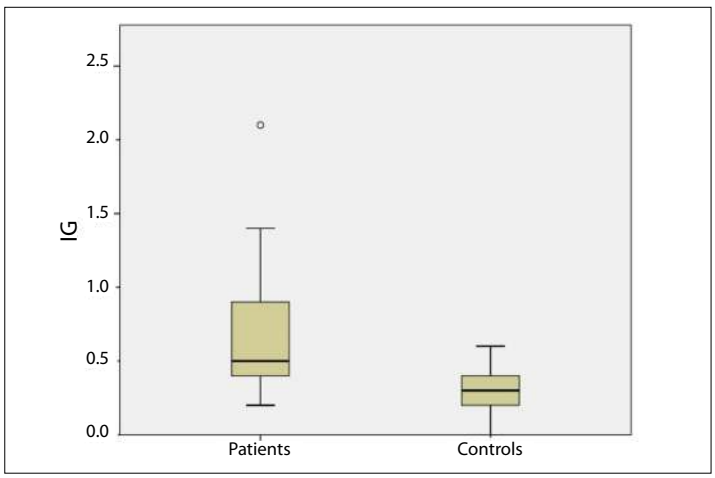

Figure 2. Box plot presentation of immature granulocytes (IG) in acute ischemic stroke (AIS) patients and healthy controls.

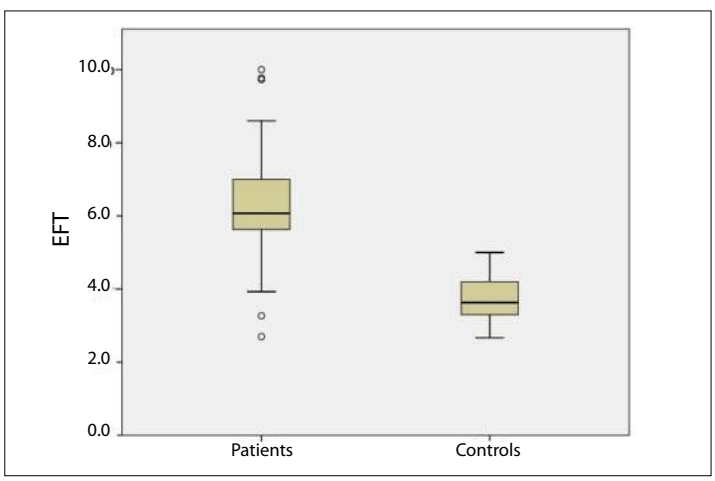

Figure 3. Box plot presentation of epicardial fat tissue thickness (EFT) in acute ischemic stroke (AIS) patients and healthy controls.

Table 1. Baseline demographic characteristics of the study population

	Ischemic stroke (n = 53)	Controls (n = 41)	P-value
Age in years, mean ± SD	71.15 ± 12.32	69.78 ± 10.31	0.427
Male, n (%)	28 (59.6)	19 (40.4)	0.533
SBP, mmHg (IQR)	160 (40)	125 (22)	< 0.001
DBP, mmHg (IQR)	85 (26)	65 (13)	< 0.001
Heart rate, beats/minute (IQR)	89 (26)	75 (15)	< 0.001
BMI in kg/m², mean ± SD	24.52 ± 2.99	25.30 ± 2.89	0.244
EFT in mm, mean ± SD	6.33 ± 1.47	3.74 ± 0.61	< 0.001
Previous history, n (%)			
Current smoker	7 (13.2)	10 (24.4)	0.162
Hypertension	36 (67.9)	17 (32.1)	0.010
Diabetes mellitus	20 (37.7)	13 (31.7)	0.544
History of CAD	24 (45.3)	23 (56.1)	0.298
Laboratory findings, mean ± SD			
WBC count (×10³/mm³)	9.18 ± 3.31	9.00 ± 2.84	0.967
Neutrophil (×10³/mm³)	6.41 ± 3.13	5.68 ± 2.61	0.215
Lymphocyte (×10³/mm³)	2.42 ± 0.41	2.34 ± 0.15	0.040
NLR	5.17 ± 0.93	3.11 ± 0.38	0.071
PLR	183.27 ± 27.72	133.68 ± 62.51	0.264
Hemoglobin, mg/dl	12.82 ± 2.12	13.18 ± 1.43	0.650
Glucose, mg/dl (IQR)	147 (75)	113 (22)	< 0.001
Blood urea nitrogen, mg/dl	22.13 ± 2.12	18.88 ± 6.64	0.506
Creatine, mg/dl	1.10 ± 0.69	0.91 ± 0.24	0.441
IG%	0.62 ± 0.36	0.28 ± 0.02	< 0.001
CRP, mg/dl	15.73 ± 4.96	6.21 ± 0.85	0.441
Mortality, n (%)	6 (11.3)	0 (0)	< 0.001

SD = standard deviation; SBP = systolic blood pressure; DBP = diastolic blood pressure; BMI = body mass index; EFT = epicardial fat tissue thickness; CAD = coronary artery disease; WBC = white blood cell; NLR = neutrophil-lymphocyte ratio; PLR = platelet-lymphocyte ratio; CRP = C-reactive protein; IG = immature granulocyte; IQR = interquartile range.

DISCUSSION

This prospective study provides the first evidence in the literature demonstrating that increased EFT and greater IG are associated with AIS disease.

Epicardial fat tissue is a true tissue layer that accumulates around the heart and around the coronary vessels. It is fed through rich microcirculation since it contains neuronal network, stromal-vascular, immune and inflammatory cells.[13,14]

In recent studies, EFT has been shown to be associated with many cardiovascular and neurovascular diseases. Chu et al. showed that EFT was usable for predicting future cardiovascular events in patients with atrial fibrillation.[15] Iacobellis et al. found high EFT values in patients with chronic atrial fibrillation and correlated this with development of heart failure.[16] Wang et al. found significantly higher EFT values in patients who had gone through acute myocardial infarction, in comparison with a control group (5.6 ± 1.1 versus 4.1 ± 1.0 mm; P < 0.001). They also reported that high EFT values may indicate higher risk of mortality among patients with acute myocardial infarction.[17] Tanındı et al. showed that patients with EFT values of 7 mm and above presented an association with death arising from cardiovascular events.[18] Sagmacı et al. reported that there was frequently an association between high EFT levels and pain frequency, in a study conducted among patients with migraine.[19] Akıl et al. showed that mean EFT values in AIS patients were significantly higher than those of the control group in their study (5.95 ± 1.14 versus 4.86 ± 0.68; P < 0.001).[20] In our study, we detected that EFT in patients with AIS was significantly greater than in the control group.

AIS is a condition associated with rapid loss of brain function that develops after a decrease in the blood supply to the brain. Atherosclerosis is a systemic disease that causes this process to develop. A number of cellular and molecular events associated with inflammation are known to contribute to development of atherosclerotic lesions and AIS. Therefore, increased vascular inflammation has been associated with AIS.[21,22]

In the literature, inflammatory biomarkers such as neutrophil to lymphocyte ratio (NLR) and platelet to lymphocyte ratio (PLR) have previously been used as measurements of correlations of leukocyte, neutrophil and lymphocyte counts with atherosclerotic processes and AIS.[23,24] Over recent years, with technological developments, simple and easily measurable biomarkers such as IG have been used in relation to many diseases for diagnostic and prognostic purposes.

IG is not normally detected in peripheral blood in healthy individuals. However, it can become mixed with peripheral blood under inflammatory conditions.[25,26] Use of this parameter has increased over recent years, although many doctors still do not consider it to be important. Its presence has been correlated with infection, sepsis and cardiovascular diseases. Moreover, its presence has been reported to be predictive of mortality in relation to many gastrointestinal diseases.[5,6,26]

No studies in the literature have yet examined the correlation between IG and AIS. Park et al. reported that IG might be a marker for mortality due to sepsis, and its sensitivity for mortality in their

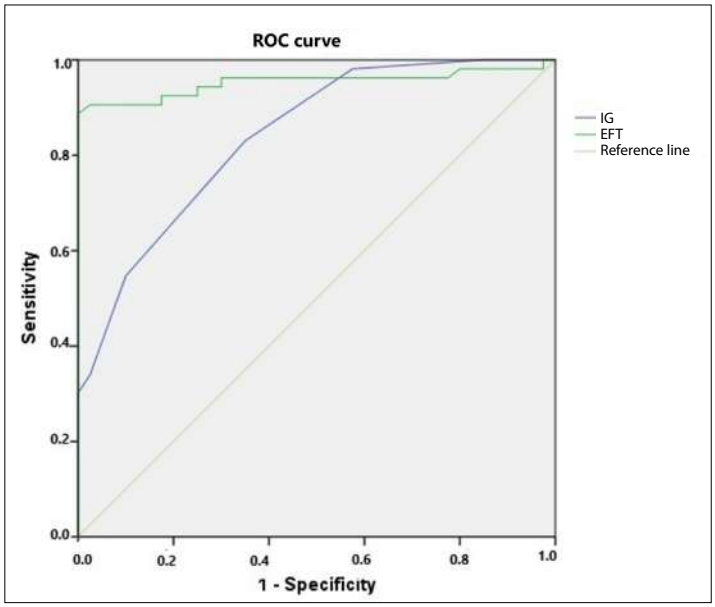

Figure 4. Receiver operating characteristic (ROC) curve analysis of immature granulocytes (IG) and epicardial fat tissue thickness (EFT) in predicting acute ischemic stroke (AIS) diagnosis.

Table 2. Receiver operating characteristic curve analysis on the echocardiographic EFT and IG findings for prediction of acute ischemic stroke

Characteristics	Value	Sensitivity	Specificity	AUC (95% CI)	P-value
IG	0.95	81.1	92.5	0.840 (0.807-0.901)	< 0.001
EFT	4.95	90.6	90	0.953 (0.926-0.974)	< 0.001

AUC = area under the curve; 95% CI = 95% confidence interval; IG = immature granulocytes; EFT = epicardial fat tissue thickness.

Table 3. Multivariate logistic regression analysis to assess predictors of ischemic stroke

Variables	OR	95% CI	P-value
Gender	1.297	0.573-2.936	0.217
Hypertension	2.990	1.281-6.79	0.011
Glucose	10.450	3.935-27.749	< 0.001
IG%	1.782	1.624-1.937	< 0.001
EFT (in mm)	2.733	2.559-2.907	< 0.001
Atrial fibrillation	1.612	1.112-1.887	0.008

OR = odds ratio; 95% CI = 95% confidence interval; IG = immature granulocytes; EFT = epicardial fat tissue thickness.

study was found to be 70%; specificity was found to be 78%.[27] In a study conducted by Zeng et al., it was shown that there is a correlation between increasing IG values and positive blood cultures.[28] In a recent study that we conducted, we found a correlation between increasing IG levels and positive indications for appendectomy.[29] İncir et al. showed that use of IG together with hemogram indices enabled detection of inflammation and infection in the early period.[30] In a study conducted by Karakulak et al., high IG values were shown to detect both disease severity and mortality in patients with acute pancreatitis.[31] In our present study, we showed the correlation between increasing IG levels and AIS, for the first time in the literature.

Our study had some limitations. The first limitation was that even though our study was prospectively designed, it was conducted with only a small number of patients because of the coronavirus disease 19 (COVID-19) pandemic. Another limitation was that the EFT measurements of the patients included in our study were linear measurements, and our results could not be compared with the tomography and magnetic resonance imaging methods. Although echocardiographic measurements are dependent on the individual, they can be performed more easily and repeatably than can tomography and MRI. One of our major limitations was that the measurements were made by a single practitioner and also that these images were not interpreted by another researcher.

CONCLUSION

This study showed the correlations between EFT, IG and AIS for the first time. IG and echocardiographic EFT are clinical markers that can be used to predict AIS risk.

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Oxidative stress in maternal milk and cord blood in gestational diabetes mellitus: a prospective study

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ABSTRACT

BACKGROUND: Reduced antioxidant defenses may reflect a poor protective response against oxidative stress and this may be implicated in progression of gestational diabetes mellitus (GDM). Oxidative stress induced by hyperglycemia plays a major role in micro and macrovascular complications, which imply endothelial dysfunction.

OBJECTIVE: Our aim in this study was to investigate the association between GDM and oxidative stress markers measured in plasma, with regard to revealing changes to total antioxidant capacity (TAC) and total oxidant status (TOS) among mothers showing impairments in oral glucose tolerance tests (OGTTs).

DESIGN AND SETTING: Prospective study at a university hospital in Turkey.

METHODS: The study group consisted of 50 mothers with GDM, and 59 healthy mothers served as controls. Umbilical cord blood samples were taken from all mothers during delivery and breast milk samples on the fifth day after delivery. TAC, TOS, thiol and disulfide levels were measured.

RESULTS: No statistically significant relationship between the blood and milk samples could be found. An analysis on correlations between TAC, TOS and certain parameters revealed that there were negative correlations between TOS and total thiol ($r = -0.386$; $P < 0.001$) and between TOS and disulfide ($r = -0.388$; $P < 0.001$) in milk in the control group. However, these findings were not observed in the study group.

CONCLUSION: Our findings suggested that a compensatory mechanism of oxidative stress was expected to be present in gestational diabetes mellitus and that this might be ameliorated through good glycemic regulation and antioxidant supplementation.

INTRODUCTION

There is a balance between the reactive oxygen compounds produced by different mechanisms and the antioxidant systems generated by the enzymatic and non-enzymatic processes that neutralize these oxygen compounds.[1] The term "oxidative stress" expresses the imbalance between oxidant/antioxidant molecules in favor of oxidants that cause aging and diseases.[2,3] Oxidative stress has also been implicated in the pathogenesis of vascular diseases, such as atherosclerosis, diabetes and hypertension, which result from an imbalance between increased formation of reactive oxygen species (ROS) and synthesis of anti-oxidative defense mechanisms.

Under diabetic conditions, the end products of abnormal glucose metabolism lead to increased synthesis of ROS. Formation of advanced glycation end products, activation of hexosamine biosynthetic pathway, increased lipid peroxidation and an impaired antioxidant defense system result in accumulation of free radicals, eventually.[5] Experimental studies have revealed increased levels of free oxygen radicals in diabetic pregnancy.[6] Furthermore, it was observed in an animal study that antioxidant supplementation can decrease occurrence of malformations in offspring.[7] Both experimental and clinical studies have shown that in the presence of gestational diabetes, there is enhanced oxidative stress, which is detectable in maternal and neonatal blood samples, placental tissue and amniotic fluid.[5]

It has been suggested that oxidative stress plays a role in maternal and fetal complications of diabetic pregnancies.[8-10] Pregnancy alone may represent an oxidative stress condition.[11] Associations between gestational diabetes mellitus (GDM) and oxidative stress markers measured in plasma have already been reported, with limited consensus. This suggests that oxidative stress may be implicated in GDM progression and/or pathogenesis and that reduced antioxidant defenses may reflect a poor protective response against oxidative stress.[9,12,13]

Oxidant agents may be produced either endogenously or exogenously, as in the case of ultraviolet rays, active smoking or passive exposure to cigarettes.[14] Although oxidant agents can be

measured one-by-one, it is widely preferred to measure total oxidant status (TOS) because the individual measurement approach involves increased work time and complicated techniques for each agent.[15] Similarly, antioxidants can be measured through the total antioxidant capacity (TAC) rather than one-by-one.

When a balance between TAC and TOS is needed, there may be a shift in favor of TOS; this is called oxidative stress (OS). One of the most important reasons for OS is impaired glucose tolerance, which manifests as obvious diabetes mellitus (DM) or through impairment seen in an oral glucose tolerance test (OGTT) during pregnancy. Previous studies revealed that the levels of oxidant molecules increase in diabetic animals, while antioxidants have been found to be decreased in diabetic patients.[16,17]

Oxidative stress induced through hyperglycemia plays a major role in micro and macrovascular complications, which imply endothelial dysfunction. Appropriate glycemic control prevents complications related to increased OS during pregnancy.[18]

OBJECTIVE

Our aim in this study was to investigate GDM-related OS markers in maternal milk and cord blood, and to increase awareness of possible measures to be taken, through revealing the changes in TAC and TOS and in the OS index levels of mothers with impairments seen in OGTTs.

METHODS

Pregnant women were routinely assessed by means of a 50 g glucose loading test at gestational ages of between 24 and 28 weeks. Those whose blood glucose levels were < 140 mg/dl were accepted as normal and were included in the control group. Those whose blood glucose levels were ≥ 140 mg/dl were given a 100 g glucose loading test. In accordance with the suggestions of the American Diabetes Association (ADA), patients with high glucose levels in two out of four tests conducted at hours 0, 1, 2 and 3 were diagnosed as having GDM and were included in the study group.

The patients in the study group attended consultations at the department of endocrinology in order to regulate their blood glucose levels. Their hemoglobin A1c (HbA1c) levels were measured, and their blood glucose levels were monitored. Out of the 50 patients, the blood glucose levels of 47 of them were regulated only through dietary control. One patient took oral antidiabetic medication, and two patients needed insulin treatment for appropriate glucose regulation. The exclusion criteria of this study were situations of histories of chronic disease, histories of infection during pregnancy, smoking during pregnancy, substance abuse history, pregnancy at ages < 18 years or > 35 years, consanguineous marriage or previous histories of complications of pregnancy or delivery.

The participants were asked about medication or food supplementation use that could have affected their antioxidant capacity (gingko, guelder-rose, vitamin C, vitamin E, coenzyme Q-10, resveratrol, lipoic acid, etc.).

Informed parental consent was obtained for the cord blood collection and the study was approved by the Ethics Committee of the School of Medicine of Istanbul Medipol University, in Istanbul, Turkey (approved on January 23, 2015; number 23.01.2015/10840098-06).

After delivery of the baby, the umbilical cord was clamped and cut. Cord blood was drawn from the umbilical vein within two minutes of delivery. In order to standardize breast milk sample collection, these samples were collected from all mothers on the fifth day. Serum samples collected from the study and control groups were immediately separated from the cells through centrifugation at 3,000 g for 10 minutes. They were then stored at –80 °C until further analysis of the native thiol, total thiol, disulfide, TOS, TAC and OSI.

Measurement of total oxidant status

Serum TOS values were measured through an assay based on an automated measurement method developed by Erel.[15] Oxidants present in the sample oxidize ferrous ions to ferric ions. Ferric ions are manifested through a colored complex with xylenol orange in an acidic medium. The color intensity, which can be assessed spectrophotometrically, is dependent on the total quantity of oxidant molecules present in the sample. TOS values are expressed in terms of micromolar hydrogen peroxide (H_2O_2) equivalents per liter (μmol H_2O_2 eq/l). Serum thiol and disulfide levels are expressed as micromoles per liter (μmol/l).

Measurement of total antioxidant capacity

The total antioxidant capacity (TAC) of the serum samples was assayed using a method developed by Erel.[19] In this method, the characteristic blue color of the 2, 2'-azino-bis (3-ethyl benzothiazoline-6-sulfonic acid) (ABTS) cation is converted back to its neutral form by any antioxidant present in the sample. This reaction is also monitored spectrophotometrically. The assay results are expressed in mmol Trolox equivalent per liter.

Measurement of plasma oxidative stress index

There are several indexes for measuring OS in humans and most of them make it possible to diagnose and differentiate OS related to human health and disease. The oxidative stress index (OSI) is one of these indexes, and this has been proven to be reliable and practical.[20]

The OSI was calculated using the following formula: OSI (arbitrary unit) = TOS (μmol H_2O_2 eq/l)/TAC (mmol Trolox eq/l) x 100 (to represent a percentage ratio).

For the analysis of the present study, in addition to descriptive statistical methods (i.e. frequency, percentage, mean and standard deviation), the Kolmogorov-Smirnov test was used to assess whether the data showed normal distribution. Independent-sample t tests were applied to compare quantitative data between pairs of groups. Pearson correlation analysis was used to evaluate relationships between the groups' quantitative data. The results were evaluated at a 95% confidence interval, and $P < 0.005$ was considered to be significant in the two-way analyses.

RESULTS

A total of 109 participants with a mean age of 28.8 ± 7.2 years were included in our study. There were 50 patients with GDM, with a mean age of 25.2 ± 6.4 years. The healthy control group included 59 patients, with a mean age of 27.6 ± 7.6 years. The normal spontaneous delivery rate was 12% (n = 6) in the study group and 11.8% (n = 7) in the control group. There were no significant differences with regard to age, body mass index (BMI) or obstetric history between the groups (for all parameters, $P > 0.05$). Two of the pregnant women with GDM required insulin treatment, and one patient needed to take oral antidiabetics. For 47 patients, their blood glucose levels were regulated through dietary control. The mean HbA1c level of the patients was 5.2% ± 0.4%.

The values for laboratory findings (TAC, TOS, OSI, disulfide, total thiol and native thiol) in the groups are presented in **Table 1**. No statistically significant relationship between blood and milk samples could be found. Although the TAS-milk, milk total thiol, milk N-thiol and milk disulfide levels in the study group were higher than those of the healthy group, there were no statistically significant differences between the groups ($P > 0.05$) (**Figures 2** and **3**). The OSI level in milk was lower in the study group than in the control group but the OSI in blood was higher ($P > 0.05$) (**Figures 1** and **4**). An analysis on the correlation between TAC, TOS and certain parameters in the control group revealed that there were negative correlations between TOS and total thiol ($r = -0.386$; $P < 0.001$) and between TOS and disulfide ($r = -0.388$; $P < 0.001$) in milk. However, these findings were not seen in the study group (**Table 2** [control group] and **Table 3** [study group]).

The detailed histories of the patients revealed that 94% of them had been taking preparations that included multivitamins (n = 48), 15% had been taking medications that included omega-3 (n = 8) and 15% had been consuming foodstuffs that had antioxidant properties (although the levels and contents of these antioxidant molecules were not well known).

DISCUSSION

Studies investigating the association between gestational diabetes mellitus (GDM) and oxidative stress have reported inconsistent findings. Although there is no irrefutable proof of a relationship between GDM and oxidative stress, it has been shown that the levels of molecules that give rise to OS are influenced by GDM.[4,21] In spite of evidence that oxidative stress plays an important role in the pathogenesis of DM, there is debate on its role in GDM and the impact of the oxidant/antioxidant balance.[11]

Although the parameters in both cord blood and maternal milk samples in the gestational diabetes group of our study were than those of the healthy group, there were no statistically significant differences between the groups in our study. In addition, the OSI

Table 1. Mean scores of oxidant and antioxidant levels of women with gestational DM and healthy pregnant women

	Gestational diabetes mellitus group Mean (SD)	Control group Mean (SD)	P
Antioxidant levels			
Blood total thiol (µmol/l)	0.409 (0.05)	0.425 (0.055)	> 0.5
Milk total thiol (µmol/l)	0.167 (0.02)	0.160 (0.018)	
Blood N-thiol (µmol/l)	0.326 (0.05)	0.343 (0.055)	
Milk N-thiol (µmol/l)	0.078 (0.02)	0.075 (0.019)	
Oxidant levels			
Blood disulfide (µmol/l)	0.041 (0.003)	0.041 (0.003)	> 0.5
Milk disulfide (µmol/l)	0.044 (0.004)	0.042 (0.005)	
Total oxidant status			
Blood (µmol H_2O_2/l)	8.288 (4.35)	9.792 (5.08)	> 0.5
Milk (µmol H_2O_2/l)	2.025 (0.94)	2.052 (0.82)	
Total antioxidant capacity			
Blood (mmol Trolox eq/l)	4.455 (3.43)	5.572 (4.25)	> 0.5
Milk (mmol Trolox eq/l)	0.756 (0.79)	0.616 (0.47)	
Oxidative stress index			
Blood	48.69 (113.31)	38.30 (44.52)	> 0.5
Milk	54.92 (50.21)	92.72(139.58)	

SD = standard deviation.

Table 2. Correlation analysis on TAC, TOS, OSI, total thiol, N-thiol and disulfide in healthy pregnant women

Oxidant and antioxidant levels in blood and milk	Blood TOS	Blood TAC	Blood total thiol	Blood N-thiol	Blood disulfide	Milk TOS	Milk TAC	Milk total thiol	Milk N-thiol
Blood TOS	**1**								
Blood TAC	**0.983****								
Blood total thiol	0.345	0.277							
Blood N-thiol	0.254	0.184	**0.992****						
Blood disulfide	**0.467***	**0.471***	-0.035	-0.159					
Milk TOS	0.329	0.342	-0.084	-0.121	0.301				
Milk TAC	0.105	0.018	0.132	0.092	0.308	**0.294***			
Milk total thiol	-0.381	-0.405	-0.119	-0.085	-0.262	**-0.386****	-0.156		
Milk N-thiol	-0.,374	-0.321	-0.,248	-0.235	-0.087	-0.146	-0.119	**0.807****	
Milk disulfide	0.054	-0.048	0.220	0.252	-0.270	**-0.388****	-0.052	0.276*	**0.345****

*P < 0.5; **P < 0.01.

TOS = total oxidant status; TAC = total antioxidant capacity; OSI = oxidative stress index.

Table 3. Correlation analysis on TAC, TOS, OSI, total thiol, N-thiol and disulfide in women with gestational diabetes mellitus

Oxidant and antioxidant levels in blood and milk	Blood TOS	Blood TAC	Blood total thiol	Blood N-thiol	Blood disulfide	Milk TOS	Milk TAC	Milk total thiol	Milk N-thiol
Blood TOS	1								
Blood TAS	0.983**								
Blood total thiol	0.462*	0.414*							
Blood N-thiol	0.456*	0.401*	0.993**						
Blood disulfide	-0.207	-0.115	0.257	0.139					
Milk TOS	0.123	0.155	-0.047	-0.065	0.123				
Milk TAC	-0.120	-0.037	-0.222	-0.257	0.222	0.081			
Milk total thiol	-0.044	-0.032	0.229	0.246	-0.077	-0.013	-0.021		
Milk N-thiol	-0.067	-0.048	0.271	0.294	-0.107	0.126	-0.042	0.934**	
Milk disulfide	0.067	0.044	-0.207	-0.232	0.137	-0.326	0.050	0.429**	0.080

*P < 0.5; **P < 0.01.

TOS = total oxidant status; TAC = total antioxidant capacity; OSI = oxidative stress index.

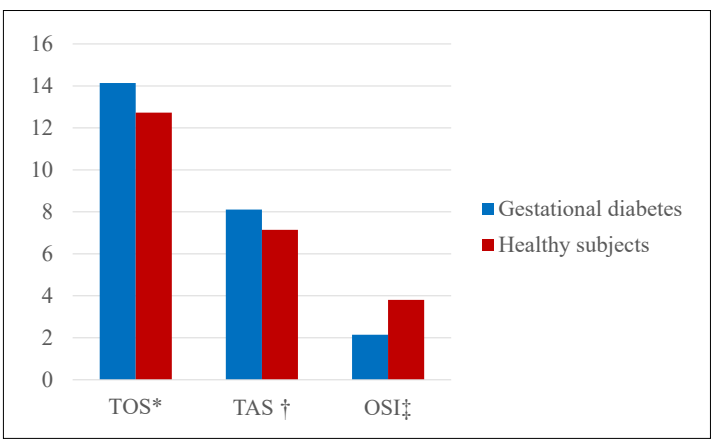

Figure 1. Comparison of blood total oxidant status (TOS) *(µmol H_2O_2 eq/l), total antioxidant capacity (TAS) †(mmol Trolox eq/l) and oxidative stress index (OSI) ‡(CarrU/(mmol HClO/ml) levels between women with gestational diabetes mellitus and healthy pregnant women.

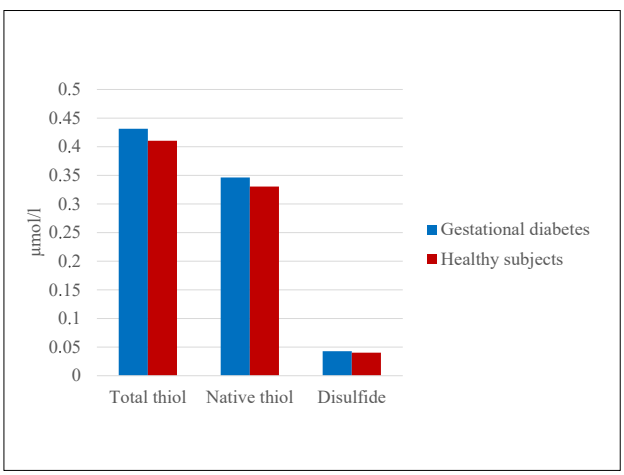

Figure 2. Comparison of blood thiol total, thiol native and disulfide levels in women with gestational diabetes mellitus and healthy pregnant women.

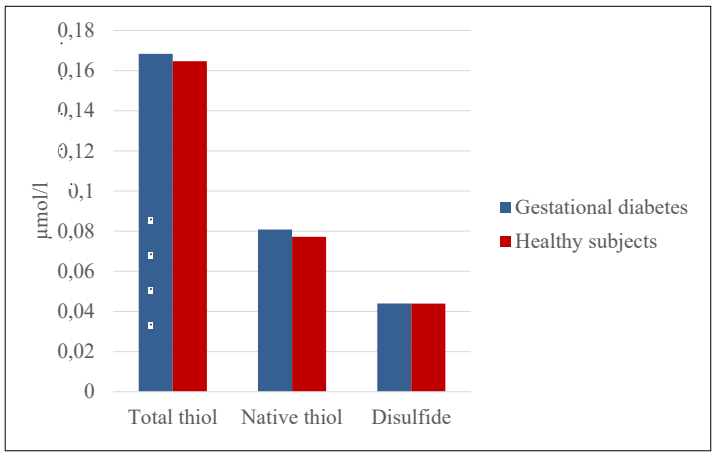

Figure 3. Comparison of milk thiol total, thiol native and disulfide levels in women with gestational diabetes mellitus and healthy pregnant women.

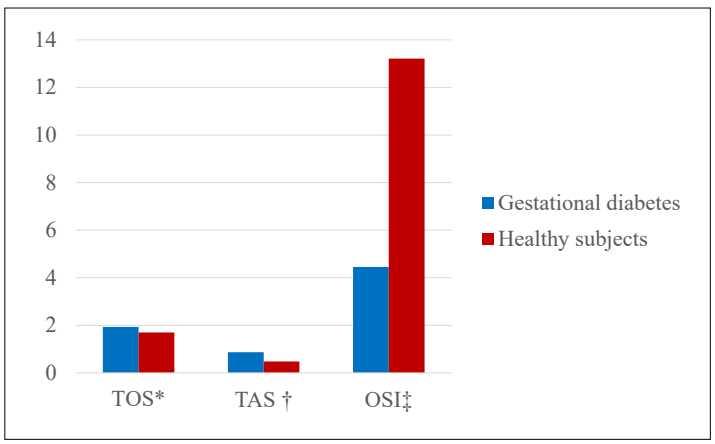

Figure 4. Comparison of milk total oxidant status (TOS) *(µmol H_2O_2 eq/l), total antioxidant capacity (TAS) †(mmol Trolox eq/l) and oxidative stress index (OSI) ‡(CarrU/mmol HClO/ml) levels in women with gestational diabetes mellitus and healthy pregnant women.

did not differ. On the other hand, the milk TOS level was negatively correlated with the milk thiol level in the non-GDM pregnant group, whereas increased milk TAC occurred as a compensation. This finding was interpreted as a sign that this compensation condition was impaired in GDM.

Oxidative stress impacts mortality and morbidity in all age groups. This may lead to disturbances during pregnancy and the postnatal period. One of the factors increasing the OSI during pregnancy is impaired glucose tolerance.[18] Reactive oxygen species (ROS) and nitrogen production can alter several cellular components, as well as the redox state. All of these are maintained by complex mechanisms that lead to insulin resistance, b-cell dysfunction, glucose intolerance and type 2 DM (T2DM).[22] Animal experiments have shown that hyperglycemia increases the oxidative damage of deoxyribonucleic acid (DNA) and plays a role in the pathogenesis of DM complications.[18,23] It has also been shown that TAC, TOS and OSI were significantly increased in the cord blood of infants of diabetic mothers, compared with healthy controls.[24] Several studies have reported occurrences of impaired antioxidant/oxidant balance in GDM caused by increased levels of reactive oxygen species, such as protein glycation, glucose oxidation and lipid peroxidation.[6,9,10]

Fluctuations in TAC and TOS levels can both result from and cause hyperglycemia. Decreased TAC levels in GDM patients increase the amount of insulin required for adequate glycemic control.[25] However, findings regarding the role of the antioxidant system in this imbalance have been conflicting. Some of the studies reported decreased maternal TAC levels in GDM but others did not.[8-10] The presence of oxidant stress in GDM was explained by Biri et al.[6] in terms of impaired antioxidant defense mechanism and increased free radical production. This result means that there is a compensatory increase in the activity of antioxidant system, to cope with the elevated free radical production.

Concerning the possible molecular mechanisms leading to oxidant stress in GDM, the result have, however, been divergent. For instance, Sarıkabadayı et al. suggested that impaired glycemic control is responsible for elevated oxidative activity in infants of diabetic mothers rather than decreased antioxidant enzyme defense systems.[8] However, since OSI was still higher than in the control group, this elevation in TAC was not enough to establish an impaired oxidant-antioxidant balance.[26] Some researchers have argued that the increase in oxidative activity in GDM is not secondary to a deficiency in the antioxidant defense, but is due to impaired glycemic control.[26] It has been suggested that oxidative stress, which mainly arises from hyperglycemia, is implicated in the development of diabetic complications. Moreover, impairment of the antioxidant system may also play a role in occurrences of oxidant stress in GDM.[6]

Studies have found that mothers experience increased OS and inflammatory responses during late gestation and lactation. These symptoms are likely to affect not only the wellbeing of the mothers, but also the health of their offspring.[27] Impairment of the balance of oxidants and antioxidants can also affect newborns, because this period of development is a more sensitive phase with low antioxidant capacity.[28] In our study, neither the cord blood of infants of diabetic mothers nor the milk TAC, TOS or OSI levels were significantly different between the groups. In a similar study, the TAC levels were similar, and the result was interpreted as a compensatory response (24). Human milk contains many bioactive antioxidant compounds that are part of the body's defense system against the actions of various free radicals, such as superoxide dismutase, glutathione peroxidase, vitamins C, A and E and α-carotenes.[29] Moreover, in diabetic animal models, antioxidant therapy has been shown to be

effective for alleviating the deleterious effects of GDM on the fetus.[30] It is still unclear whether a diet rich in antioxidants, or antioxidant supplementation of the diet, might improve oxidative stress in GDM.[11]

We could not find any relationship between the TAC, TOS and OSI levels of the study and control groups, and we have two explanations for this result. Firstly, as the mean HbA1c level was 5% ± 0.4%, we can state that blood glucose levels were regulated adequately and that these results were reached under conditions of good glycemic regulation in the study group. Additionally, consumption of antioxidant foods was not taken into account, and this was a limitation of our study that may have affected the results. Moreover, antioxidants can be used to decrease OSI, besides decreasing TOS. For example, omega-3 fatty acids have been shown to decrease malondialdehyde (MDA) levels in GDM patients.[31] In the literature, it was shown that when lipid peroxidation products (MDA) increased, levels of glutathione peroxidase (GPX) and superoxide dismutase were decreased in pregnant women with GDM.[9,32] However, Orhan et al. found significantly increased erythrocyte selenium (Se)-GPX activity in insulin-dependent diabetic pregnancy.[12] An animal study by Kemse et al. revealed that supplementation of micronutrients such as folic acid, B12 and omega-3 fatty acids can decrease OS and inflammation related to preeclampsia.[33] Use of supplementation of antioxidant molecules was reported by Gurkan et al., in situations of high MDA and low selenium levels in infants with acute bronchiolitis.[34] In another animal study, resveratrol, a plant-derived antioxidant, was reported to prevent embryopathy resulting from exposure to high glucose levels and increased OSIs.[35]

Among our subjects, 94% of our study group declared that they had been taking some medications. This may have influenced our results.

Moreover, many mothers significantly modify their diets during pregnancy and lactation: in particular, they demand products that are safe and free from synthetic additives. To meet consumer needs, the food industry has begun to use natural antioxidant extracts as food preservatives.[36] Thus, another limitation of our study was that we did not record the participants' food consumption: these foods might have had oxidant/antioxidant effects.

CONCLUSION

Impaired glucose tolerance, one of the most common problems experienced during pregnancy, can produce many complications in newborns through increasing oxidative stress. Thus, in this study, we sought to emphasize the importance of proper glucose regulation for preventing health problems among newborns. Our findings suggested that a compensatory mechanism of oxidative stress was expected to be present in gestational diabetes mellitus and that this might be ameliorated through good glycemic regulation and antioxidant supplementation.

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Relationship between dialysis quality and brain compliance in patients with end-stage renal disease (ESRD): a cross-sectional study

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AUTHORS' KEY WORDS:
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ABSTRACT

BACKGROUND: The high number of patients with end-stage kidney disease (ESRD) on hemodialysis makes it necessary to conduct studies aimed at improving their quality of life.

OBJECTIVES: To evaluate brain compliance, using the Brain4care method for intracranial pressure (ICP) monitoring, among patients with ESRD before and at the end of the hemodialysis session, and to correlate ICP with the dialysis quality index (Kt/V).

DESIGN AND SETTING: Cross-sectional study conducted at a renal replacement therapy center in Brazil.

METHODS: Sixty volunteers who were undergoing hemodialysis three times a week were included in this study. Brain compliance was assessed before and after hemodialysis using the noninvasive Brain4care method and intracranial pressure wave morphology was analyzed.

RESULTS: Among these 60 ESRD volunteers, 17 (28%) presented altered brain compliance before hemodialysis. After hemodialysis, 12 (20%) exhibited normalization of brain compliance. Moreover, 10 (83%) of the 12 patients whose post-dialysis brain compliance became normalized were seen to present good-quality dialysis, as confirmed by Kt/V > 1.2.

CONCLUSIONS: It can be suggested that changes to cerebral compliance in individuals with ESRD occur frequently and that a good-quality hemodialysis session (Kt/V > 1.2) may be effective for normalizing the patient's cerebral compliance.

INTRODUCTION

The number of patients with end-stage kidney disease (ESRD) has been increasing over the years and the treatment method that is most used is hemodialysis (HD).[1,2] Although the development of HD by Willem Kolff and Belding Scribner has revolutionized the treatment of renal failure, mortality remains significantly high, with high rates of comorbidities and low quality of life.[3] Several factors influence the risk of mortality among patients undergoing HD,[4] but high mortality rates may be related to aspects of the dialysis procedure.[5]

To assess the quality of dialysis, the dialysis quality index (Kt/V) is measured, which can be calculated in several ways.[6] This index needs to be at least 1.2 per dialysis session in order to verify that the session was of good quality. Observational studies have demonstrated a relationship between Kt/V and mortality and morbidity. Patient survival is longer when Kt/V is greater than 1.0.[7-9]

Studies have focused on prevention of and intervention in frequent HD-related complications, given that these complications reduce patients' quality of life and can contribute to mortality.[10,11] Approximately 30% of HD sessions have some type of complication,[11] which may include hypotension, muscle cramps and post-dialysis complaints such as headache, fatigue and inability to concentrate.[12]

Frequent symptoms that are present in dialysis patients, such as headache, nausea and muscle cramps, can represent milder forms of dialysis disequilibrium syndrome (DDS), and this often remains undiagnosed.[13] DDS occurs due to a sudden drop in urea levels, which results in an osmotic imbalance, with consequent failure of self-regulation of the cerebral circulation, thus leading to cerebral edema. Its clinical presentations depend on the brain region involved: for

example, edema in the occipitoparietal subcortical white matter can result in sudden loss of vision and other neurological symptoms.[14]

To prevent this syndrome, it is recommended that high-risk patients should be identified, rapid correction of metabolic acidosis using bicarbonate should be avoided and the urea clearance rate and decrease in plasma osmolality should be controlled.[13] Considering that cerebral edema and the consequent decrease in cerebral compliance are involved in the pathophysiology of DDS,[15–17] assessment of intracranial pressure (ICP) could also contribute to a more positive outcome by helping to identify individuals with mild forms of DDS that are hard to recognize.[13]

OBJECTIVE

The aim of this study was to evaluate the brain compliance of patients with ESRD before and at the end of the hemodialysis session, and to correlate the results obtained with the dialysis quality index (Kt/V).

METHODS

This was a cross-sectional study at a renal replacement therapy (RRT) center in Brazil. It was conducted after authorization had been received from the Research Ethics Committee of Universidade Estadual de Ponta Grossa (UEPG) (procedural number: 2174527; approved on June 26, 2014).

Participants

Sixty patients aged 18 years or over, with ESRD, who were undergoing hemodialysis three times a week for 3-4 hours in each session, were included. To define the sample size, studies with similar variables were consulted. However, given that the intracranial pressure variable remains poorly studied, the sample size was defined according to the number of volunteers available. The exclusion criteria were presence of acute infections, chronic viral diseases and pregnancy. All the participants received information about the study and provided written informed consent.

Clinical and dialysis characteristics

The clinical and dialysis characteristics of the participants were obtained through the computerized system of the RRT center at the Santa Casa de Misericórdia de Ponta Grossa Hospital.

Assessment of brain compliance

Brain compliance was assessed through the Brain4care method, using equipment provided by Brain4care (São Paulo, SP, Brazil) for non-invasive ICP monitoring. The method is based on measuring volumetric changes in the skull that are detected by a sensor attached to a bandana that is kept in contact with the patient's head. The equipment filters, amplifies and digitizes the signal coming from the sensor and sends it to a computer. This method has been patented and validated through comparison with the invasive method for monitoring ICP,[18,19] and it has been registered with the Brazilian National Health Surveillance Agency (ANVISA; registration number 81157910004).

The results are obtained through analysis on the ICP wave pulse morphology. The curve obtained has three peaks: i) P1 is a percussion peak that results from transmission of blood pressure from the choroid plexus; ii) P2 varies according to brain compliance; and iii) P3 is related to closure of the aortic valve in the heart. In situations of intracranial compliance, the amplitudes of the peaks P1, P2 and P3 decrease sequentially.[20,21] On the other hand, if the cranial adaptive capacity decreases, there is an increase in the ICP, as well as a change in the ICP pulse waveform because the amplitude of the P2 peak becomes higher than those of P1 and P3.[22]

To numerically represent the volunteers' brain compliance, the ratio between the amplitude of the peaks P1 and P2 was defined as P1/P2 (ratio R = AmpP1/AmpP2). Ideally, the result from this relationship should be > 1.10. A ratio between 1.00 and 1.10 indicates that the patient is on the threshold of abnormality. Values < 1.00 indicate abnormality, i.e. P2 > P1.

Pre-dialysis monitoring was performed before the patient started the hemodialysis session, and the patient was asked to remain immobile for 15-20 minutes of monitoring. At the end of the hemodialysis session, the same procedure was performed.

Statistical analysis

The Kolmogorov-Smirnov test was used to assess the normality of the data. Since most of the continuous variables did not present normal distribution, these were presented as the median and interquartile range. Categorical variables were presented as absolute numbers (n) and relative frequency (%). The paired parameters obtained pre and post-dialysis were analyzed by means of the Wilcoxon test, and the McNemar test was used to assess the significance of normal and altered ICPs. Possible differences between the groups were showed by means of the chi-square test (χ^2) for categorical variables and the Mann-Whitney test for continuous variables. The ICP was also evaluated through a classification and regression tree (CART) model using the dialysis quality index (Kt/V) as the dependent variable and the pre and post-dialysis ICPs as independent variables. In all analyses, the significance level was set at $P < 0.05$. The data were evaluated using the statistical program SPSS 20.0 (SPSS, Chicago, United States).

RESULTS

The clinical parameters of the patients included in this study are shown in **Table 1** and the parameters associated with hemodialysis are shown in **Table 2**.

Figure 1 shows the absolute distributions of individuals with and without ICP changes from before to after hemodialysis. Before

Table 1. Clinical characteristics of the subjects with end-stage renal disease (ESRD)

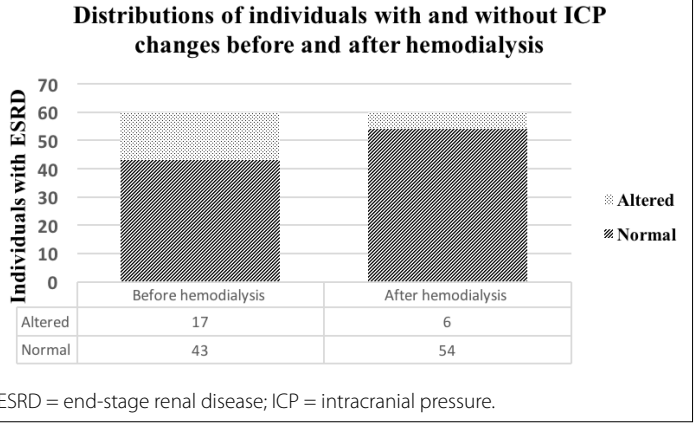
Clinical parameters	ESRD (n = 60)
Age, in years, mean (range)	60 (50-67)
Gender, n (%)	
Male	32 (53)
Female	28 (47)
Underlying diseases relating to CKD, n (%)	
Hypertensive nephrosclerosis	35 (59)
Diabetic nephropathy	23 (38)
Polycystic kidney disease	2 (3)
Length of time on dialysis, in months, mean (range)	45 (30-68)

Values are expressed as the mean and range or the absolute number (n) and relative frequency (%).
CKD = chronic kidney disease.

Table 2. Dialysis characteristics of subjects with end-stage renal disease (ESRD)

Dialysis patients' characteristics	ESRD (n = 60)		
	Pre-dialysis	Post-dialysis	P-value
Weight (kg)	70 (62-78)	67 (58-76)	< 0.0001*
BMI (kg/m²)	26 (22-30)	25 (21-30)	< 0.0001*
SBP (mmHg)	155 (128-181)	151 (122-170)	0.212
DBP (mmHg)	79 (70-92)	78 (69-88)	0.335
MBP (mmHg)	104 (91-123)	103 (88-113)	0.185
BPM	76 (67-85)	72 (64-81)	0.050
Urea (mg/dl)	102 (88-123)	32 (29-42)	< 0.0001*
Kt/V			
> 1.20	-	39 (65)	-
< 1.20	-	21 (35)	-

Values are expressed as the median and interquartile range or absolute number (n) and relative frequency (%); *statistical difference between the groups studied, from Wilcoxon text (P < 0.05); BMI = body mass index; SBP = systolic blood pressure; DBP = diastolic blood pressure; MBP = mean blood pressure; BPM = beats per minute; Kt/V = dialysis quality index.



ESRD = end-stage renal disease; ICP = intracranial pressure.

Figure 1. Bar diagram showing the numbers of subjects with ESRD who had normal and altered intracranial pressure before and after hemodialysis.

the hemodialysis session, 17 individuals (28%) presented ICP changes, while 43 (72%) had normal ICP. After the hemodialysis session, six individuals (10%) were identified as presenting altered ICP and 54 (90%) had normal ICP. Thus, there was a statistical difference (P = 0.035) from before to after hemodialysis for patients with ESRD. It is important to highlight that, out of the 17 patients who presented altered ICP pre-dialysis, 12 presented normal brain compliance after the session, while five continued to present altered ICP. Among the 43 patients who had normal pre-dialysis ICP, one exhibited abnormal ICP after the session.

Table 3 presents a comparison of the clinical and dialysis parameters of the patients with ESRD and their cerebral compliance (normal or altered), to show whether there was any parameter that was related to patients with worse cerebral compliance.

A comparison of the P1/P2 ratio of ICP before and after dialysis, for patients with ESRD who presented normal and altered brain compliance, is shown in **Figure 2**. Individuals with normal brain compliance showed median values for the P1/P2 ratio of 1.67 (range, 1.43-1.83) and those with altered cerebral compliance, 0.87 (range, 0.67-1.00). This difference in the pre-dialysis evaluation was statistically significant (P < 0.001). After dialysis, the patients with normal cerebral compliance exhibited median values of 1.60 (range, 1.33-1.77) and those with altered compliance, 1.34 (range, 1.02-1.50), which was also a statistical difference (P = 0.004). The increase in the P1/P2 ratio after dialysis may indicate an improvement in brain compliance in patients who presented changes in ICP parameters prior to hemodialysis treatment.

Through a classification and regression tree (CART) (**Figure 3**), it was visualized that, out of the 60 volunteers with ESRD, 21 (35%) had a Kt/V < 1.20 and 39 (65%) had a Kt/V > 1.20. After the hemodialysis session, there were six individuals with abnormal brain compliance: three with a Kt/V < 1.20 and three > 1.20. Among these six individuals, five of them showed altered brain compliance before the hemodialysis session. One of them presented normal brain compliance before the dialysis and altered compliance afterwards. Also in relation to pre-dialysis brain compliance, out of the 12 individuals whose brain compliance was altered before dialysis and became improved through it, 10 of them had a Kt/V >1.20. Therefore, it can be suggested that efficient dialysis may have helped in improving cerebral compliance.

DISCUSSION

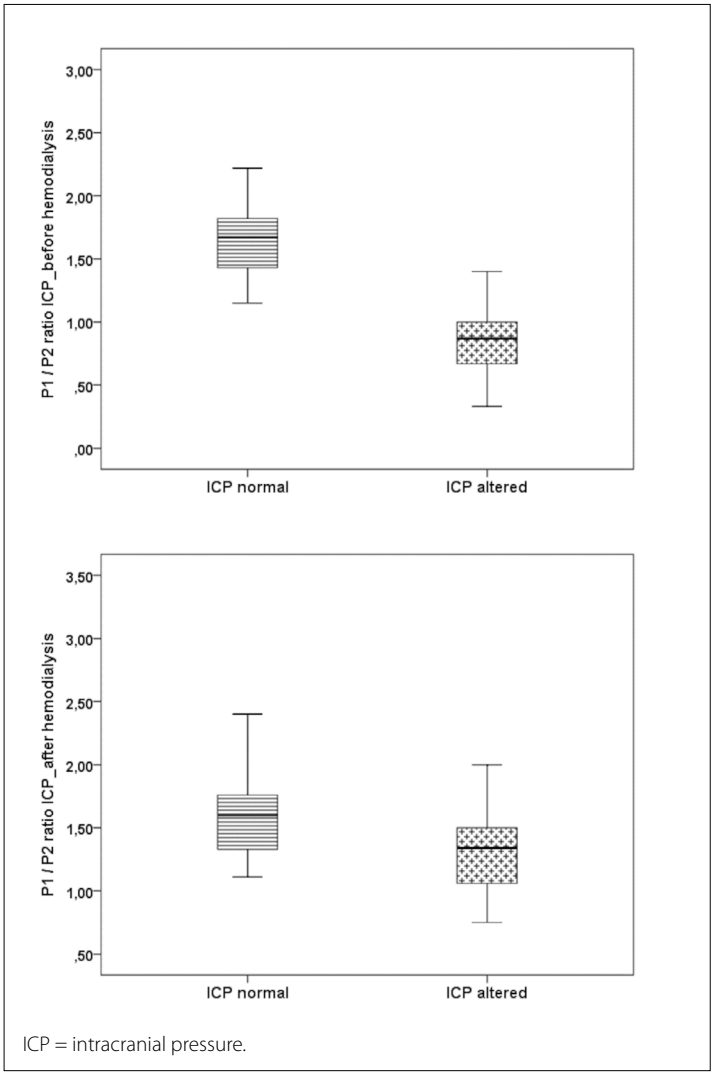
In the present study, the main finding was that the alteration in pre-dialysis brain compliance that was observed in 28% of the volunteers showed a tendency to become normalized, as seen immediately after the dialysis. Moreover, this study presented the possibility that this result may have been related to HD quality.

Previous studies that assessed the ICP of patients with renal failure did so through correlating the altered results with DDS.[16,23,24] However, no studies had previously assessed the brain

Table 3. Comparison of clinical and dialysis variables of subjects with end-stage renal disease (ESRD), according to intracranial pressure: normal or altered

Parameters	ESRD		
	ICP parameters		P-value
	Normal (n = 42)	Altered (n = 18)	
Clinical			
Age, in years[a]	62 (55-67)	50 (44-66)	0.021*
Gender, n (%)[b]			
Male	25 (60)	7 (39)	0.235
Female	17 (40)	11 (61)	
Underlying diseases relating to CKD, n (%)[c]			
Hypertensive nephrosclerosis	23 (55)	12 (67)	-
Diabetic nephropathy	17 (40)	6 (33)	-
Polycystic kidney disease	2 (5)	0 (0)	-
Time on dialysis, in months[a]	41 (30-65)	49 (32-84)	0.345
Dialytic			
Weight (kg)[a]			
Pre-dialysis	69 (63-82)	71 (56-77)	0.425
Post-dialysis	66 (60-80)	68 (54-75)	0.429
BMI (kg/m²)[a]			
Pre-dialysis	25 (23-31)	24 (21-29)	0.302
Post-dialysis	25 (23-30)	24 (20-28)	0.379
SBP (mmHg)[a]			
Pre-dialysis	157 (135-190)	134 (121-159)	0.031*
Post-dialysis	153 (119-175)	142 (124-154)	0.220
DBP (mmHg)[a]			
Pre-dialysis	79 (70-91)	79 (67-95)	0.771
Post-dialysis	77 (68-86)	80 (68-91)	0.942
MBP (mmHg)[a]			
Pre-dialysis	106 (93-128)	103 (85-113)	0.208
Post-dialysis	102 (88-118)	103 (90-110)	0.463
BPM[a]			
Pre-dialysis	74 (63-84)	82 (74- 92)	0.023*
Post-dialysis	70 (62-77)	79 (71-91)	0.008*
Urea (mg/dl)[a]			
Pre-dialysis	102 (84-127)	99 (89-116)	0.910
Post-dialysis	37 (28-45)	32 (29-39)	0.375
Kt/V[b]			
< 1.20	16 (38)	5 (28)	0.637
> 1.20	26 (62)	13 (72)	

Values are expressed as the median and interquartile range or absolute number (n) and relative frequency (%); [a]Mann-Whitney test; [b]chi-square χ^2 test; [c]descriptive statistics; *statistical difference between the groups studied (P < 0.05); CKD = chronic kidney disease; BMI = body mass index; SBP = systolic blood pressure; DBP = diastolic blood pressure; MBP = mean blood pressure; BPM = beats per minute; Kt/V = dialysis quality index.



ICP = intracranial pressure.

Figure 2. Boxplot showing the median and interquartile range of the P1/P2 ratio for ICP, before and after hemodialysis, among subjects with end-stage renal disease who presented normal and altered intracranial pressure.

compliance of routine HD patients, because the technique most used is highly invasive.

Noninvasive assessment of cerebral compliance through the Brain4care method could be useful in monitoring patients with ESRD in HD. It would constitute an additional tool for clinical evaluation of these patients and would possibly help in early detection of complications. This method has already been used in several situations such as epilepsy,[25] hydrocephalus,[26] cryptococcal meningitis associated with HIV infection,[27] traumatic brain injury,[19] hemorrhagic stroke[28] and assessment of cerebral compliance in the elderly,[29] among others.

ICP refers to the pressure inside the skull, which is influenced by blood and cerebral parenchyma and by the circulatory dynamics of cerebrospinal fluid (CSF). If there is an increase in the proportion of one of these components (blood, fluid or parenchyma) and the cerebral adaptive capacity is exceeded, the ICP increases.[30,31]

The aim of HD is to simulate the process of glomerular ultrafiltration. It is based on the principle of diffusion, in which clearance or removal of a high concentration of uremic toxins present in the blood is achieved by means of migrating the blood through

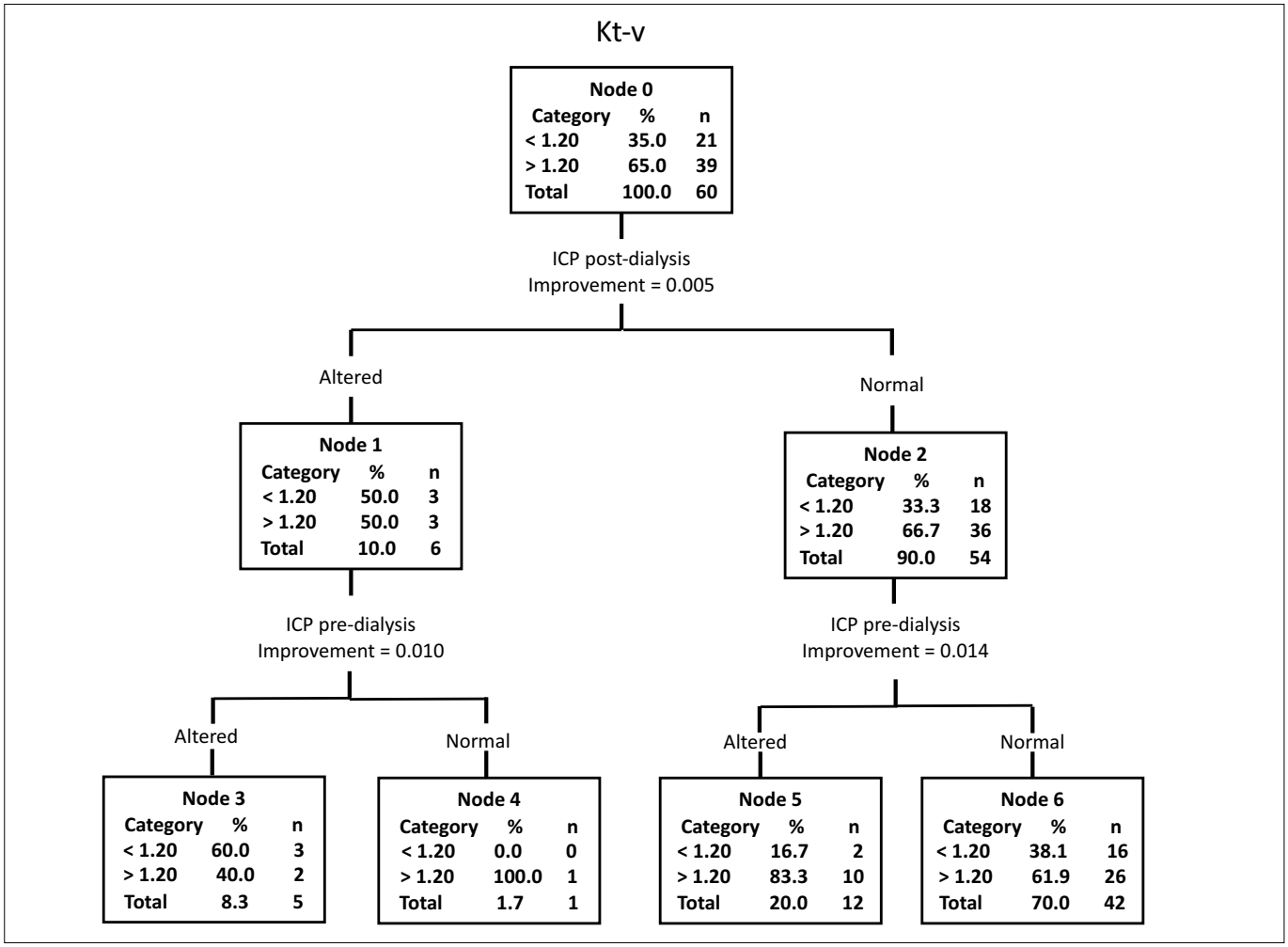


Figure 3. Classification and regression tree (CART) for dialysis quality (Kt/V) in relation to pre and post-dialysis intracranial pressure.

a semipermeable membrane (the dialyzer or filter), to form a solution of lower concentration, called the dialyzed solution.[32]

A normal ICP waveform (**Figure 3**) was observed in 90% of the patients in the present study after dialysis. Considering the mechanisms of HD and the pathophysiology of changes to ICP, it can be suggested that the removal of fluids that occurs in HD directly influences the maintenance of the balance between blood, CSF and cerebral parenchyma, thereby normalizing ICP.

According to Robertson et al.,[33] patients with intracranial hypertension often have elevated blood pressure due to sympathetic hyperactivity, especially in cases of head trauma. However, in the present study, patients with altered ICP parameters had lower systolic blood pressure (SBP). At the same time, it was found that patients with changes to their cerebral compliance had higher heart rate values, both pre and post-dialysis. Dimitri et al.[34] suggested that there was direct interaction or communication between the heart and the brain, with the observation that when the ICP rose, the heart rate also increased. This is important information, because it can assist in validation of the ICP parameters for assessing cerebral compliance.

Another important result from the present study was the relationship between cerebral compliance and the best-quality dialysis, as shown by Kt/V > 1.20. It can be suggested that good-quality dialysis can help to improve the post-dialysis ICP parameters of patients with ESRD who presented altered brain compliance before HD.

In this study, one volunteer showed changes to his brain compliance only after dialysis. No complications were reported in this patient's medical records during his HD session, and his Kt/V was 1.25. In addition, it was not possible to establish any relationship between this finding and the patient's clinical and dialysis parameters.

In the formula of the Kt/V ratio, K refers to the dialyzer urea clearance, which is multiplied by the treatment time (t) and divided by the patient's urea distribution volume (V). K depends on the blood flow rate, size of the dialyzer and flow of the dialysate, t varies from three to four hours and the urea distribution volume of

the patient (V) corresponds to approximately 55% of the individual's body weight.[35] This volume can be more accurately estimated through an anthropometric equation that uses an individual's gender, age, height and weight (e.g. the Watson equation).[36] Based on the above, several factors can influence the quality of HD and be reflected in the Kt/V ratio, such as the duration of the session and the interdialytic weight gain, which needs to be controlled by the patient through dietary care.

Inadequate hemodialysis (Kt/V < 1.20) can occur due to low adherence or non-adherence to treatment recommendations (such as fluid restriction, regular frequency of dialysis sessions and adherence to 240-minute sessions) and because of the clearance limitations of the conventional HD technique. It has been shown that not attending just one dialysis session is associated with a 25%-30% increase in the risk of death. This is a common problem with regard to HD.[12]

According to Kimata et al.,[37] small changes in the way of conducting HD sessions, such as increasing the blood flow rate to 200 ml/min and the treatment time to four hours for some patients, can decrease the percentage of patients with Kt/V < 1.20, with a consequent increase in survival among these patients.

However, four-hour dialysis sessions do not guarantee good-quality dialysis. It is necessary to consider the residual renal function, the Kt/V ratio normalized according to body surface area and the expected ultrafiltration rate. In addition, it is important to consider the patients' acceptance of the duration of dialysis to which they are subjected, since this directly impacts their quality of life and treatment adherence.[38]

Madero and Sarnak[39] questioned whether the hemodialysis procedure might be partly responsible for brain structural and cognitive changes, such as cerebral edema, which leads to increased ICP. However, our results suggested that an adequate hemodialysis session, with Kt/V > 1.20, may be effective in normalizing the ICP of individuals who have presented changes.

Lastly, it is worth mentioning that the clinical assessment is superior to any Kt/V formula and should serve as the basis for determining the adequacy of dialysis.[6] Thus, the importance of clinical assessment of cerebral compliance among patients undergoing HD can be emphasized.

CONCLUSION

Through noninvasive assessment of the ICP parameters by means of the Brain4care® method, it could be seen that changes to cerebral compliance among patients on hemodialysis can occur frequently. Moreover, it can be suggested that good-quality hemodialysis (i.e. when Kt/V is greater than 1.20) may help to normalize the ICP parameters. Thus, the importance of maintaining a Kt/V ratio of at least 1.20 can be emphasized, in order to ensure good-quality dialysis for patients with ESRD.

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Association between frailty and depression among hemodialysis patients: a cross-sectional study

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ABSTRACT

BACKGROUND: Frailty is consensually understood to be a clinical syndrome in which minimal stressors can lead to negative outcomes such as hospitalization, early institutionalization, falls, functional loss and death. Frailty is more prevalent among patients with chronic kidney disease (CKD), and those on dialysis are the frailest. Depression contributes towards putting patients with CKD into the frailty cycle.

OBJECTIVE: To assess frailty and its relationship with depression among patients with CKD undergoing hemodialysis.

DESIGN AND SETTING: Observational and quantitative cross-sectional study conducted in a renal therapy unit, located in the interior of the state of São Paulo, Brazil.

METHODS: This investigation took place in 2019, among 80 patients. The following instruments were applied: a sociodemographic, economic and health condition characterization and the Subjective Frailty Assessment (SFA) and Patient Health Questionnaire-9 (PHQ-9).

RESULTS: Among the patients, there was higher prevalence of females, individuals with a steady partner and retirees, and their mean age was 59.63 (± 15.14) years. There was high prevalence of physical frailty (73.8%) and depression (93.7%). Depression was associated with frailty, such that patients with depression were 9.8 times more likely to be frail than were patients without depression (odds ratio, OR = 9.80; 95% confidence interval, CI, 1.93-49.79).

CONCLUSION: Based on the proposed objective and the results achieved, it can be concluded that depression was associated with the presence of frailty among patients with CKD on hemodialysis.

INTRODUCTION

Frailty is consensually understood to be a clinical syndrome in which minimal stressors can lead to negative outcomes such as hospitalization, early institutionalization, falls, functional loss and death.[1] There are several models for frailty, but the two that have been most widely used are the deficit model, developed by Mitnitski, Mogilner and Rockwood,[2] and the phenotype-based model, developed by Fried et al.[3]

Because of the high incidence and prevalence of physical and cognitive impairment among patients with chronic kidney disease (CKD), these individuals are more likely to develop frailty early.[4] According to a systematic review carried out by Chowndhuty et al.,[5] frailty is very prevalent in patients with CKD, and those on dialysis are the frailest. Furthermore, frailty has been shown to be associated with increased risk of hospitalization and mortality among patients with CKD. An association between frailty and higher risk of mortality among patients seen at rapid diagnostic clinics (RDCs) has been found in other studies in the literature.[4,6,7]

According to Wu et al.,[6] in addition to sociodemographic factors and health issues, depression contributes towards putting patients with CKD into the frailty cycle. A study by Perez et al.[7] among frail and non-frail adults with diabetes mellitus (type 1 and type 2) and with chronic kidney disease (stages 1 to 5) demonstrated that frail participants presented depression more often ($P \leq 0.005$) than did the non-frail participants. In addition, depression has been found to be the most recurrent psychiatric condition among patients with CKD, with a rate of 20 to 30% among patients on hemodialysis.[8]

OBJECTIVE

The aim of the present study was to assess frailty and its association with depression among patients with CKD undergoing hemodialysis.

METHODS

Design

The present study was of observational and quantitative cross-sectional nature. The investigation took place in 2019, at a dialysis service located in the interior of the state of São Paulo, Brazil.

Sample

The final sample was 80 patients who met the following inclusion criteria: having a medical diagnosis of CKD, being on hemodialysis and possessing preserved oral communication. The exclusion criterion was presentation of dementia that could be verified in the medical records. An initial non-probabilistic convenience sample of 180 patients was used; seventy patients were excluded from the study, 46 did not meet the study's eligibility criteria and 64 did not agree to participate in the study.

Data collection

The data collection process took place as follows. At the initial contact with the patients, the research project was explained to them and they were invited to participate in the study. Patients who agreed to participate signed a free and informed consent statement. At their next hemodialysis session, and specifically within the first two hours (during which patients present with fewer hemodynamic changes), evaluations were started using a sociodemographic, economic and health condition characterization and the Subjective Frailty Assessment (SFA) and Patient Health Questionnaire-9 (PHQ-9).

The sociodemographic, economic and health condition characterization consisted of asking about the following characteristics: sex, age, marital status, ethnicity, comorbidities, education level, income, time on hemodialysis and number of medications.

The SFA was validated in Brazil by Nunes,[9] based on the components of the frailty phenotype proposed by Fried et al.,[10] which was considered to be the gold standard. The frailty phenotype consists of the following five criteria: unintentional weight loss, reported fatigue, reduced grip strength, reduced walking speed and low physical activity. Nunes[9] elaborated and validated items for subjective evaluation, especially through dichotomous questions about each component. The SFA can have a total score of 5 points, and individuals are considered non-frail if they do not have any of the criteria evaluated. They are considered pre-frail if they have one or two of these components and frail if they have three or more components.

The PHQ-9 is a screening scale for depression that was validated by Kroenke, Spitzer and Williams[11] and adapted for use in Brazil by Santos et al.[12] It is an instrument with nine questions based on the clinical diagnostic criteria for depressive episodes (Diagnostic and Statistical Manual of Mental Disorders, fourth edition, DSM-IV), which form the evaluation module for depressive disorders in the Patient Health Questionnaire (PHQ). The symptoms evaluated are the following: depressed mood; anhedonia (loss of interest or pleasure in doing things); problems with sleep, tiredness or lack of energy; changes in appetite or weight; feelings of guilt or worthlessness; problems with concentration; feelings of slowness or restlessness; and thoughts of suicide. The frequency of each symptom over the past two weeks is assessed on a Likert scale from 0 to 3 corresponding to the answers "no days", "less than a week", "more than a week" and "almost every day", respectively. The questionnaire also includes a tenth question that assesses the interference of these symptoms in performing daily activities, such as working and studying. If the final score reaches 5, the individual is characterized as having mild depression; 10, moderate; 15, moderately severe; and 20 or more, severe.

Data analysis

The statistical treatment of the data was performed with support from the Statistical Package for the Social Sciences (SPSS) software, version 22.0 (IBM Corporation, Armonk, New York, United States). Descriptive analyses were performed, with preparation, including central trend data (average, minimum and maximum) and dispersion measurements (standard deviation). The Kolmogorov-Smirnov test was performed to ascertain whether the data showed normal distribution. This showed that nonparametric tests needed to be used.

To compare continuous variables according to the level of physical frailty, with subjective frailty assessment (non-frail, pre-frail or frail), the Kruskal-Wallis test was used. To compare categorical variables according to the level of physical frailty, with subjective frailty assessment (non-frail, pre-frail or frail), the Pearson test was adopted. The significance level for the statistical tests was taken to be 5% ($P \leq 0.05$).

To investigate the association between depression and frailty, multivariate logistic regression analysis was performed. Initially, univariate logistic regression analyses were performed using sociodemographic, economic and health condition variables. Statistically significant variables ($P < 0.25$) in the univariate regression analyses were selected for the multivariate analysis. The model was adapted for sex, age and education. The respective odds ratios (OR) and 95% confidence intervals (CI) were calculated.

Ethical considerations

The protocol was approved by the ethics committee of our institution (CAAE: 18828419.0.0000.5504; number 3.535.236; date: August 27, 2019). Participants needed to sign an informed consent statement before entering the study.

RESULTS

Regarding frailty assessed according to the SFA scale, it was found that only four participants were non-frail, while 17 (21.2%)

were pre-frail and 59 (73.8%) were frail. The sociodemographic, economic and comorbidity profile did not show any statistically relevant difference between the levels of frailty. The majority of the participants were female, white and retired, and had a steady partner. Their average age was less than 61 years; their average schooling level did not exceed 8.5 years; and their average per capita income did not exceed 1.5 minimum monthly wages (**Table 1**).

Regarding the comorbidities of diabetes and hypertension, **Table 1** shows that most participants reported having these comorbidities, regardless of their level of frailty. The number of continuous-use medications was 6.50 for non-frail, 6.06 for pre-frail and 6.41 for frail individuals.

Regarding depression, it was noted that overall, 83.7% of the participants had depression, at levels ranging from mild to severe. When compared according to frailty levels, there was a statistically significant difference between the groups. Non-frail and pre-frail patients had mean scores of 5 and 8.88, respectively, thus characterizing a mild degree of depression. Frail participants, on the other hand, had an average score of 15.92, thus characterizing moderately severe depression, according to the PHQ-9 instrument (**Table 1**).

Table 1. Sociodemographic variables, economic characteristics, comorbidities and depression measured through the patient health questionnaire-9 (PHQ-9), according to levels of frailty. São Carlos (SP), Brazil, 2019 (n = 80)

Variable	Categories	Non-frail (n = 4)	Pre-frail (n = 17)	Frail (n = 59)	P-value
Sex	Male	3	8	25	0.44
	Female	1	9	34	
Ethnicity	White	4	13	35	0.52
	Black	0	2	18	
	Brown	0	2	5	
	East Asian	0	0	1	
Marital status	With a fixed partner	4	10	38	
	No fixed partner	0	7	20	0.59
Occupation	Retired	4	8	45	
	Absent*	0	4	6	0.11
	Housewife	0	3	7	
	Other	0	2	0	
Comorbidities	Diabetes				0.67
	Yes	2	12	36	
	No	2	5	23	
	Hypertension				
	Yes	2	7	26	0.95
	No	2	10	33	
	Other types of comorbidities				
	Yes	3	17	52	0.21
	No	1	0	7	

Variable	Categories	Mean	P-value
Age**	Non-frail (n = 4)	60.25	
	Pre-frail (n = 17)	55.88	0.59
	Frail (n = 59)	60.66	
Education level**	Non-frail (n = 4)	8.50	0.15
	Pre-frail (n = 17)	7.24	
	Frail (n = 59)	6.37	
Income***	Non-frail (n = 4)	1,226.75	
	Pre-frail (n = 17)	996.88	0.20
	Frail (n = 59)	930.50	
Number of medications	Non-frail (n = 4)	6,50	
	Pre-frail (n = 17)	6.06	0.57
	Frail (n = 59)	6.41	
Depression (PHQ-9)	Non-frail (n = 4)	5.00	
	Pre-frail (n = 17)	8.88	0.001
	Frail (n = 59)	15.92	

*Absent from work, as approved by the National Institute of Social Security. **In years; ***In reais; Kruskal-Wallis test; Pearson's test.

In the analysis on depression as a predictor of frailty, it was noted that depression was associated with frailty. Patients with depression were 9.8 times more likely to be frail than were patients without depression (OR = 9.80; 95% CI, 1.93-49.79). For the other variables analyzed (sex, age and education level), there was no statistically relevant relationship (**Table 2**).

DISCUSSION

In the present study, among the sociodemographic, economic and health condition characteristics of the participants, female sex was more prevalent, the mean age was 59.63 years and the mean schooling level was 6.66 years. It was noted that 83.7% of the patients presented depression. This profile has also been found in other studies conducted in Brazil and in other countries among patients with CKD.[4,13,14]

Assessing the occurrence rates of frailty and depression syndromes is challenging given the diversity of models and measurements that have been used in studies to identify their prevalences. In this, the heterogeneity of the conditions themselves needs to be borne in mind: their expression of signs and symptoms varies according to age and socioeconomic context.[5]

With regard to frailty, 21.3% of the patients were considered pre-frail in the present study. However, in the study by Gesualdo et al., in which the objective was to evaluate frailty and identify its associated factors among adults and elderly people with CKD who were undergoing hemodialytic treatment, most of the adults were pre-frail (54.84%). Their study corroborates the findings of the present study regarding the prevalence of frailty.[4]

Another study that addressed factors associated with frailty was conducted by Duarte et al.[17] In that study, the aim was to describe the prevalence of frailty among elderly people, in order to analyze associated factors and the evolution of the syndrome. A total of 1,399 older adults aged 60 years or older, living in the state of São Paulo, Brazil, participated in the study. Frailty syndrome was evaluated based on the phenotype proposed by Fried et al.,[10] composed of five components: unintentional weight loss, self-reported fatigue, reduction in strength, low walking speed and low level of physical activity. Duarte et al.[17] found that out of the total number of elderly subjects (n = 1,399), 8.5% were frail and presented factors associated with age, functional impairment, cognitive decline, hospitalization and multimorbidity. Over a four-year period, 3.3% of the non-frail and 14.7% of the pre-frail elderly people became frail. In the present study, we found that the prevalence of frailty among the patients was 73.8%.

Table 2. Multivariate logistic regression analysis on frailty (n = 80)

	Frailty		
Variables	**P-value**	**OR***	**95% CI**
Sex**	0.105	2.27	1.93-49.79
Age	0.601	1.01	0.97-1.05
Education level	0.574	0.96	0.83-1.11
Depression***	0.006	9.80	1.93-49.79

*Risk ratio for frailty/subjective frailty assessment; **women; ***presence of depression. OR = odds ratio; CI = confidence interval.

Although most definitions of frailty focus primarily on physical and biological indicators of vulnerability, it is significant to consider the risk of adverse health outcomes associated with mental disorders. As well as frailty, depression among the elderly has been characterized in terms of diminished reserve capacity, thus representing a lack of resources to respond to stressors.[15] Hence, depression can serve to accentuate physical frailty: not because it is associated with any disease process or model of frailty in particular, but because it represents a lack of psychological and social coping resources.[15,16]

The association between frailty and depression demonstrated in the present study was also found in other studies, such as the one by Tavares et al.[17] In that study, the aim was to describe the socioeconomic variables of older adults with indications of depression according to sex; investigate the association between frailty status and sex; and describe the component of the frailty phenotype most impacted among elderly people with indications of pre-frail and frail depression. A total of 418 elderly people with indications of depression living in the city of Uberaba, Minas Gerais, Brazil, participated in that study. The results showed that the prevalence of frailty among older adults with indications of depression was 27.8%, while 51.7% presented pre-frailty. That study corroborated the findings of the present study, in which depression was associated with frailty, thus indicating that patients with depression were 9.8 times more likely to be frail than were patients without depression. In the light of this exposure, it is possible to infer that patients with CKD who are undergoing hemodialysis treatment are more vulnerable to becoming affected by depressive symptoms.

Another study that addressed depression in CKD patients was conducted by Pretto.[18] The aim in that study was to investigate associations shown by sociodemographic variables, clinical factors, lifestyle habits and functional capacity in relation to indications of depression among chronic renal patients on hemodialysis. The study participants were 238 patients, who were attending the reference renal units for the Northwest and Missions regions of Rio Grande do Sul, Brazil. Beck's Depression Inventory (BDI) was used to assess depression. It was found that the prevalence of symptoms indicative of depression among the patients was 60.3% (111): mild in 36.4% (67), moderate in 22.3% (41) and severe in 1.6% (3).

Depression scores are three to four times higher among patients with CKD than in the general population and two to three times higher than among people with other chronic diseases. In the present study, it was found that 83.7% of the patients had depressive symptoms, i.e. more than half of the patients presented depression, just as in the study by Pretto.[18]

Nursing plays an important role in this scenario. Nurses are the professionals who establish greatest contact and bonding with these patients, given the long periods of time spent with them.

Health education plays a fundamental role in promoting quality of life for patients affected by CKD. Moreover, this raises awareness among patients, family members and the nursing team, thereby stimulating changes to behavioral strategies.

Future research should continue to explore the reasons for comorbidities and the implications of comorbidities in predicting adverse health outcomes. Therapies to combat depression can prevent or mitigate the symptoms of frailty.

The limitation of the present study was that its sample was selected for convenience.

CONCLUSION

From the proposed objective of this study and the results achieved, it can be concluded that depression was associated with the presence of frailty among patients with CKD who were undergoing hemodialysis. Thus, it is important to highlight the need for early screening of frailty in this population.

In addition, there is an urgent requirement to create healthcare policies that meet the social and psychological needs of these patients, given that this is a predictable and preventable syndrome. In addition, professionals must pay attention to the signs and symptoms of depression that can lead patients to frailty, in addition to other outcomes.

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Relationship between depressive symptoms, burnout, job satisfaction and patient safety culture among workers at a university hospital in the Brazilian Amazon region: cross-sectional study with structural equation modeling

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ABSTRACT

BACKGROUND: Workplaces can be sources of mental distress. In healthcare services, this can also affect patients.

OBJECTIVE: To assess the prevalence of and factors associated with depressive symptoms, burnout, job satisfaction and patient safety culture and the relationships between these constructs, among healthcare workers.

DESIGN AND SETTING: Cross-sectional study in a university hospital in Manaus, Brazil.

METHODS: Randomly selected workers were interviewed based on Brazilian-validated tools. We calculated the prevalence ratio (PR) and 95% confidence interval (CI) of depressive symptoms and burnout using Poisson regression with robust variance; and the β-coefficient of safety culture and job satisfaction using linear regression. Outcome relationships were assessed using partial least-squares structural equation modeling.

RESULTS: 300 professionals were included; 67.3% were women. The prevalence of depressive symptom was 19.0% (95% CI: 14.5; 23.5%) and burnout, 8.7% (95% CI: 5.2; 12.3%). Lack of work stability increased depression (PR = 1.88; 95% CI: 1.17; 3.01) and burnout (PR = 2.17; 95% CI: 1.03; 4.57); and reduced job satisfaction (β = -11.93; 95% CI: -18.79; -5.07). Depressive symptoms and burnout were positively correlated, as also were job satisfaction and safety culture (P < 0.001); job satisfaction was negatively correlated with burnout (P < 0.001) and depression (P = 0.035).

CONCLUSION: Impermanent employment contracts increased depression and burnout and reduced job satisfaction. Job satisfaction reduced poor mental health outcomes and increased safety culture. Job satisfaction and safety culture were directly proportional (one construct increased the other and vice versa), as also were depression and burnout. Better working conditions can provide a virtuous cycle of patient safety and occupational health.

INTRODUCTION

Negative psychological effects are frequently experienced by healthcare professionals, due to distress relating to patients' sorrows, health-threatening diseases and poor working environments. Workplace conditions within healthcare affect both the health of the workers and the safety of care.[1] Adverse events and harm to patients cause physical, psychological and professional distress and require proper organizational support for the team.[2] Frequent exposure to this insalubrious setting affects the emotional health of workers, who face high prevalence of mental health problems.[3,4] In this highly human-mediated activity, practitioners' and patients' health are mutually influenced.[5]

Chronic workplace stress that is not properly managed has been recognized as a cause of burnout syndrome, which was included as a morbidity in the International Classification of Diseases for Mortality and Morbidity Statistics, eleventh revision, in 2019.[6] Satisfaction and engagement with labor are central to wellbeing and good performance at both the individual and the collective level.[7]

The relationships between work conditions, workers' health and patient safety need further investigation. A review on professional burnout, depression, employee commitment, patient outcomes and safety culture interplays identified that even though safety culture and clinical errors

were both associated with burnout, few studies had focused on engagement and individual-level factors that impact patient safety culture.[8] A previous Brazilian survey observed an inverse causal relationship between burnout syndrome and job satisfaction, while depressive symptoms were predictors for burnout.[9] Real-world evidence about the connections between safety culture, job satisfaction, depression and burnout could enlighten the discussions and improve both workplace and patient safety.

Safety culture was deemed to be fragile, with predominance of individual culpability standards, in a previous survey at a university hospital in the Brazilian Amazon region.[10] This scenario motivated an investigation on how patient safety culture, workers' engagement and outcomes interact in this setting.

OBJECTIVE

The aim of this study was to assess the prevalence of and factors associated with depressive symptoms, burnout, job satisfaction and patient safety culture and the relationships between these constructs, among healthcare workers at a university in the Brazilian Amazon region.

METHODS

Study design and setting

This was a cross-sectional study conducted among healthcare workers at Getulio Vargas University Hospital from July to November 2016. This is a teaching hospital belonging to the Universidade Federal do Amazonas (UFAM), and it forms part of the Brazilian National Health System (Sistema Único de Saúde, SUS). At the time of this study, the hospital had 159 beds (11 in intensive care) and 1,222 employees.

Participants

Individuals who had been employees for at least three months and who were working in the main building of the hospital were eligible for inclusion in this study. The sample size was calculated as 300 participants, considering the potential total population of 863 eligible employees, a frequency of positive answers regarding safety culture of 50%, a confidence level of 95%, design effect of 1 (random sampling) and addition of 10% to compensate for losses. From the hospital's human resources spreadsheet, we randomly selected 300 employees for interviews and 300 employees to serve as replacements in cases of refusals.

Variables

The primary outcomes were the prevalences of depressive symptoms and burnout, and the perceptions of job satisfaction and patient safety culture.

The independent variables were the following: sex (women or men); age (in years, categorized as 18-35, 35-50 or 51 or over); skin color/race (whites [white and Asian] or non-whites [black, indigenous and brown, i.e. Brazilian mixed race]); marital status (married or single [single, separated, divorced or widowed]); body composition (normal, overweight or obese); educational level (high school or less, higher education or postgraduate school); economic classification (A, B1, B2 or C/D/E); profession (physicians, nursing staff [nurses and nursing technicians], other healthcare professionals [psychologists, nutrition/radiology/laboratory technicians, physiotherapists, social workers and nutritionists] or technical support [maintenance/cleaning and administrative staff, i.e. office assistant/secretary/receptionist]); working in direct contact with patients (yes or no); length of time working in the hospital (in years, categorized as < 1, 1-2, 3-4, 5-10, 11-20 or ≥ 21); work contract stability (permanent [statutory, contracted through consolidation of labor laws] or temporary [contracted through a support foundation or residency scholarship]); number of jobs (1, 2 or ≥ 3); and weekly workload (in hours, categorized as 20-40, 41-60 or ≥ 61).

Data sources and measurement

The participants who had been drawn for inclusion were contacted by the research team and were asked to answer a questionnaire using electronic devices (Samsung Tab-3 SM-T110). The KoboToolbox software (https://www.kobotoolbox.org/) was used to configure the questionnaire, with mandatory answers for each question. The software worked offline and filled-out forms were submitted automatically once an internet connection was available.

Depressive symptoms were measured using the nine-item Patient Health Questionnaire (PHQ-9), in its version validated for use in Brazil.[11] We adopted the cutoff point of ≥ 9 to define presence of depressive symptoms.[12] The Brazilian version of the Maslach Burnout Inventory Human Services Survey (MBI-HSS) was used to assess burnout, which was taken to be present in cases of higher scores for emotional exhaustion (≥ 27) and depersonalization (≥ 13), and lower scores for personal accomplishment (≤ 31).[13]

Job satisfaction was assessed through the validated version of the Job Satisfaction Survey (JSS) for the Brazilian context.[14] The score range of 36-108 was taken to indicate dissatisfaction; 144-216, satisfaction; and 109-143, ambivalence. Safety culture was measured through the Brazilian validated version of the Safety Attitudes Questionnaire (SAQ). After reversing the scores of negatively worded items, the positivity percentage was calculated and results of 75% or more were considered to denote the presence of a positive safety culture.[15]

The economic classification was based on reported possession of comfort items and on the education level of the head of the family, which led to a classification from A (richest) to E (poorest).[16] Weight and height were self-reported by the participants and body mass index (BMI) was obtained by dividing the weight in kilograms (kg) by the square of the height in meters (m^2). BMI was classified as normal (≤ 24.9 kg/m^2), overweight

(25-29.9 kg/m²) or obese (≥ 30 kg/m²). Weekly workload and the number of jobs were obtained from the Brazilian National Registry of Healthcare Establishments by searching for the professional's name.[17] Information on work contract stability was obtained from the hospital's human resources spreadsheet.

Statistical methods

We calculated descriptive statistics for all variables, and obtained frequencies, means, standard deviations and 95% confidence intervals (CI), according to the nature of the data. The instruments' reliability was calculated by means of Cronbach's alpha, using a cutoff of ≥ 0.6.

The prevalence ratio (PR) and 95% CI of depressive symptoms and burnout were calculated by means of Poisson regression with robust variance. Factors associated with safety culture and job satisfaction were calculated through linear regression to obtain the coefficient β and 95% CI. We adopted the significance level of $P < 0.05$ to identify presence of associations between the outcomes and independent variables.

The relationships between depressive symptoms, burnout, job satisfaction and patient safety culture were assessed using partial least-squares structural equation modeling (PLS-SEM). The significance and the relationships between the constructs were established through the path coefficient, expressed as values between -1 and 1. Values close to 0 indicate weak relationships and Pearson's coefficient of determination was classified as showing a large effect if $R^2 \geq 26\%$.[18] We tested all relationships between constructs and kept significant associations ($P < 0.05$) in the final PLS-SEM. All analyses were done using the Stata software, version 14.2 (StataCorp, College Station, Texas, United States).

Ethics

This project was approved by the Research Ethics Committee of the UFAM, through opinion report no. 1,564,841, dated May 30, 2016 (Certificate of Submission for Ethical Appreciation: 44286115.0.0000.5020, on the Brazil Platform); and by the university hospital's teaching and research management (research monitoring number 03/2016 NP0 25/2015). Participation in the research was voluntary, and only took place after the individual had read and signed an informed consent statement.

RESULTS

Participants' characteristics

Out of the 1,222 active employees, 863 were eligible and 300 were included. There were 23 refusals among the first 300 invitees, thus yielding initial response rate of 93% (**Figure 1**).

The majority were women (67%), aged 36-50 years (48%) and married (65%), had nonwhite skin color (black, brown or indigenous; 66%), had a postgraduate degree (53.3%), were overweight (42%) and belonged to economic classification B2 (33%) (**Table 1**).

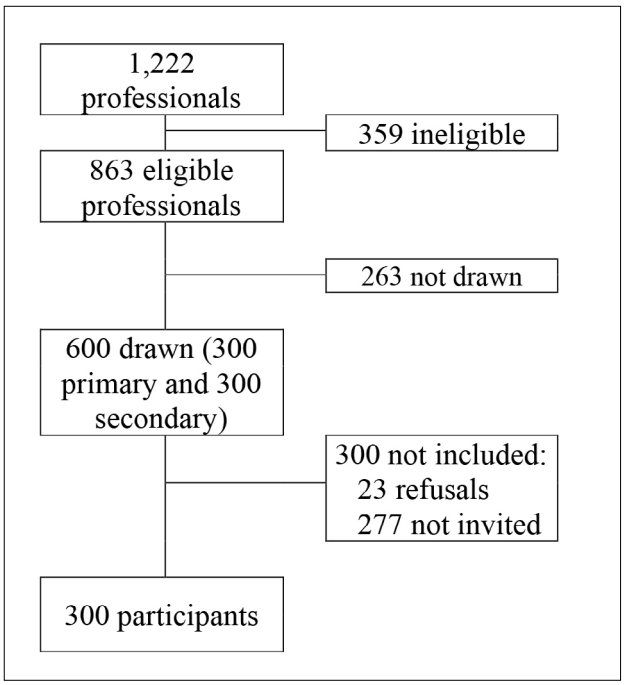

Figure 1. Process of study participant selection and inclusion.

Frequencies of depressive symptoms, burnout, job satisfaction and safety culture

Depressive symptoms were present in 19.0% (95% CI: 14.5; 23.5%; α = 0.845). The highest prevalences in our sample were noted among people who had been working in the hospital for fewer than five years and among those with higher education, one job and weekly workload of up to 40 hours. Burnout affected 8.7% of the participants (95% CI: 5.2; 12.3%; α = 0.908). Higher prevalence was noted among the participants in higher social classes, physicians, people who had been working in the hospital for 3-4 years and those with higher educational attainment (**Table 1**).

Job satisfaction scores were ambivalent in the whole sample (131.3; 95% CI: 128.4; 134.2; α = 0.884). Higher scores were observed among nursing staff, people who had been working in the hospital for 5-10 years, those with high school education or less and those with two job contracts. The level of safety culture did not reach the criterion for a positive safety culture that had been defined (i.e. 75%) (62.7%; 95% CI: 60.7; 64.6%; α = 0.920). Higher scores for safety culture were observed among men and professionals with weekly workloads of over 60 hours (**Table 1**).

Factors associated with outcomes

Workers with temporary contracts (PR = 1.88; 95% CI: 1.17; 3.01) and with higher education (PR = 2.19; 95% CI = 1.33; 3.59) had higher prevalence of depressive symptoms than workers with stable contracts (**Table 2**). Those with two jobs (PR = 0.39; 95% CI: 0.19; 0.79) and with weekly workloads of 61 hours or

Table 1. Sociodemographic and professional characteristics of participants and frequencies of depressive symptoms, burnout, job satisfaction and safety culture (n = 300)

Variables	n	%	Depressive symptoms	Burnout	Job satisfaction	Safety culture
			n (%)	n (%)	Mean ± SD	% ± SD
Sex						
Women	202	67.3	42 (20.8)	15 (7.4)	131.0 ± 25.6	60.8 ± 13.5
Men	98	32.7	15 (15.3)	11 (11.2)	131.9 ± 25.7	67.3 ± 14.7
Age (years)						
18-35	82	27.3	17 (20.7)	12 (14.6)	127.7 ± 23.3	62.0 ± 12.1
36-50	144	48.0	27 (18.8)	9 (6.3)	132.8 ± 27.0	62.8 ± 15.3
≥ 51	74	24.7	13 (17.6)	5 (6.8)	132.5 ± 25.0	63.2 ± 14.1
Skin color						
White	103	34.3	21 (20.4)	9 (6.3)	127.4 ± 25.7	60.7 ± 15.6
Nonwhite	197	65.7	36 (13.1)	17 (8.6)	133.4 ± 25.4	63.7 ± 13.1
Marital status						
Single	105	35.0	24 (22.9)	15 (8.7)	129.1 ± 28.5	61.8 ± 13.1
Married	195	65.0	33 (16.9)	11 (8.6)	132.5 ± 23.9	63.1 ± 14.7
Body composition						
Normal	101	33.7	20 (19.8)	11 (10.9)	129.9 ± 23.6	61.8 ± 11.9
Overweight	126	42.0	25 (19.8)	12 (9.5)	131.8 ± 26.1	63.8 ± 15.1
Obese	73	24.3	12 (16.4)	3 (4.1)	132.5 ± 27.5	62.0 ± 15.2
Economic class						
A	77	25.7	17 (22.1)	14 (18.2)	122.9 ± 26.0	62.5 ± 15.6
B1	69	23.0	15 (21.7)	6 (8.7)	130.9 ± 21.7	61.5 ± 13.5
B2	100	33.3	17 (17.0)	5 (5.0)	135.6 ± 25.6	62.9 ± 13.5
C/D/E	54	18.0	8 (14.8)	1 (1.9)	136.1 ± 27.1	64.4 ± 13.8
Profession						
Physician	73	24.3	14 (19.2)	11 (15.1)	119.7 ± 22.8	62.8 ± 14.4
Nursing staff	123	41.0	21 (17.1)	5 (4.1)	138.4 ± 24.6	63.4 ± 12.8
Other healthcare professional	57	19.0	11 (19.3)	6 (10.5)	127.5 ± 27.5	61.9 ± 16.2
Technical support	47	15.7	11 (23.4)	4 (8.5)	135.5 ± 23.0	60.4 ± 15.3
Contact with patient						
No	252	84.0	9 (18.8)	2 (4.2)	132.3 ± 26.8	56.5 ± 16.7
Yes	48	16.0	48 (19.1)	24 (9.5)	131.1 ± 25.4	63.5 ± 13.6
Time working in the hospital (years)						
< 1	13	4.3	4 (30.8)	1 (7.7)	134.5 ± 21.7	62.8 ± 12.4
1-2	28	9.3	7 (25.0)	3 (10.7)	130.2 ± 26.2	62.3 ± 15.0
3-4	32	10.7	8 (25.0)	7 (21.9)	125.9 ± 18.5	61.8 ± 11.0
5-10	56	18.7	9 (16.1)	5 (8.9)	136.6 ± 25.1	65.6 ± 12.5
11-20	88	29.3	15 (17.1)	4 (4.6)	130.0 ± 25.3	63.7 ± 15.0
≥ 21	83	27.7	14 (16.9)	6 (7.2)	131.2 ± 28.8	60.0 ± 15.4
Contract stability						
Permanent	233	77.7	37 (15.9)	16 (6.9)	134.0 ± 26.2	62.9 ± 14.4
Temporary	67	22.3	20 (29.9)	10 (14.9)	122.1 ± 20.9	62.0 ± 13.1
Education						
Postgraduate degree	160	53.3	24 (15.0)	11 (6.9)	130.3 ± 26.7	61.5 ± 14.8
University/college	73	24.3	24 (32.9)	13 (17.8)	128.2 ± 25.6	63.0 ± 13.3
High school or less	67	22.3	9 (13.4)	2 (3.0)	137.1 ± 22.0	65.4 ± 13.1
Number of jobs						
1	172	57.3	43 (25.0)	18 (10.5)	129.8 ± 24.2	60.8 ± 13.6
2	82	27.3	8 (9.7)	6 (7.3)	136.4 ± 28.6	63.9 ± 16.2
≥ 3	46	15.3	6 (13.0)	2 (4.4)	128.3 ± 24.0	66.6 ± 11.9
Weekly workload (hours)						
20-40	143	47.7	32 (22.4)	12 (8.4)	132.4 ± 23.7	60.9 ± 14.1
41-60	101	33.7	20 (19.8)	11 (10.9)	128.6 ± 27.5	62.3 ± 15.2
≥ 61	56	18.7	5 (8.9)	3 (5.4)	133.4 ± 26.7	67.4 ± 11.1

SD = standard deviation.

Table 2. Factors associated with depressive symptoms and burnout, calculated by means of Poisson regression with robust variance; and with patient safety culture and job satisfaction, calculated by means of logistic regression

Variables	Depressive symptoms	Burnout	Job satisfaction	Safety culture
	PR (95% CI)	PR (95% CI)	β (95% CI)	β (95% CI)
Sex				
Women	Reference	Reference	Reference	Reference
Men	0.74 (0.42; 1.26)	1.51 (0.72; 3.17)	0.88 (-5.33; 7.09)	**6.47 (2.27; 10.68)**
Age (years)				
18-35	Reference	Reference	Reference	Reference
36-50	0.90 (0.52; 1.55)	0.43 (0.19; 0.97)	5.15 (-1.82; 12.11)	0.86 (-3.69; 5.40)
≥ 51	0.85 (0.44; 1.62)	0.46 (0.17; 1.25)	4.85 (-3.21; 12.92)	1.18 (-4.28; 6.63)
Skin color				
White	Reference	Reference	Reference	Reference
Nonwhite	0.90 (0.55; 1.45)	0.99 (0.46; 2.14)	6.03 (-0.07; 12.12)	3.01 (-1.03; 7.05)
Marital status				
Single	Reference	Reference	Reference	Reference
Married	0.74 (0.46; 1.18)	0.39 (0.19; 0.83)	3.49 (-2.60; 9.58)	1.30 (-2.74; 5.35)
Body composition				
Normal	Reference	Reference	Reference	Reference
Overweight	1.00 (0.60; 1.70)	0.87 (0.40; 1.90)	1.88 (-4.86; 8.62)	2.00 (-2.52; 6.51)
Obese	0.83 (0.43; 1.59)	0.38 (0.11; 1.31)	2.57 (-5.19; 10.32)	0.23 (-4.90; 5.35)
Economic class				
A	Reference	Reference	Reference	Reference
B1	0.98 (0.53; 1.82)	0.48 (0.19; 1.18)	7.96 (-0.24; 16.17)	-1.04 (-6.38; 4.3)
B2	0.77 (0.42; 1.40)	0.28 (0.10; 0.73)	**12.64 (5.14; 20.15)**	0.34 (-4.74; 5.43)
C/D/E	0.67 (0.31; 1.44)	0.10 (0.01; 0.75)	**13.18 (4.40; 21.97)**	1.92 (-4.17; 8.02)
Profession				
Physician	Reference	Reference	Reference	Reference
Nursing staff	0.90 (0.48; 1.64)	0.27 (0.10; 0.75)	**18.75 (11.62; 25.88)**	0.64 (-4.04; 5.32)
Other health professional	1.01 (0.49; 2.04)	0.70 (0.27; 1.78)	7.87 (-0.66; 16.40)	-0.88 (-6.95; 5.20)
Technical support	1.22 (0.60; 2.45)	0.56 (0.19; 1.67)	**15.87 (6.85; 24.90)**	2.35 (-9.12; 4.41)
Contact with patient				
No	Reference	Reference	Reference	Reference
Yes	1.01 (0.53;1.93)	2.29 (0.56;9.38)	-1.20 (-9.14;6.74)	**7.03 (1.05; 13.00)**
Time working in the hospital (years)				
< 1	Reference	Reference	Reference	Reference
1-2	0.81 (0.28; 2.29)	1.39 (0.16; 12.19)	-4.28 (-21.20; 12.64)	-0.49 (-12.07; 11.08)
3-4	0.81 (0.29; 2.24)	2.84 (0.39; 20.95)	-8.59 (-25.17; 7.99)	-0.98 (-12.29; 10.34)
5-10	0.52 (0.18; 1.43)	1.16 (0.15; 9.14)	2.15 (-13.37; 17.67)	2.82 (-7.90; 13.55
11-20	0.55 (0.21; 1.41)	0.59 (0.07; 4.90)	-4.50 (-19.47; 10.48)	0.88 (-9.70; 11.46)
≥ 21	0.55 (0.21; 1.41)	0.94 (0.12; 7.21)	-3.26 (-18.29; 11.78)	-2.85 (-13.36; 7.67)
Contract stability				
Permanent	Reference	Reference	Reference	Reference
Temporary	**1.88 (1.17; 3.01)**	**2.17 (1.03; 4.57)**	**-11.93 (-18.79; -5.07)**	-0.84 (-5.40; 3.72)
Education				
Postgraduate degree	Reference	Reference	Reference	Reference
University/college	**2.19 (1.33; 3.59)**	**2.59 (1.22; 5.51)**	-2.07 (-9.15; 5.01)	1.40 (-3.24; 6.04)
High school or less	0.90 (0.43; 1.83)	0.43 (0.10; 1.73)	6.77 (-0.52; 14.06)	3.83 (-1.21; 8.86)
Number of jobs				
1	Reference	Reference	Reference	Reference
2	0.39 (0.19; 0.79)	0.70 (0.29; 1.70)	6.60 (-0.13; 13.33)	3.04 (-1.62; 7.70)
≥ 3	0.52 (0.23; 1.15)	0.42 (0.10; 1.73)	-1.48 (-9.81; 6.83)	**5.80 (0.70; 10.89)**
Weekly workload (hours)				
20-40	Reference	Reference	Reference	Reference
41-60	0.88 (0.54; 1.46)	1.30 (0.60; 2.83)	-3.83 (-10.38; 2.71)	1.45 (-2.85; 5.75)
≥ 61	**0.40 (0.16; 0.97)**	0.64 (0.17; 2.18)	1.01 (-6.92; 8.96)	**6.57 (1.36; 11.77)**

β = beta coefficient; PR = prevalence ratio; CI = confidence interval.

more had fewer depressive symptoms (PR = 0.40; 95% CI: 0.16; 0.97). The prevalence of burnout was lower among people aged 36 to 50 years (PR = 0.43; 95% CI: 0.19; 0.97), married people (PR = 0.39; 95% CI: 0.19; 0.83), individuals in lower economic strata (PR = 0.10; 95% CI: 0.01; 0.75) and nursing staff (PR = 0.27; 95% CI: 0.10; 0.75), in comparison with the respective reference. It was significantly higher among workers with temporary contracts (PR = 2.17; 95% CI: 1.03; 4.57) and with higher education (PR = 2.59; 95% CI: 1.22; 5.51).

Workers within the fields of nursing (β = 18.7; 95% CI: 11.62; 25.88) and technical support (β = 15.9; 95% CI: 6.85; 24.90) had greater job satisfaction than did physicians. People in lower economic strata had higher job satisfaction than did those in higher strata (P < 0.003); and those with temporary contracts had lower satisfaction than permanent workers (β = -11.93; 95% CI: -18.79; -5.07). Men (β = 6.47; 95% CI: 2.27; 10.68), professionals in direct contact with patients (β = 7.03; 95% CI: 1.05; 13.00), those with three or more jobs (β = 5.80; 0.70; 10.89) and those with weekly workloads exceeding 60 hours (β = 6.57; 95% CI: 1.36; 11.77) had significantly higher perception of safety culture, compared with their reference categories.

Relationships between depressive symptoms, burnout, job satisfaction and safety culture

Burnout was negatively correlated with job satisfaction (path coefficient = -0.342; P < 0.001) and it was positively correlated with depressive symptoms (path coefficient = 0.520; P < 0.001); 56% of burnout variance was explained by these factors (**Table 3** and **Figure 2**). Job satisfaction reduced depressive symptoms (path coefficient = -0.125; P = 0.035) and burnout increased these symptoms (path coefficient = 0.614; P < 0.001); 48% of the variability of the depressive symptoms was explained by the predictive variables.

Job satisfaction increased patient safety culture (path coefficient = 0.741; P < 0.001) and explained 55% of its variance. Safety culture increased job satisfaction (path coefficient = 0.590; P < 0.001) and burnout reduced it (path coefficient = -0.310; P < 0.001); 62% of job satisfaction variance was explained by these predictors. Other path coefficients between the constructs were not significant (P < 0.05) and were removed from the PLS-SEM.

DISCUSSION

The healthcare workers in this university hospital in the Amazon region had high prevalences of depressive symptoms and burnout, while patient safety culture and job satisfaction were sub-optimal. Burnout and depressive symptoms were directly proportional: the presence of one outcome potentiated the occurrence of the other. Job satisfaction was a protective factor for depression and burnout and was negatively affected by these constructs. Satisfaction with work increased patient safety culture, which in turn improved job satisfaction; and it reduced the frequency of poor mental health outcomes.

Although we used a causality technique to assess how the constructs were related to each other (structural equation modeling), the cross-sectional design did not allow us to draw conclusions regarding causal relationships. No adjustments for confounding factors in regression analyses were performed in order to investigate individual associations: instead, we aimed to study the relationships between constructs.

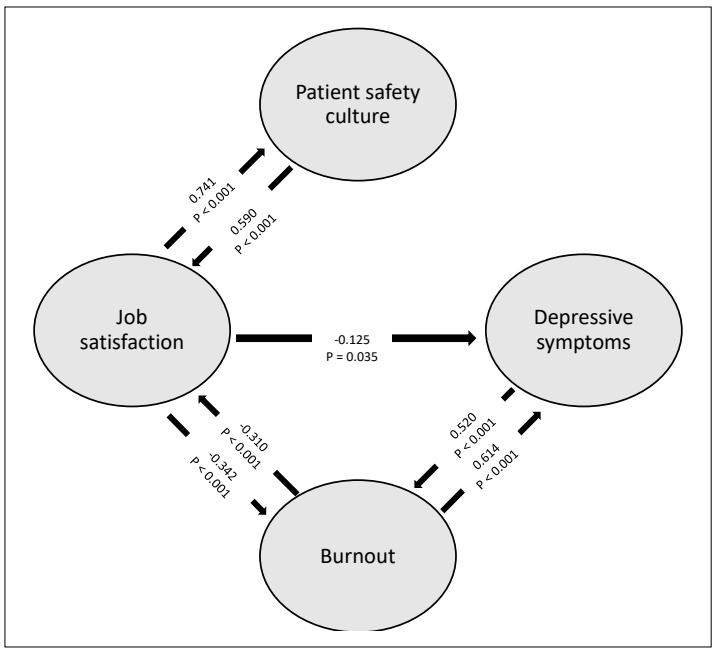

Figure 2. Correlation path of depressive symptoms, burnout, job satisfaction and safety culture, obtained through partial least-squares structural equation modeling (PLS-SEM).

Table 3. Correlation between depressive symptoms, burnout, job satisfaction and safety culture

Predictive variable	Endogenous variable	Path coefficient	P-value	R^2 (%)
Depressive symptoms	Job satisfaction	-0.125	0.035	48.0
	Burnout	0.614	< 0.001	
Burnout	Job satisfaction	-0.342	< 0.001	56.0
	Depressive symptoms	0.520	< 0.001	
Job satisfaction	Safety culture	0.590	< 0.001	62.0
	Burnout	-0.310	< 0.001	
Safety culture	Job satisfaction	0.741	< 0.001	54.8

The present results may have been affected by survival bias, since individuals with severe emotional problems could have been on sick leave or could have taken early retirement, which would thus have rendered them ineligible for this study. Nonetheless, the random sampling adopted and the consequent high response rate increased the level of confidence in the representativeness of this study.

The conclusions from the present study were supported only through quantitative data. Comprehension of the constructs would become better explained if these were supplemented with qualitative data. Measurements were based on a self-administered questionnaire that asked sensitive questions about the workplace and the participants' mental health of participant. The nature of these questions might have led to non-response bias, but the high reliability that was found for the constructs showed that this risk was low. One contextual factor that could not be avoided was that at the time of data collection, there was co-occurrence of physical renovation and managerial changes, which may have impacted the professionals' perceptions.

Safety culture and job satisfaction had a positive and reciprocal relationship. Job satisfaction reduced depressive symptoms and burnout and the latter reduced satisfaction. Depressive symptoms and burnout were positively and mutually associated. In a previous survey conducted in a hospital in the state of São Paulo, Brazil, similar relationships between job satisfaction, depressive symptoms and burnout were observed.[9] An adequate labor environment favors better mental outcomes and safety culture, thereby contributing to the quality of the care provided.

Almost one-fifth of the workers presented depressive symptoms and one-tenth, burnout. The prevalence of depressive symptom was almost three times greater than the 7% observed in the adult population of Manaus Metropolitan Region in 2015,[19] while the burnout rates were similar to global trends among nurses (10%) and ranged from 9 to 60% among physicians, using the same instruments and cutoffs that we adopted.[20, 21] Poor mental health outcomes seem to occur more frequently among healthcare workers, and this represents a risk factor for unsafe care.[3-5] Lack of stability in work contracts doubled the prevalence of both depression and burnout, and significantly reduced job satisfaction in our sample. Concerns about job loss frequently lead to emotional distress.[22] This situation will probably deteriorate in Brazil with a major labor reform that has been implemented in Brazil, which has increased the occurrence of precarious contracts and has reduced workers' rights.[23] Higher pressures on public healthcare, and on healthcare personnel, are also expected because of a freeze on government investments in healthcare and other social areas for 20 years that was started in 2017.[24]

People with two work contracts and with higher weekly workloads had fewer depressive symptoms. This was inconsistent with previous research, in which higher rates of depression due to reduced time for eating, leisure, rest, sleep and social and family contact were observed.[25,26] The possible explanations for our findings are that coping skills may have been developed through more job experience; or that it may have been a limitations of our cross-sectional design due to reverse causality, i.e. people who fell ill reduced their workload; or that it may have been related to survival bias, as mentioned earlier. Higher workload was also associated with better perception of safety in our sample and in some previous studies,[27,28] but this was seen to have a negative effect in other settings.[29,30] The divergences regarding this finding may reflect the labor market and safety culture perceptions of each geographical area,[31] or possible desensitization to safety issues in situations of longer working hours.

The score for patient safety culture was only slightly above the midpoint and was therefore not considered to be positive culture. The 2015 survey conducted in this hospital also found weaknesses in safety culture, using another instrument.[10] Men, workers in direct contact with patients and people with greater workloads and more jobs had better perceptions of safety culture. Men also had more favorable perceptions regarding teamwork climate, job satisfaction, management and working conditions in a survey held in the United States using SAQ.[32] Men tend to present positive reactivity and, according to the model of patterns of change, need more time to perceive potential errors. Thus, they may classify performance as excellent, while this might be considered substandard by women.[33] A survey among 1,408 professionals also observed higher safety culture among professionals who work in direct contact with patients than among those without contact.[28] This may reflect the skills developed in adjusting professional and technical issues to achieve a capacity to interrelate with patients.[34]

Job satisfaction was ambivalent in our sample as a whole. Professionals of lower social class and in the field of nursing had higher job satisfaction and lower prevalence of burnout. Job satisfaction probably acted as a protective factor against burnout in these categories. Higher satisfaction in lower social classes is counterintuitive, in that higher income is generally associated with greater satisfaction with payment.[35] One possible explanation for this is that people of lower economic level place greater value on the type of work performed in hospitals, while those with higher incomes feel disregarded in relation to their working conditions. It is also possible that wealthier people occupy positions that bring more responsibilities, dissatisfaction with work and professional burnout. Lower levels of satisfaction and high prevalence of burnout among both nurses and physicians is a common finding, but comparisons of satisfaction across healthcare professions in the same sample are scarce.[36] Local reasons can also explain these findings.

More than half of the professionals presented overweight or obesity, without any association with patient safety, job satisfaction, depressive symptoms or burnout. A previous investigation found positive associations between eating problems and burnout,[37] between burnout and obesity,[38] and between depression and obesity.[39] Most of our sample were black, brown or indigenous, and ethnicity was not associated with the constructs in our study, which was a pattern differing from what had previously been observed.[40]

CONCLUSION

Job satisfaction increased safety culture, which also positively affected satisfaction, and reduced depressive symptoms and professional burnout. Depressive symptoms, burnout, job satisfaction and safety culture were partially explained by and individually associated with lack of work stability, number of jobs, weekly workload and sociodemographic factors. Improving the institution's patient safety culture involves enhancement of working conditions and empowerment of workers, in order to provide a healthy environment for professionals and safe care for patients. Job satisfaction has a central role in relation to safer care for patients and better mental health among workers.

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Association between falls and cognitive performance among community-dwelling older people: a cross-sectional study

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ABSTRACT

BACKGROUND: Falls among older people have a negative impact on health and therefore constitute a public health problem. Cognitive decline can also accompany the aging process, and both conditions lead to significant increases in morbidity and mortality in this population.

OBJECTIVE: To analyze the cognitive performance of older people, classified as non-fallers, sporadic fallers and recurrent fallers, and investigate the relationship between falls and cognition.

DESIGN AND SETTING: Cross-sectional study conducted in the interior of the state of São Paulo, Brazil.

METHODS: Evaluations on 230 older people were conducted. They were divided into three groups: non-fallers, sporadic fallers (one fall) and recurrent fallers (two or more falls). The Mini-Mental State Examination, Consortium to Establish a Registry for Alzheimer's Disease (CERAD), Brief Cognitive Screening Battery (BCSB), Cambridge Examination for Mental Disorders of the Elderly (CAMDEX) similarities subtest and digit span test were applied.

RESULTS: In multinomial logistic regression, being a recurrent faller was significantly associated with lower scores in the CERAD word list (odds ratio, OR = 0.92; 95% confidence interval, CI, 0.86-0.98; P = 0.01), in CERAD constructive praxis (OR = 0.88; 95% CI, 0.79-0.98; P = 0.02), in BCSB figure list memory (OR = 0.94; 95% CI, 0.89-0.99; P = 0.02) and in verbal fluency (OR = 0.89; 95% CI, 0.81-0.97; P = 0.01). Recurrent fallers also had lower scores in these same tests, compared with sporadic fallers.

CONCLUSION: Cognitive impairment, especially in the domains of memory and executive functioning, can influence occurrences of recurrent falls.

INTRODUCTION

Occurrence of falls among older people constitutes a public health problem due to their negative impact on quality of life in this population. Falls can contribute to loss of independence, social isolation, institutionalization and mortality.[1] The World Health Organization (WHO)[2] defines a fall as "inadvertently coming to rest on the ground, floor or other lower level, excluding an intentional change in position to rest on furniture, a wall or other objects".[2]

The prevalence of falls among older Brazilians ranges from 10 to 35%, depending on the region analyzed.[3] Falls may occur due to osteoarticular and/or neurological decline related to the aging process or due to an adverse clinical condition that affects mechanisms of balance and stability.[4] The risk factors for falls may be intrinsic or extrinsic. Intrinsic factors comprise characteristics or clinical factors relating to older people, such as dizziness, weakness and chronic health conditions.[5] Extrinsic factors include characteristics of the surrounding environment, such as uneven surfaces and inadequate lighting.[5]

Like falls, cognitive impairment can significantly increase morbidity and mortality among older people. Indeed, these two events often coexist in this population and contribute to significantly increased healthcare expenditures and reduction of quality of life. It has also been reported that individuals with cognitive decline exhibit gait deficits.[6,7] Thus, altered cognitive capacity may have a negative impact on postural stability, thus leading to greater risk of falls.[6]

Despite the evidence accumulated to date, the mechanisms involved in the association between cognitive impairment and the risk of falls have not yet been fully clarified.[6] These two conditions have always been addressed as distinct geriatric syndromes, which hinders the understanding of cognitive-motor interactions.[8] Studies have indicated that cognitive decline, specifically with regard to attention and executive functions, can compromise gait and contribute to occurrences

of falls in the older population.[9,10] Moreover, the prevalence of gait disorders is higher among older people with major neurocognitive disorder (MND), and this impairment increases as cognitive performance diminishes.[11]

A prospective population-based study conducted among 9,279 community-dwelling elderly people sought to investigate whether their experience of falls in the past two years was responsible for cognitive function, after adjusting for all possible confounding variables.[12] The results from this study demonstrated that elderly people with an experience of falling had a cognitive performance estimate that was 0.13% lower (95% confidence interval, CI: 0.023 to 0.002; P-value: 0.017) than the estimate for those without a fall experience.[12] Hence, evaluating the cognitive performance of elderly fallers becomes essential. Complementarily, it was sought in another study to assess cognitive function and its relationships with balance, history of falls and fear of falling, among 250 elderly people.[13] Elderly people with cognitive decline were found to have greater fear of falling than elderly people without cognitive decline (P = 0.008).[13] These results corroborate the importance of evaluating the presence of new falls in this population.

Few studies have specifically investigated the most altered cognitive domains in the presence of falls. A cross-sectional study involving 462 older people investigated the association between cognitive capacity and falls and found that the prevalence of falls among those with cognitive impairment evaluated using the Mini-Mental State Examination was 42%, which confirmed the strong association between these variables.[11] Thus, a more comprehensive assessment of altered cognitive domains among older people with a history of falls may contribute towards planning interventions aimed at preventing occurrences of both of these negative outcomes in specific domains, to enable maintenance of physical and cognitive health throughout the aging process.

Some studies have addressed the importance of assessing the frequency of falls, considering that the number of falls may predict greater health risks.[14] A study evaluating 325 community-dwelling older people who suffered at least one fall in the previous year found that greater numbers of falls were associated with higher frequency of risk factors, such as fractures, loss of mobility due to the fear of falling again and hospitalizations. These were considered to be predictors of reduced quality of life of this population.[14]

In this light, assessment of cognitive performance and numbers of falls in the older population can help broaden our understanding of the association between falls and cognition and may assist in planning early interventions.

OBJECTIVE

Therefore, the aim of the present study was to analyze cognitive performance among older people classified as non-fallers, sporadic fallers and recurrent fallers.

METHODS

Setting and participants

This study was conducted in the city of São Carlos, in the state of São Paulo, Brazil. This city has 28,696 residents aged 60 years or older, corresponding to 12.92% of the total population.[15] The study was conducted in the area of coverage of one family health unit: in this area there were 317 residents aged 60 years and older, according to data from that health unit. There was no potential difference in this area in relation to other regions of the city. The exclusion criteria were situations of diagnoses of MND, severe mental disorders or intellectual disability. In addition, potential subjects were excluded if they had any other serious health problem that made it impossible for them to respond to the tests.

During home visits, 28 individuals were not encountered at their homes or were found to no longer live at the address, five declined to participate in the study, two were bedridden and 23 did not answer the questionnaire addressing falls. Furthermore, 25 elderly people who had been diagnosed with MND, two with schizophrenia and two with intellectual disabilities were excluded. Thus, 230 older people were included in the present study. Data were collected between March 2016 and February 2017.

Groups

The individuals were allocated to different groups based on the numbers of falls, which were investigated using the following question: "How many falls have you suffered in the last 12 months?" Depending on the answer to this question, the participants were classified as non-fallers (those who had not suffered any falls in the previous year), sporadic fallers (those who had suffered a single fall in the previous year) or recurrent fallers (those who had suffered two or more falls in the previous year). During the interviews, a companion of the elderly subject was always present. For example, this could be someone who lived together with this subject (husband or wife or children).

Cognitive assessment

The cognitive assessment was performed by means of the following battery of tests:

- Mini-Mental State Examination (MMSE): This is a widely used screening tool for evaluating overall cognition, with scores ranging from 0 to 30 points, which assesses temporal and spatial orientation, memory (registration and recall), language, attention and calculation.[16]
- Consortium to Establish a Registry for Alzheimer's Disease (CERAD): This battery is composed of the following tests: verbal fluency (animal naming), Boston naming (15 items),

recall of a list of words, constructional praxis, recognition list and recall of praxis. In the evaluation of memory, a list of ten words is presented to the participant, who is asked to remember as many words as possible within a maximum recall time of 90 seconds (free recall). The procedure is repeated two more times and the score is obtained as the sum of the words recalled during the three trials. Constructional praxis is evaluated through copying four figures. Delayed recall of the list of words presented previously is then performed for a maximum of 90 seconds. Next, the initial ten words are presented together with ten distractors and the participant is asked to recognize which words were in the original list. Lastly, the participant is asked to reproduce the four drawings that had previously been copied.[17]

- Brief Cognitive Screening Battery (BCSB): This battery involves verbal fluency (animals), a clock drawing test and a figure memory test (incidental, immediate recall, learning, delayed recall and recognition). The BCSB has been shown to have good accuracy for populations with high rates of illiteracy or low levels of schooling. Memory is assessed through presentation of ten figures (frog, spoon, comb, tree, turtle, key, airplane, house, book and bucket), which the participants are asked to name immediately. The figures are then presented two more times, followed by immediate recall. After the verbal fluency test and the clock drawing test (both of these form part of the BCSB), the participant is asked to remember the ten figures (delayed recall). Lastly, there is a recognition test (consisting of the ten target figures plus ten distracting figures).[18]
- Similarities subtest of the Cambridge Mental Disorders of the Elderly Examination (CAMDEX): This test consists of four questions to assess the abstraction capacity of the participant, based on the similarity between two things or objects; for example: What do an apple and banana have in common?[19]
- Digit span test (forward and backward): This test is composed of seven pairs of numerical sequences with different quantities of digits applied in forward and backward order. The sequences have three to nine numbers in the forward order and two to eight numbers in the backward order. The test ends after the participants err in two consecutive sequences.[20] The maximum quantity of numbers repeated without error is recorded for each version (forward and backward).

Procedures

This study received approval from the institutional review board of the Federal University of São Carlos (October 29, 2015; certificate number: 48602515.5.0000.5504). All the volunteers who agreed to participate in the study signed a statement of informed consent prior to the interviews.

Five trained gerontologists conducted the interviews in the participants' homes, during which sociodemographic and clinical data were collected and the cognitive tests were applied. The presence of polypharmacy was also assessed, and was defined as the use of five or more medications.[21] Information relating to medical conditions (histories of stroke, diabetes mellitus, hypertension, heart disease and dyslipidemia) was obtained through self-reports. The evaluation took 60 to 90 minutes. Furthermore, these participants were evaluated within a maximum of 30 days by three psychiatrists, who performed a diagnostic evaluation based on the criteria of the Diagnostic and Statistical Manual of Mental Disorders, 5th edition (DSM-5).

Statistical analysis

Descriptive analysis was performed on the variables, considering the overall sample and the three fall classification groups (non-fallers, sporadic fallers and recurrent fallers). The chi-square test, ANOVA or the Kruskal-Wallis test was used to compare differences among the groups, depending on the distribution of the sample and the type of variable. Associations between the groups of fallers and cognitive performance were analyzed using multinomial logistic regression. Variables for which statistically significant differences were found between groups were adjusted in the regression model. The odds ratio (OR) and its respective 95% confidence interval (95% CI) were calculated for each cognitive test. The variance inflation factor (VIF) was used to detect multicollinearity. All VIF values were found to be less than 2, which showed that multicollinearity did not affect the model. All analyses were conducted with the aid of the Statistical Package for the Social Sciences (SPSS), version 21.0 (International Business Machines Corp., Armonk, New York, United States). The significance level was set at 5% ($P \leq 0.05$).

RESULTS

The 230 older people included in this study were classified as non-fallers (n = 159), sporadic fallers (n = 38) or recurrent fallers (n = 33). The sociodemographic and clinical characteristics of the groups are displayed in **Table 1**. A significant difference among the groups was found with regard to the occurrence of polypharmacy (P = 0.03).

The multinomial logistic regression results are shown in **Table 2**. After adjusting for polypharmacy, the regression data indicated that being a recurrent faller was significantly associated with lower scores in the CERAD word list (OR = 0.92; 95% CI 0.86-0.98; P = 0.01), in CERAD constructive praxis (OR = 0.88; 95% CI 0.79-0.98; P = 0.02), in BCSB figure list memory (OR = 0.94; 95% CI 0.89-0.99; P = 0.02) and in verbal fluency (OR = 0.89; 95% CI 0.81-0.97; P = 0.01), compared with non-fallers. In addition, being a recurrent faller was significantly associated with lower scores in

the CERAD word list (OR = 0.89; 95% CI 0.82-0.96; P = 0.01), in CERAD constructive praxis (OR = 0.87; 95% CI 0.76-1.00; P = 0.05), in BCSB figure list memory (OR = 0.93; 95% CI 0.87-1.00; P = 0.05) and in verbal fluency (OR = 0.87; 95% CI 0.77-0.98; P = 0.02), compared with sporadic fallers.

DISCUSSION

In the present study, we evaluated the cognitive performance of older people in relation to their numbers of falls. We found that cognitive impairment could influence the number of falls, considering that statistically significant differences were found between non-fallers/sporadic fallers and recurrent fallers regarding the cognitive domains of immediate recall, praxis (visuospatial skills) and executive functions (verbal fluency). The group of recurrent fallers performed more poorly in these domains. It was also noteworthy that no significant differences were found between the non-fallers and sporadic fallers regarding any of the cognitive tests applied.

Investigation of specific cognitive domains according to the number of falls has been little explored in the recent literature. The current evidence sustains the notion that compromised executive functions may predict occurrences of falls.[22] In the present study, recurrent fallers performed worse in the tests that evaluated this domain, even after adjusting for polypharmacy. Hsu et al.[22] conducted a review of the literature to identify cognitive domains associated with the risk of falls among

Table 1. Sociodemographic and clinical variables of the total sample and of the three fall groups

Variables	Total (n = 230)	Non-fallers (n = 159)	Sporadic fallers (n = 38)	Recurrent fallers (n = 33)	P
	Mean (standard deviation)				
Age	69.74 (± 7.30)	69.25 (± 6.97)	70.45 (± 8.46)	71.27 (± 7.38)	0.36
Years of schooling	3.39 (± 3.12)	3.68 (± 3.36)	2.87 (± 2.34)	2.64 (± 2.52)	0.29
	Percentage				
Illiterate					
Yes	23.8%	23.6%	23.8%	25%	0.98
No	76.2%	76.4%	76.2%	75%	
Sex					
Female	59.1%	57.2%	63.2%	63.6%	0.68
Male	40.9%	42.8%	36.8%	36.4%	
Retired					
Yes	72.2%	74.2%	71.1%	63.6%	0.46
No	27.8%	25.8%	28.9%	36.4%	
Marital status					
Married/with partner	60.4%	64.8%	52.6%	48.5%	0.12
Polypharmacy					
Yes	29.6%	25.2%	47.4%	30.3%	0.03*
No	70.4%	74.8%	52.6%	69.7%	
Systemic arterial hypertension					
Yes	61.3%	58.5%	65.8%	69.7%	0.40
No	38.7%	41.5%	34.2%	30.3%	
Dyslipidemia					
Yes	29.1%	28.3%	34.2%	27.3%	0.75
No	70.9%	71.7%	65.8%	72.7%	
Cardiac disease					
Yes	18.3%	16.4%	18.4%	27.3%	0.34
No	81.7%	83.6%	81.6%	72.7%	
Stroke					
Yes	9.1%	9.4%	10.5%	6.1%	0.79
No	90.9%	90.6%	89.5%	93.9%	
Arthritis					
Yes	22.6%	19.5%	26.3%	33.3%	0.19
No	77.4%	80.5%	73.7%	66.7%	
Current smoker					
Yes	12.2%	13.8%	5.3%	12.1%	0.35
No	87.8%	86.2%	94.7%	87.9%	

*Significant at P < 0.05.

older people and found that 12 studies reported an association between executive functions and the risk of falls, whereas only three studies did not find such an association. These authors suggested that changes to gait might be usable as markers of cognitive decline.

Using methods similar to those in the present study, Holtzer et al.[23] analyzed the association between cognition and falls among older people, who were classified as single (sporadic) fallers or recurrent fallers, based on self-reported falls. A neuropsychological battery was used to assess executive functions, processing speed, attention and memory. The regression analyses revealed that an increase in the standard deviation of the attention and processing speed tests was associated with an approximately 50% reduction in the risk of falls. Lower executive functioning scores were associated with an increase in falls only in the group of recurrent fallers. These findings are concordant with the results from the present study, which revealed differences between sporadic fallers and recurrent fallers. In contrast, Holtzer et al.[23] found that there was no association between the memory domain and an increased risk of falls, either among single fallers or among recurrent fallers.

Studies investigating the association between cognition and falls among older people have generally not found any increased risk of falls in relation to declining memory.[23-26] Anstey et al.[24] and Herman et al.[25] reported that executive functions, processing speed (executive functioning) and visuospatial skills (constructional praxis) were the main predictors of falls, which is in agreement with the present findings. However, memory decline was also found in the group of recurrent fallers in the present study, but not in the other two groups. Likewise, Al-Sari et al.[27] found that subjective memory complaints might predict fall events among older women. In that study, self-reported complaints of forgetfulness were seen to be associated with increased risk of more restricted mobility, as well as a greater risk of falls and fractures.[27] In another study, it was found that participants with episodic memory impairment were at 24 to 29% greater risk of falls than those without impairment, over an eight-year follow-up period.[28] In a prospective study, it was also found that deficits in immediate recall and diminished verbal capacity and processing speed were associated with increased risk of falls.[24]

Concerning the divergent results relating to memory, there is the possibility that non-amnestic cognitive impairment can co-occur with amnestic deficits.[29] Moreover, memory decline directly affects one's correct recall regarding the number of falls in the previous year.[30] Thus, reporting bias may occur when investigating the association between falls and memory among older people, considering that falls are self-reported in the majority of studies.

Recurrent falls present as a chronic disorder. Risk factors that are not previously resolved and identified can result in recurrent falls and negatively affect the quality of life of elderly people.[31] Therefore, it is important to consider the usefulness of investigating some risk factors, such as histories of falls, injuries and comorbidities among elderly people who have already fallen at some point. Within primary care, screening for visual, balance and gait deficits, investigation of the medications used, guidance on the choice of appropriate footwear and investigation of environmental factors form viable alternative approaches that can optimize the

Table 2. Multinomial regression analysis of cognitive domains in relation to each group of falls

Variables	Groups					
	Sporadic fallers versus non-fallers (reference)		Recurrent fallers versus non-fallers (reference)		Recurrent fallers versus sporadic fallers (reference)	
	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
MMSE	1.02 (0.95-1.10)	0.61	0.95 (0.88-1.01)	0.11	0.98 (0.91-1.06)	0.10
Word list memory (CERAD)	1.04 (0.98-1.10)	0.25	0.92 (0.86-0.98)	0.01**	0.89 (0.82-0.96)	0.01**
Delayed recall (CERAD)	1.06 (0.93-1.22)	0.38	0.97 (0.85-1.12)	0.70	0.92 (0.77-1.09)	0.32
Recognition (CERAD)	1.07 (0.95-1.21)	0.28	0.98 (0.87-1.09)	0.67	0.91 (0.77-1.06)	0.23
Constructional praxis (CERAD)	1.01 (0.90-1.12)	0.90	0.88 (0.79-0.98)	0.02*	0.87 (0.76-1.00)	0.05*
Figure list memory (BCSB)	1.01 (0.95-1.06)	0.83	0.94 (0.89-0.99)	0.02*	0.93 (0.87-1.00)	0.05*
Delayed recall (BCSB)	1.02 (0.90-1.17)	0.73	0.90 (0.80-1.01)	0.09	0.88 (0.75-1.03)	0.11
Recognition (BCSB)	1.15 (0.97-1.36)	0.11	0.93 (0.83-1.04)	0.20	0.81 (0.67-0.98)	0.38
Abstraction subtest (CAMDEX)	1.04 (0.89-1.20)	0.64	0.96 (0.82-1.12)	0.61	0.93 (0.76-1.13)	0.44
Clock drawing test	1.03 (0.94-1.13)	0.56	0.92 (0.83-1.02)	0.12	0.90 (0.79-1.02)	0.09
Verbal fluency	1.02 (0.93-1.12)	0.65	0.89 (0.81-0.97)	0.01**	0.87 (0.77-0.98)	0.02*
Boston naming test	1.08 (0.95-1.23)	0.22	0.96 (0.85-1.07)	0.45	0.88 (0.76-1.03)	0.12
Digit extension test (forward)	0.94 (0.74-1.20)	0.63	0.85 (0.66-1.08)	0.18	0.90 (0.66-1.23)	0.50
Digit extension test (backward)	1.20 (0.91-1.60)	0.20	0.98 (0.74-1.29)	0.88	0.82 (0.57-1.16)	0.26

OR = odds ratio; CI = confidence interval; MMSE = Mini-Mental State Examination; CERAD = Consortium to Establish a Registry for Alzheimer's Disease; BCSB = Brief Cognitive Screening Battery; CAMDEX = Cambridge Examination for Mental Disorders of the Elderly; *significant at $P < 0.05$; **significant at $P < 0.01$.

reduction of modifiable risk factors for recurrent falls.[31,32] A care plan developed between the professional and the elderly individual, regarding prevention of falls, can also be an important facilitator for gaining knowledge of their clinical history, as well as for the activities and interventions that can be implemented, according to their interests and needs, while always aiming to heed the uniqueness of each case.[33]

In terms of clinical variables, the only significant difference among the groups was in relation to polypharmacy, although other clinical conditions can also increase the risk of falls.[30] The consensus in the literature is that polypharmacy increases the risk of falls even after excluding other associated factors. Moreover, social factors and demographic differences need to be taken into consideration, along with the type and dose of medications, when interpreting this association.[1,33] Visual impairment is another variable that can increase the possibility of falls among older people.[34] Thus, altered constructional praxis may reflect not only impaired executive functioning, but also an individual's capacity for visual perception. Therefore, one limitation of the present study was that this variable may not have been evaluated adequately, considering that the results may have been influenced by some unidentified visual deficit.

Other limitations of this study should be considered, such as the way in which falls were assessed and classified, which was based on a simple question that was subject to recall bias. Moreover, the cross-sectional design precluded establishment of a causal relationship between the variables studied.

The strength of this study was its use of more specific memory tests, which may have favored the finding regarding the association between memory and falls. Nonetheless, longitudinal studies are needed in order to confirm this finding and evaluate this domain specifically. Moreover, older people who experienced a single fall in the previous 12 months exhibited no evidence of poorer cognitive performance, in comparison with non-fallers, given that these two groups did not show any significant differences in terms of cognition.

CONCLUSION

The prevalences of falls and cognitive impairment among the elderly are factors of concern for researchers and healthcare professionals. We found that elderly people who suffered recurrent falls had impaired memory and executive functioning, compared with non-fallers and sporadic fallers. Furthermore, having suffered only one fall in the last year did not seem to be associated with worse performance in cognitive tests. Hence, interventions need to be planned so that elderly sporadic fallers will not suffer more falls and thus evolve to become recurrent fallers.

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A randomized clinical trial on inhaled ciclesonide for managing acute asthma in the emergency room

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ABSTRACT

BACKGROUND: Use of inhaled corticosteroids for managing acute asthma exacerbations has been tested since the 1990s.

OBJECTIVE: To compare high doses of inhaled ciclesonide with systemic hydrocortisone for managing acute asthma exacerbations in the emergency department.

DESIGN AND SETTING: Double-blind, randomized clinical trial in the public healthcare system of the city of São Paulo.

METHODS: Fifty-eight patients with moderate or severe asthma with peak flow < 50% of predicted were randomized into two groups. Over the course of four hours, one group received 1440 mcg of inhaled ciclesonide plus hydrocortisone-identical placebo (ciclesonide + placebo), while the other received 500 mg of intravenous hydrocortisone plus ciclesonide-identical placebo (hydrocortisone + placebo). Both groups received short-acting bronchodilators (fenoterol hydrobromide and ipratropium bromide). The research protocol included spirometry, clinical evaluation, vital signs and electrocardiogram monitoring. Data were obtained at 30 (baseline), 60, 90, 120, 180, and 240 minutes. We compared data from baseline to hour 4, between and within groups.

RESULTS: Overall, 31 patients received ciclesonide + placebo and 27 received hydrocortisone + placebo. Inhaled ciclesonide was as effective as intravenous hydrocortisone for improving clinical parameters (Borg-scored dyspnea, P = 0.95; sternocleidomastoid muscle use, P = 0.55; wheezing, P = 0.55; respiratory effort, P = 0.95); and spirometric parameters (forced vital capacity, P = 0.50; forced expiratory volume in the first second, P = 0.83; peak expiratory flow, P = 0.51).

CONCLUSIONS: Inhaled ciclesonide was not inferior to systemic hydrocortisone for managing acute asthma exacerbations, and it improved both clinical and spirometric parameters.

TRIAL REGISTRATION: RBR-6XWC26 - Registro Brasileiro de Ensaios Clínicos (http://www.ensaiosclinicos.gov.br/rg/RBR-6xwc26/).

INTRODUCTION

Asthma is a chronic inflammatory disease of the airways that affects approximately 300 million people worldwide. In the United States, between 2001 and 2003, asthma exacerbations caused 4,210 deaths, 504,000 hospitalizations and 1.8 million emergency room visits.[1]

Since the mid-1990s, inhaled corticosteroids have been tested for managing asthma exacerbations in emergency-room settings. They have systemic corticosteroid-sparing potential and avoid the need for venipuncture, which is sometimes a difficult procedure.[2-4] These drugs exert vasoconstrictor effects on the mucosa by reducing neuronal reuptake of noradrenaline at the neuromuscular junctions of mucosal vessels, thus reducing secretions and facilitating the delivery of beta-2 agonists to their target receptors. Their onset of action is rapid, with peak vasoconstriction occurring in 30 minutes and lasting up to 90 minutes after inhalation.[5,6]

Ciclesonide is a prodrug that is activated at the site of action (bronchial cells and lining fluid of the bronchus) by bronchial esterases. These convert ciclesonide to desisobutyryl ciclesonide, which has 100-fold greater affinity for the glucocorticoid receptor than ciclesonide itself.[7] Because of this peculiar property, common side effects such as hoarseness, dysphonia, oral candidiasis and suppression of the hypothalamic-pituitary-adrenal axis are much less frequent with ciclesonide than with other high-dose inhaled corticosteroids, as it is inactive outside the lung.[7-11]

The anti-inflammatory action of intravenous corticosteroids occurs via a genomic mechanism. This reduces expression of proinflammatory mediators such as interleukins[12] and upregulates

expression of beta-adrenoceptors in bronchial smooth muscle tissue. This effect is also shared by inhaled corticosteroids and begins four to six hours after administration,[13] although some studies have shown that systemic corticosteroids administered to severely ill patients up to one hour after emergency department admission yields clinical benefits, such as reduced hospitalization rate and shorter length of emergency department stays.[14,15] Data from double-blind randomized controlled trials have suggested that, compared with systemic corticosteroids, inhaled corticosteroids can decrease admission rates and allow earlier discharge from the emergency department. Peak flow levels and forced expiratory volume in the first second (FEV1) also rise more quickly in patients who are given inhaled corticosteroids.[16-19]

OBJECTIVE

To the best of our knowledge, this was the first double-blind randomized clinical trial with the objective of comparing high doses of inhaled ciclesonide with use of injectable hydrocortisone for managing acute asthma in emergency settings. This trial was justified by the expected potential for fewer side effects with inhaled ciclesonide, the supposed benefit of its rapid onset of action and the need for more inhaled drugs to be available for clinicians dealing with asthma exacerbation in the emergency department.

METHODS

Population and setting

We studied patients with asthma aged 13 years or older, of both sexes, in the city of São Paulo, Brazil. Patients were recruited from the emergency department of Hospital São Paulo (a teaching hospital that is part of the Universidade Federal de São Paulo [UNIFESP]) and from two freestanding public urgent care centers affiliated with the hospital: Assistência Médica Ambulatorial (AMA) Santa Cruz and AMA Sacomã.

We included patients with a previous diagnosis of asthma (dyspnea, coughing, wheezing and chest tightness, associated with allergen exposure or cold air)[20,21] who received follow-up at outpatient clinics within the catchment area of the Hospital São Paulo emergency department and had a peak flow < 50% of the predicted flow. All participants had a longstanding history of asthma, with repeated exacerbations and emergency room visits. The patients who we included had had at least two years of moderate or severe asthma, with a mean peak flow immediately before intervention of 163 liters/min.

We excluded patients with body temperature ≥ 37.8 °C, smokers, pregnant women, patients undergoing psychiatric treatment, patients with a history of heart, liver, kidney or other disease that might contraindicate corticosteroid therapy, patients who had undergone lung resection, patients undergoing treatment for tuberculosis or mycotic infections of the lungs and patients with tracheotomy or mechanical obstruction of the trachea. We also excluded patients with myopathies or neurological conditions (such as sequelae of stroke or encephalopathies) and patients with body mass index (BMI) > 40 kg/m².

Ethical matters

This study was approved by the Research Ethics Committee of UNIFESP (judgment number 0974/09; date: September 18, 2009). All patients provided written informed consent for participation, in accordance with international regulations for human subject research. When patients were underage (< 18 years of age), consent was obtained from their parents or legal guardians.

This study was registered in the Brazilian Registry of Clinical Trials (http://www.ensaiosclinicos.gov.br/) under accession number RBR-6XWC26; date: January 5, 2016.

Sample

We studied 31 patients in the ciclesonide group and 27 patients in the hydrocortisone group. We calculated the sample size as prescribed by Greenberg,[22] considering a FEV1 improvement of 0.37 ± 0.85 liters after intervention, thus resulting in 65 patients for each group.

Study design

This was a double-blind, placebo-controlled, randomized clinical trial that was designed to compare the efficacy of inhaled ciclesonide versus intravenous hydrocortisone for managing moderate or severe acute asthma in an emergency department setting.

Blinding

Both blinding and randomization were done centrally at the Neuro-Sono Sleep Center, São Paulo, Brazil. Blinding of active ingredients and their respective placebos was achieved by random allocation of four letters (A, B, C and D) to each of the following products: hydrocortisone, ciclesonide, hydrocortisone-identical placebo and ciclesonide-identical placebo. After random allocation of letters to designate each product, we defined two product pairs: Inhaled Active Ingredient + Intravenous Placebo; and Intravenous Active Ingredient + Inhaled Placebo. This was done in random combinations that enhanced the safety of blinding. Both the intravenous placebo and the inhaled placebo were identical to their active counterparts.

Information about the intervention that each patient randomized to the study would receive was distributed in opaque numbered envelopes, which were only opened at the time of use. The nursing staff prepared the medications for administration as instructed in the numbered envelopes. The staff who prepared the medications, the providers who administered them and all researchers involved were blinded to the active pharmaceutical ingredients of interest.

Randomization

Patients included in the sample were recorded consecutively in a logbook and were assigned a serial number.

The 58 patients were divided into two groups: study (ciclesonide) and control (hydrocortisone), in accordance with two computer-generated random number tables. Each table contained an ascending sequence of numbers. Patients were allocated to one or the other according to the serial number attributed at the time of enrollment, which ensured that neither staff nor patients were aware of the intervention to which each patient would be allocated.

Ciclesonide group

Patients received ciclesonide at a dose of 160 mcg/puff. The first dose was administered five minutes after inclusion in the trial, and consisted of three puffs (480 mcg); the second dose at 20 minutes (480 mcg); and the third dose at 40 minutes (480 mcg). Thus, the total dose was 1440 mcg. Patients in this group also received hydrocortisone-identical placebo at five minutes. Since there was no standard recommendation, we took the total dose of 1440 mcg to represent a high dose, in accordance with the Global Initiative for Asthma (GINA) (www.ginasthma.org). This also needed to be given during the first hour after admission to the emergency room.

Hydrocortisone group

Patients in this group received 500 mg of hydrocortisone intravenously and ciclesonide-identical placebo at 5, 20 and 40 minutes.

Both groups

Both groups received short-acting bronchodilators (fenoterol hydrobromide and ipratropium bromide) at 0, 10 and 30 minutes.

Measurements

We used the spirometric variables FEV1 and peak expiratory flow (PEF) as primary outcome measurements, along with the clinical variables of dyspnea, wheezing and accessory muscle use during breathing (as assessed through observation of the sternocleidomastoid muscle). As secondary outcomes, we evaluated the heart rate, respiratory rate, blood pressure and pulse oximetry.

These parameters were measured every 30 minutes from the time of patient admission until the second hour and every 60 minutes thereafter until the fourth hour in the emergency department, thus making a total of six measurements. In this manner, we ensured rigorous monitoring throughout the patient observation period. We analyzed all six measurements, and no statistical difference was observed in comparisons of each paired time point (**Table 1**). For the purposes of this study, we analyzed and showed data from 30 minutes (baseline) and from the fourth hour, as we felt that these assessments were sufficient to represent the patients' course, among the six measurements obtained.

Procedures

The emergency room nurse applied the Manchester triage system and measured oxygen saturation, blood pressure and breathing pattern. The emergency room physician then confirmed the diagnosis of asthma exacerbation and notified the investigators, who performed an initial assessment by measuring peak flow and explained the study to the patient. Patients with a peak flow less than 50% of the predicted flow were invited to participate in the study (**Figure 1**), as the sample was designed to include only severe patients.

Once the patient had been included, the investigators worked with the emergency department staff to provide all the necessary care and perform the measurements required for the study.

Spirometric parameters were measured in an Easy One model 2009 spirometer (NDD Medizintechnik AG, Zurich, Switzerland). The best of three successive expiratory curves was considered valid and was used for the analysis, as recommended by the American Thoracic Society. Peak flow was estimated using the Mini-Wright Peak Flow meter (Clement Clarke, Hanlon, United Kingdom). Again, the highest of three measurements was used for the analysis.

Dyspnea was assessed subjectively as perceived shortness of breath, using the Borg scale: this is a visual analogue scale from

Table 1. Comparison of spirometric variables between the two study treatments. Data from 30 minutes (baseline), 60 minutes, 90 minutes, 120 minutes, 180 minutes and 240 minutes

FVC												
Time	**30′**		**60′**		**90′**		**120′**		**180′**		**240′**	
	C	**H**	**C**	**H**	**C**	**H**	**C**	**H**	**C**	**H**	**C**	**H**
	2.82 ± 0.99	2.49 ± 0.80	2.93 ± 1.0	2.75 ± 0.88	2.95 ± 1.02	2.66 ± 0.83	2.96 ± 1.02	2.67 ± 0.77	2.90 ± 1.07	2.69 ± 0.79	2.83 ± 0.99	2.67 ± 0.81
FEV1												
Time	**30′**		**60′**		**90′**		**120′**		**180′**		**240′**	
	C	**H**	**C**	**H**	**C**	**H**	**C**	**H**	**C**	**H**	**C**	**H**
	1.80 ± 0.84	1.59 ± 0.68	1.87 ± 0.80	1.72 ± 0.64	1.93 ± 0.85	1.73 ± 0.65	1.91 ± 0.84	1.78 ± 0.63	1.89 ± 0.87	1.80 ± 0.64	1.82 ± 0.84	1.78 ± 0.61

FVC = forced vital capacity; FEV1 = forced expiratory volume in the first second; C = ciclesonide; H = hydrocortisone.

0 to 10, on which 0 represents absence of dyspnea and 10 is the maximum dyspnea. During the initial assessment and at each time point for reassessment, we evaluated wheezing and accessory muscle use. Wheezing was assessed through pulmonary auscultation and was ranked from 0 to 3 on an ascending scale of severity (0: no wheezing; 1: slight wheezing; 2: moderate wheezing; 3: severe wheezing). Accessory muscle use was also measured on a scale of increasing intensity (0: no accessory muscle activity: 1: slight activity; 2: moderate activity; 3: marked accessory muscle activity). When there was little wheezing or a silent chest plus marked accessory muscle use or signs of muscle fatigue, dyspnea was classified as severe. Individual and pooled analyses were performed for all parameters.

Criteria for improvement

Patients were evaluated for improvement at all assessment time points, to ensure patient safety and detect any possible need for additional interventions other than those provided for in the study protocol. For the purposes of this study, we considered the following definitions of improvement: 1) FEV1 and PEF ≥ 70% of those predicted for the age, sex, weight and height; and 2) improvement of dyspnea: a) Borg score < 2;[23] b) reduction of wheezing severity from baseline; and c) no accessory muscle use, as determined through observation of the sternocleidomastoid muscles.

Interim analysis

We planned to conduct an interim analysis at the time when the number of patients included had reached approximately half the predicted sample size, in order to decide whether to continue or to terminate inclusion. This analysis was carried out at the randomization and blinding center (Neuro-Sono Sleep Center) by a committee that was established specifically for this purpose. After inclusion of 58 patients, this interim analysis committee suggested that recruitment for the study should be halted, since no difference between the treatments had been detected.

Adverse events

We actively evaluated the more frequent adverse events, such as dry mouth, tremor, palpitations, anxiety and headache, and recorded any other patient-reported events.[24,25] These variables were evaluated in terms of intention to treat (ITT).

Statistical analysis

The sample size was calculated considering a change in FEV1 of 0.37 liters, after treatment, as an indicator of improvement; a standard deviation of 0.85 liters; a significance level of 5%; and a statistical power of 80%.[16,22] This resulted in a sample size of n = 130 patients, i.e. 65 patients in each group. As noted above, interim analyses were carried out as planned after enrollment of 30 patients in each group; at that time, in view of the results, the interim analysis committee recommended termination of enrollment.

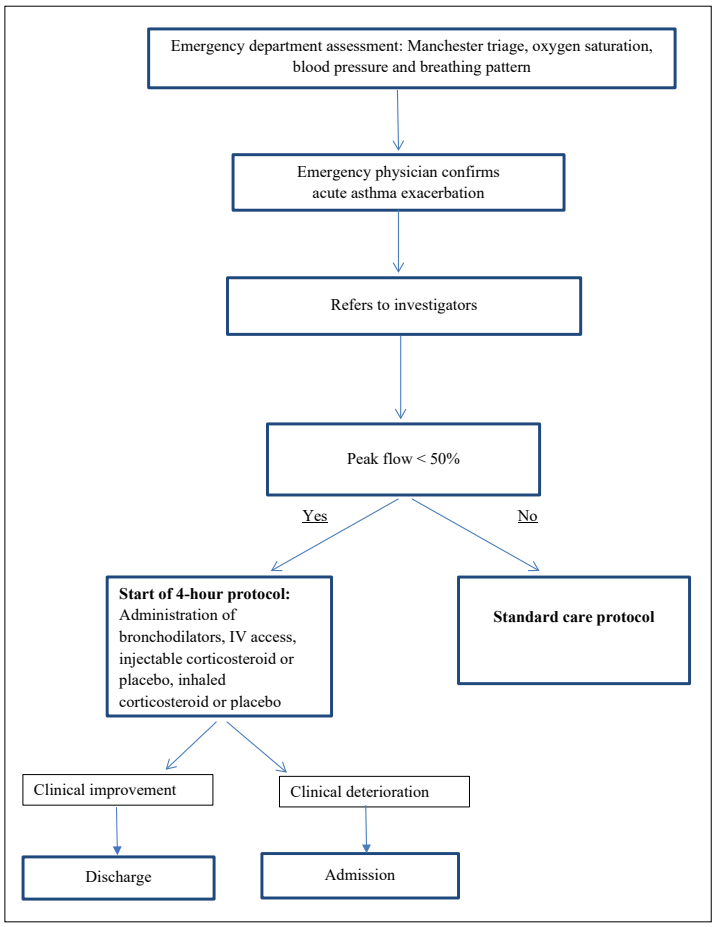

Figure 1. Flow diagram of patient inclusion and care.

Quantitative variables were expressed as the mean ± SD, and categorical variables, as n (%). We used Student's t test for independent samples for normally distributed data, the Mann-Whitney U test for asymmetrically distributed data and Pearson's chi-square test or Fisher's exact test for categorical data.[26,27] Outcomes were assessed using ITT, considering the worst scenario, i.e. losses in the study group were considered treatment failures and losses in the control group, successful treatment. P-values < 0.05 were considered statistically significant.

Availability of data and materials

All the data generated and analyzed during this study are available upon contact with authors.

RESULTS

Thirty-one patients in the ciclesonide group and 27 patients in the hydrocortisone group were analyzed using ITT.

Demographic data

The ciclesonide and hydrocortisone groups (**Table 2**) were similar with regard to age, systolic blood pressure (SBP), diastolic blood pressure (DBP) and the proportions of smoking, hypertension (HTN), diabetes mellitus (DM) and alcohol use. There were more women in the ciclesonide group ($P < 0.001$).

The ciclesonide and hydrocortisone groups did not differ regarding vital signs and pulse oximetry (**Table 3**). In within-group assessments, as expected, heart rate (HR) and respiratory rate (RR) were lower at hour 4, which was consistent with the clinical improvement observed in both groups. In between-group analyses, pulse oximetry and vital signs did not differ at hour 4.

Clinical variables

All the clinical parameters evaluated in this study showed improvement at hour 4, compared with entry to the emergency room. There was also no difference between the effects of ciclesonide and those of hydrocortisone at hour 4, i.e. both treatments were equally effective in improving respiratory effort, accessory muscle use, wheezing and Borg dyspnea scale scores (**Table 4**).

Spirometric variables

The patients treated with inhaled ciclesonide and those treated with hydrocortisone exhibited similar forced vital capacity (FVC), FEV1 and PEF values and similar progression of these

Table 2. Clinical and demographic characteristics of the ciclesonide and hydrocortisone groups

Variable	Group		P-value
	Ciclesonide (n = 31)	Hydrocortisone (n = 27)	
Sex: female, n (%)	23 (74)	18 (66)	< 0.001
Age (years), mean ± SD	38.3 ± 13.58	39.0 ± 18.99	0.826
BMI (kg/m^2), mean ± SD	25.9 ± 5.75	28.0 ± 5.44	0.094
SBP (mmHg), mean ± SD	120 ± 14.27	127 ± 14.55	0.057
DBP (mmHg), mean ± SD	78 ± 12.34	75 ± 17.85	0.477
Smokers (current and former), n (%)	9 (29.0)	8 (30)	0.960
HTN, n (%)	3 (10)	6 (22.0)	0.175
DM, n (%)	0 (0.0)	0 (0.0)	0.180
Alcoholism, n (%)	1 (3.2)	1 (3.4)	0.920
Obesity, n (%)	7 (22.58)	10 (37.03)	0.727

BMI = body mass index; SBP = systolic blood pressure; DBP = diastolic blood pressure; HTN = hypertension; DM = diabetes mellitus; SD = standard deviation.

Table 3. Response to treatments using hydrocortisone and ciclesonide, at hour 4, considering heart rate (HR), respiratory rate (RR), oxygen saturation (SpO2), systolic blood pressure (SBP) and diastolic blood pressure (DBP)

Variable	Ciclesonide (n = 31) (mean ± SD)	Hydrocortisone (n = 27) (mean ± SD)	P	Absolute effect size (95% CI)
HR	86 ± 23.14	90 ± 11.30	0.404	4.0 (-13.19; 5.19)
RR	18 ± 3.74	18 ± 5.52	0.679	0.0 (-2.46; 2.46)
SpO2	97 ± 3.19	95 ± 3.40	0.144	2.0 (0.30; 3.70)
SBP	120 ± 15.64	127 ± 14.32	0.092	7.0 (-14.71; 0.71)
DBP	75 ± 9.07	79 ± 12.31	0.208	4.0(-9.64; 1.64)

SD = standard deviation; CI = confidence interval.

Table 4. Effect of treatment on the variables of respiratory effort, accessory muscle use, wheezing and Borg dyspnea scale score at hour 4

Variable	Ciclesonide (n = 31)		Hydrocortisone (n = 27)		P	Relative effect size OR (95% CI)
	n (%)	Absolute effect size (95% CI)	n (%)	Absolute effect size (95% CI)		
Respiratory effort	1 (3)	32/1000	1(3)	28/1000	0.95	0.87 (0.05; 14.56)
Accessory muscle use	1 (3)	32/1000	2 (6)	28/1000	0.55	0.42 (0.04; 4.87)
Wheezing	8 (25)	258/1000	9 (31)	232/1000	0.55	0.70 (0.22; 2.16)
Borg ≥ 8	0 (0)	0/1000	0 (0)	0/1000	0.99	NE

SD = standard deviation; CI = confidence interval; OR = odds ratio; NE = not estimable.

parameters (**Table 5**). At hour 4, neither the FVC nor the FEV1 values had changed from baseline in either group. PEF increased significantly from 30 minutes (baseline) to hour 4 (P < 0.001) in both groups, and both treatments were equally effective when compared head-to-head at hour 4 (**Table 6**).

Adverse events

More patients in the hydrocortisone group complained of dry mouth, but there was no statistically significant difference in the frequency of any adverse effect between the groups (**Table 7**).

Hospitalization, losses and exclusions

Two patients in the ciclesonide group developed worsening bronchospasm and severe desaturation early in the course of treatment (having received only one dose of medication), and ultimately required ventilatory support.

DISCUSSION

To the best of our knowledge, this was the first double-blind randomized clinical trial to test high-dose inhaled ciclesonide for managing acute asthma in the emergency department. Our findings suggest that high-dose inhaled ciclesonide is as effective as intravenous hydrocortisone for this purpose. In this study, we tested ciclesonide as the intervention because it is a prodrug with high potency and less potential for oropharyngeal side effects than inhaled corticosteroid. This is particularly important for use in situations of acute exacerbations of asthma, a setting in which high doses of inhaled corticosteroids need to be administered.[12]

Studies have shown that use of inhaled and systemic corticosteroids can decrease the length of emergency department stay and the hospitalization rate, when administered in the first hour of an acute asthma exacerbation.[16] Nevertheless, the optimal agent, dosage and duration of observation in the emergency department remain unknown.[20,28]

Both drugs reduced expiratory effort, wheezing and accessory muscle use (**Table 4**); however, among the spirometric parameters analyzed, only PEF improved significantly from baseline at hour 4 in both groups (**Table 6**). Adverse events, such as dry mouth, palpitations, tremor, headache and anxiety, did not differ between the two groups (**Table 7**).

Table 5. Progression of spirometric variables from baseline (30 minutes) to hour 4 and comparison of the two study treatments at hour 4

Variable	Ciclesonide (Mean ± SD)		P	Hydrocortisone (Mean ± SD)		P	C x H Hour 4
	Baseline	Hour 4		Baseline	Hour 4		P
FVC	2.82 ± 0.99	2.83 ± 0.99	0.95	2.49 ± 0.80	2.67 ± 0.81	0.41	0.50
VEF1	1.80 ± 0.84	1.82 ± 0.84	0.94	1.59 ± 0.68	1.78 ± 0.61	0.28	0.83
PEF	157.58 ± 48.11	276.89 ± 100.46	< 0.001	170.18 ± 54.21	293.0 ± 92.24	< 0.001	0.51

SD = standard deviation; C x H = ciclesonide versus hydrocortisone; FVC = forced vital capacity; FEV1 = forced expiratory volume in the first second; PFE = peak expiratory flow.

Table 6. Comparison of the spirometric variables of the two study treatments at hour 4

Variable	Ciclesonide (n = 31) Mean ± SD	Hydrocortisone (n = 27) Mean ± SD	P	Absolute effect size (95% CI)
FVC	2.83 ± 0.99	2.67 ± 0.81	0.50	0.16 (-0.30; 0.62)
FEV1	1.82 ± 0.84	1.78 ± 0.61	0.83	0.04 (-0.30; 0.41)
PEF	276.89 ± 100.46	293.0 ± 92.24	0.51	-16,11 (-65.72; 33.50)

SD = standard deviation; CI = confidence interval; FVC = forced vital capacity; FEV1 = forced expiratory volume in the first second; PFE = peak expiratory flow.

Table 7. Adverse events in the two groups

Event	Ciclesonide (n = 31)			Hydrocortisone (n = 27)		
	n	%	95% CI	n	%	95% CI
Dry mouth	2	6.5	0 to 15.0	7	25.9	12.0 to 39.9
Palpitations	3	9.7	0 to 15.0	1	3.7	0 to 10.8
Tremors	11	35.5	0 to 37.4	7	25.9	12.0 to 39.6
Headache	2	6.5	0 to 15.0	3	11.1	10.4 to 11.8
Anxiety	1	3.2	3.0 to 3.4	2	7.4	0 to 17.3

CI = confidence interval.

There was no significant difference between groups; dry mouth was the only complaint that was more prevalent in the hydrocortisone group.

Clinical studies using high doses of inhaled corticosteroids such as fluticasone,[16] flunisolide[17] and ciclesonide[11] also found these agents to be effective in increasing peak flow.

Although we did not enroll a large number of patients, the groups did not differ in terms of demographic characteristics, except for the higher proportion of women in the ciclesonide group (**Table 2**). Two patients in the ciclesonide group, both with peak flow < 30% of the predicted flow, developed worsening bronchospasm and severe desaturation early in the course of treatment (having received only one dose of medication), and ultimately required invasive ventilation. Given the small sample, the likelihood of between-group differences was very high, and we judged these events to be attributable to chance.

Clinical parameters (**Table 4**) and vital signs (**Table 3**) were similar at admission to the emergency room and at hour 4. Only DBP was higher in the hydrocortisone group, possibly due to the systemic effects of the corticosteroid.[3]

FVC and FEV1 remained unchanged from baseline to hour 4, and did not differ between the two groups. In previous studies on fluticasone[16] and flunisolide,[17] improvements in these parameters were reported. In our study, we observed an increase in PEF despite no increase in FEV1. This is consistent with the well-known mismatch between FEV1 and PEF in cases of acute severe asthma,[29-31] a condition in which FEV1 is underestimated and does not correlate adequately with rises in peak flow.

In a Cochrane review, it was noted that the higher cost of inhaled corticosteroids, compared with systemic corticosteroids, was an obstacle to use of the former.[28] However, this was not an issue in our study, in which nine puffs of ciclesonide (the total dose used in the emergency department) had an estimated cost of US$ 2.47, while a single 500-mg dose of hydrocortisone had a cost of US$ 3.18, thus making ciclesonide more cost-effective. In the United States, the average cost of treatment reaches US$ 1368.00, 30 days after a severe asthmatic exacerbation.[32] Also with regard to the cost and utility of inhaled corticosteroids, the FourFold Asthma Study (FAST) showed that it is clinically safe for patients to simply quadruple their usual dose of inhaled corticosteroids at home, upon deterioration of their condition, thus aborting a severe asthma attack and obviating the need for hospitalization.[33]

The limitations of our study included the lack of follow-up (to assess for recurrence) and the small sample size. The latter had the consequence of, for instance, preventing us from determining whether dry mouth was truly more prevalent in the hydrocortisone group. The strengths of our study included its design and external validity, since we included adult patients from the general population with no restrictions regarding age, gender or ethnic group; rigorous evaluation of clinical and spirometric parameters; appropriate masking and blinding; and rigorous close monitoring of patients for a four-hour period during the study protocol.

CONCLUSION

In summary, our study suggests that high-dose inhaled ciclesonide is as effective as injectable hydrocortisone for managing acute severe asthma and had a similarly favorable adverse-event profile, with the advantage of being a prodrug that exerts topical anti-inflammatory effects while reducing the risk of long-term systemic side effects.

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Management strategies for implementing a multicenter cross-sectional study: lessons from the ADHERE Brazil study

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ABSTRACT

BACKGROUND: Epidemiological studies involving large samples usually face financial and operational challenges.

OBJECTIVES: To describe the planning and execution of ADHERE Brazil, an epidemiological study on 1,105 kidney transplant patients, and report on how the study was structured, difficulties faced and solutions found.

DESIGN AND SETTING: Cross-sectional multicenter study in 20 Brazilian kidney transplantation centers.

METHODS: Actions developed in each phase of implementation were described, with emphasis on innovations used within the logistics of this study, aimed at estimating the prevalence of nonadherence to treatment.

RESULTS: Coordination of activities was divided into four areas: general, regulatory, data collection and statistics. Weekly meetings were held for action planning. The general coordination team was in charge of project elaboration, choice of participating centers, definition of publication policy and monitoring other coordination teams. The regulatory team provided support to centers for submitting the project to ethics committees. The data collection team prepared a manual on the electronic collection system, scheduled web meetings and was available to respond to queries. It also monitored the data quality and reported any inadequacies found. Communication with the centers was through monthly reports via e-mail and distribution of exclusive material. The statistical team acted in all phases of the study, especially in creating the data analysis plan and data bank, generation of randomization lists and data extraction.

CONCLUSIONS: Through these logistics, we collected high-quality data and built a local research infrastructure for further studies. We present supporting alternatives for conducting similar studies.

CLINICAL TRIAL ANNOTATION: http://clinicaltrials.gov/ on October 10, 2013; NCT02066935.

INTRODUCTION

Nonadherence to treatment of chronic diseases is one of the main determinants of long-term complications.[1] Kidney transplantation (KT) is one of the therapies used for the most advanced phase of chronic kidney disease. It results in better survival, better quality of life and lower costs.[2] Nonadherence to immunosuppressive drugs that are necessary to avoid graft rejection is associated with an increased risk of acute rejection episodes, graft dysfunction, lower graft survival and higher healthcare costs.[2]

Over recent years, Brazil has reached the second highest position in the world, in terms of the absolute number of KTs performed in the world's largest public transplantation system.[3] However, this country has a large geographical size and great cultural, social and economic diversity, which translates into large variation in transplantation activity across the country, when normalized according to population size.[4] Evaluation of and addressing nonadherence after KT is crucial to achieve the best results. However, Brazilian studies are scarce, limited to small samples, and are therefore not representative of the general population.[5-12]

Studies on adherence to immunosuppressives after KT by our group began in 2009. In the initial project, we performed a cross-cultural adaptation and validation study of a self-report instrument for diagnosing nonadherence to immunosuppressives.[8] Then, we estimated the prevalence of and identified factors associated with nonadherence to immunosuppressives after KT in a single center in the city of Juiz de Fora, Minas Gerais.[10] Discussion on the need for a multicenter study that could better portray Brazilian epidemiology arose when these results were presented in national conferences in 2011. Over a period of two years, these discussions continued and led to planning of a project among Brazilian national researchers in collaboration with a world-reference research center that had already started a similar study on heart transplantation (Building Research Initiative Group: Chronic Illness Management and Adherence in

Transplantation - BRIGHT study).[13] Consequently, the ADHERE Brazil study was designed as a multicenter cross-sectional study with the following objectives: to estimate the prevalence of nonadherence to immunosuppressives and other aspects of nonpharmacological treatment; to evaluate multilevel factors associated with nonadherence to treatment; and to benchmark the participating centers, bring the subject up for discussion and disseminate data to support future actions towards reducing these behaviors.[14]

However, conducting such a large study, comprising twenty kidney transplantation centers and an ideal sample size of 1,139 patients, presented several challenges. This was an epidemiological study, and therefore without interventions, but which needed adequate infrastructure, in order to follow regulatory policies, and needed funding similar to that required in clinical research. For example, the study required a study coordination room, a meeting room and a specific area for archives and staff training.[15,16]

A multicenter study can be divided into four phases: 1) Planning; 2) Project development; 3) Study implementation; and 4) Dissemination. Some detailed reports on each of these phases exist, but the actions developed and the project management may vary according to the project and local characteristics.[17] Indeed, in our project we faced some difficulties, but feasible solutions were found that translated into a path towards positive results. Considering that these challenges may be experienced in similar research proposals, a report presenting a detailed list of actions may help other researchers succeed.

OBJECTIVE

Thus, our objective was to present the stages followed by the researchers responsible for conception of the ADHERE Brazil study; show how the development of the study was planned and implemented; and describe the difficulties found and solutions applied. Ultimately, reliable results could be obtained regarding the diagnosis of nonadherence to treatment and associated factors. We therefore present a description of the actions taken, especially in the project implementation phase, with emphasis on the innovative and adaptive processes employed.

METHODS

This was a cross-sectional and descriptive study on the procedures undertaken for execution and management of the ADHERE Brazil multicenter study (http://clinicaltrials.gov/ on October 10, 2013; NCT02066935).

Use of project management principles through coordination teams, for the various phases of a study, is fundamental for enabling of a multicenter study to be properly conducted.[17,18] Many of these studies are conducted by clinical research centers with professional support from companies that are in charge of managing all phases of the project.[19] In the case of the ADHERE Brazil study, because of its epidemiological nature and its objective of being representative of all types of KT services, it was not envisaged that the project would have the infrastructure of the multicenter studies mentioned above. Accordingly, a specific and local proposal was developed.

Here, we describe the organizational structure proposed at different phases of our study for actions to be elaborated, especially in the project implementation phase, so that it would be possible to reproduce in similar studies. This report was authored by the researchers who participated in the study.

RESULTS

The initial idea for the project arose in 2011. It was then designed over a two-year period. A group of co-investigators, whom we called the ADHERE Brazil study consortium, was then consolidated. The project was registered at the Research Department of the Federal University of Juiz de Fora, the institution to which the principal investigator was attached; and on the Clinical Trials website (https://clinicaltrials.gov/) under the number NCT02066935 and on the Open Science Framework scientific dissemination platform (https://osf.io/dpr2j/).

Recruitment of potential study participant centers began in 2013. The initial invitations to 20 centers were made in person at national and regional conferences. After communicating by e-mail and signing statements of confidentiality and feasibility, the participation of these centers was formalized through a contract between them and the principal investigator's center (coordinating center). Invitations were sent out in May 2013, and the final composition of the participating centers was completed in May 2016. A total of 22 centers were invited because two of the initially invited centers subsequently refused to participate. The new invitations were made using the same criteria and characteristics as for the original centers. Among the 20 centers that ultimately participated in the ADHERE Brazil study, 38.2% showed low activity (< 50 transplantations/year), 36% were moderately active (50-150 transplantations/year) and 25.8% were highly active (> 150 transplantations/year). This distribution was similar to what had been reported by the Brazilian Transplant Register[5] (**Figure 1**).

Scientific participation and authorship were defined before the study started and were described and agreed upon in a publication policy statement. The regulatory and fundraising phases began in 2014. The first sets of data were collected in December 2015 and finalized in April 2017. Dissemination of results began in 2015 and is ongoing. By April 2021, 17 presentations had been made in national/regional conferences and five in international events, and two articles had already been published.[14,20]

As mentioned above, we established an early research collaboration with the Leuven Basel Research Group (LBARG). They already had broad experience in international dissemination of knowledge and multicenter studies. The group supported all the phases

of our study, through a strengthened partnership and promotion of a better qualified process.

To make the project feasible, coordination of actions in four areas was proposed: general, regulatory, data collection and statistics.

General coordination

General coordination was performed by the principal investigator of the study, who was responsible for project elaboration, study consortium formation and sending invitations to the participating transplantation centers. She also participated in all the other phases: regulation, data collection and analysis. In addition, she prepared other reference documents for the study, such as the statements for center participation feasibility and confidentiality, as well as the publication policy of the study.

In the center recruitment phase, after the informal invitation had been accepted, the centers received a confidentiality agreement, which addressed the importance of confidentiality of the project content and of the data collected during the study. After signing, the center received the feasibility document, which contained information that would ensure that the participating center had the minimum infrastructure to be able to conduct the study. The center also received the publication policy statement on issues relating to publications generated using ADHERE Brazil study data, authorship and subprojects. Centers were only included in the study after the leading local investigator of each center signed these documents. After the regulatory phase, participation was formalized through a research contract signed by the parties involved.

Soon after regulatory approval had been obtained, applications for financial support were sent out. Support was sought from the national governmental funding agency (Conselho Nacional de Desenvolvimento Científico e Tecnológico, CNPq) and from the local state agency (Fundação de Amparo à Pesquisa do Estado de Minas Gerais, FAPEMIG); and also from private companies interested in research relating to transplantation. Unfortunately, the project was not selected for governmental support, but two pharmaceutical companies decided to join the proposal. The operational support provided by a clinical research structure and the virtual and centralized data collection system, together with interest in the potential results, seemed to contribute to the companies' decision. However, other than financial support, the companies had no role in any of the stages of the project design, data collection, analysis or writing of the manuscripts. The resources enabled personnel payments (regulatory and data collection activities), participation in events, translation/editing and printing services.

Figure 1. Geographical locations of participating centers in the ADHERE Brazil study around the country. Colors indicate the transplantation activity level: red, high activity (> 150 kidney transplantations/year); blue, moderate activity (50 to 150 kidney transplantations/year); and green, low activity (< 50 kidney transplantations/year).

Weekly meetings were held among the general coordination team and the other coordinators to schedule the following phases of the study: forwarding the participating center projects to the local ethics committees; organizing and training for data collection; checking the ongoing data collection; and performing data analysis. This coordination team was responsible for following up on all the activities necessary for development of the project, with organization and control of the information (**Figure 2** and **Table 1**).

The general coordination effectively developed the following strategic activities for project progression: 1) Weekly meetings mentioned above; 2) Definition of well-established functions among the other coordination teams; 3) Creation of an e-mail address shared by the coordinators; 4) Communication with the centers via Skype or other communication sources such as telephone or e-mail, to answer questions; 5) Monthly newsletters to the centers, which totaled 28 between 2015 and 2020, reporting on study progression; 6) Meetings with members of the participating centers at national conferences to disseminate information about the study proposals (14th Brazilian Congress on Transplantation 2015 and 17th Brazilian Congress of Nephrology 2016), along with distribution of material containing the visual identity of the study (buttons, notebooks and pens) (Table 1); 7) Provision of an appropriate physical area for meetings, a telephone line and a computer for coordinators to work on.

Regulatory coordination

Initially, the coordinating center submitted the project to the research ethics committee (REC) of the institution at which that center was located. After approval, which was granted in May 2014, the centers recruited were asked to submit the project to their local RECs. Participation of each center depended on receiving approval from the local REC. Patients were only included after they had signed an informed consent statement (ICS).

The first step for the coordinator of the regulatory team was to send all the guidelines for REC submission to the other 19

participating centers and a model of the formal documents to be included in the national REC website (Brazil Platform): research project, ICS model, budget model and schedule model. This guidance greatly facilitated REC submission by the participating centers. However, there were some difficulties, which can be gauged from the time that elapsed between obtaining the first REC approval (September 2014) and obtaining the last one (February 2017), which amounted to 30 months.

The main difficulty faced was the lack of familiarity with the Brazil Platform among the participating researchers, with regard to registering researchers, preparing documents to be included and, subsequently, formulating answers to any questions raised by the RECs. For these points, availability and participation of the regulatory team was essential for solving any issues. Another difficulty was the refusal of two RECs to evaluate the project because it was a multicenter study. These cases were then referred to and evaluated by the coordinating center's REC. This process delayed patient inclusion in these centers. Lastly, after initial approval of the project, two amendments for replacement/inclusion of two new centers had to be submitted to all RECs. The archiving of documents submitted to each local REC and final approvals from them was done by the regulatory team.

It was also the responsibility of this coordination group, after the end of data collection, to forward the final report to the coordinating center REC and to send a model to the participating centers, for them to perform the same procedure in local RECs.

This team was also responsible for registering the project on the Clinical Trials website, which ensured transparency with regard to execution and publication of results and avoidance of bias, with ethical support for the participants, since the results generated would promote scientific knowledge.[21]

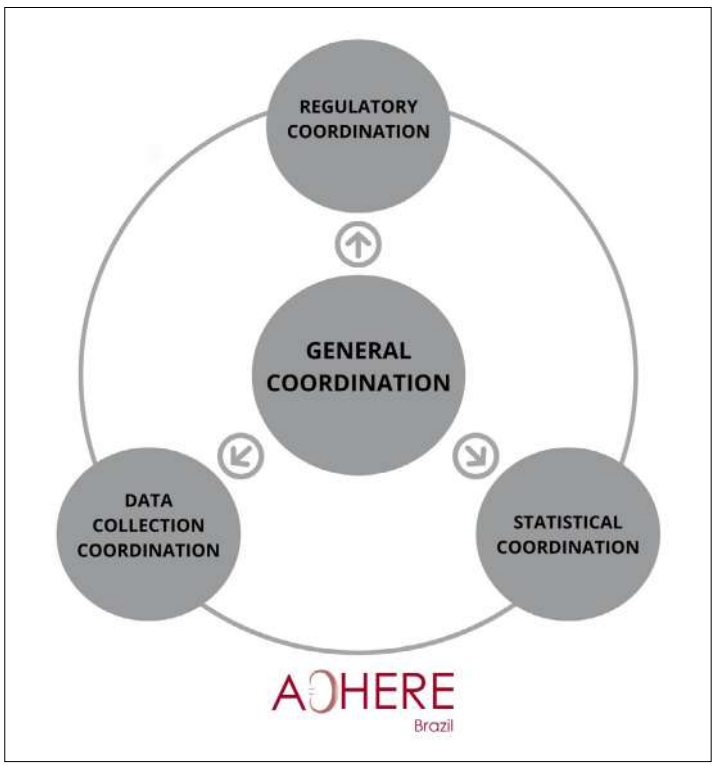

Figure 2. Organizational structure, showing the four coordination teams and flux process among them.

Data collection coordination

The two-member data collection coordination team oversaw organizing the training of personnel involved in data collection at the participating centers and monitoring and checking data inclusion during collection. The objective of this coordination team was to make available staff who had been trained to operate the data collection system, on the Research Electronic Data Capture (RedCap) platform, and who would be easily accessible to people at the centers, by telephone, e-mail or other communication platforms.

The interviews and questionnaires used in the ADHERE Brazil study were prepared based on a theoretical framework[1,14] and on previous studies.[13] The RedCap system was used for data collection. This is a safe internet-based software platform that was created at Vanderbilt University, in Nashville, United States, for the purposes of data capture and storage, which could be fed remotely by trained individuals (http://www.project-redcap.org/) at the coordinating and participating centers. The ADHERE Brazil project was created on this platform; thereafter, the data collection questionnaires were included. Each center had remote access to the project to include data and access only their respective data, thereby ensuring anonymity.

All the research coordinators of the centers participating in the ADHERE Brazil study were trained to use the RedCap system through a manual that was prepared specifically for this purpose, and through Skype meetings. In addition to training, each center was asked to include a "test patient" in the system before data collection started.

Table 1. Description of the participation of each coordination team at different phases of the ADHERE Brazil study

Phases Coordination team	Phase 1 Regulation	Phase 2 Data collection training	Phase 3 Data collection	Phase 3 Data analysis
General	X	X	X	X
Regulatory	X			
Data collection		X	X	
Statistical		X	X	X

Another data collection coordination function was to send randomization lists that had been generated by the statistics coordination team, to the participating centers so that patients could be selected to participate in the study.

Lastly, to ensure data quality, the data collection team checked the data weekly, as the patients were inserted into the RedCap system by the participating centers, to detect missing data or possible typing errors. Following this evaluation, reports were generated every week and sent to the centers, requesting them to check and correct inconsistencies if necessary. A one-week deadline was given for checking the data. Thereafter, the data were checked again.

One of the biggest challenges of the data collection coordination team was to adjust for missing/inconsistent data from each center. Data collection was only considered finalized after inconsistencies had been checked and corrected. To solve such problems after a report had been sent more than once, telephone contact was made to detect possible process difficulties. Using this strategy, data collection began in December 2015 and was completed in April 2017 (a total of 17 months), with inclusion of 1,105 patients, which corresponded to 97% of the ideal sample size.

Statistical coordination

The statistical coordination team participated in all study phases, from conception to analysis and dissemination of data.

More specifically, it was responsible for the sampling strategy, and for defining the sample size and the criteria for patient distribution in each center. It created the database in the RedCap system and sent access passwords to the 20 participating centers. RedCap access levels differ, depending on the function: the centers only had access to their own information, while the data collection and statistics coordination teams and the principal investigator had broader access. Additionally, the statistical coordination team was responsible for generating randomization lists using computer software to select patients for the study. These lists included information provided by the centers, such as the number of patients scheduled per day in the post-transplantation clinic and the number of patients to be included in each period.

Another function of this coordination team was to organize data extraction after data collection through the RedCap system had been completed, thus generating the initial ADHERE Brazil study database. The entire analysis plan was defined by this coordination team in agreement with the researchers' consortium. It was also responsible for defining and guiding the tests performed and for performing more complex analyses.

DISCUSSION

The ADHERE Brazil study involved data collection from 20 Brazilian KT centers. Active participation by the general, regulatory, data collection, and statistical coordination teams was essential for enabling effective collection and reliable analysis of the information generated. Consequently, these integrated actions enabled inclusion of 97% of the ideal sample size, which was accomplished with high-quality data collection and integration among the coordination teams. The strength of our study that we can highlight is that we were able to accomplish a high-complexity study using low-cost available solutions, thus generating unpublished data with great potential to contribute towards healthcare practice.

The general coordination team conducted activities in conjunction with the other coordination teams in a harmonious fashion. Based on the basic principles of project management in research,[21] weekly updates with discussion of the problems that arose, together with short and medium-term planning of solutions, aligned project progression and established the actions and functions to be fulfilled.[21] This proposal was essential for solving problems in our study, as previously described in health and educational research settings.[21-24] Management skills among health researchers are not fully available because such abilities are not systematically developed during undergraduate courses. Additionally, investigators are frequently involved in other activities such as teaching or healthcare assistance outside of research, and thus have limited time to dispend on further educational and training efforts.[25]

In line with international standards,[17] all research in Brazil involving human beings has to be evaluated by a REC to ensure respect for and protection of research subjects.[21] The role of RECs is to ensure that the principles of research ethics are respected in the study and that the rights of those involved are preserved.[21] Although there is clear legislation on regulatory procedures for this type of research, the effectiveness and operational particularities of RECs still vary across Brazil.[26,27] For multicenter studies, which involve multiple REC evaluations, some avoidable inefficiencies have been reported, mainly due to discrepancies in the opinions of these multiple committees.[27] This can be demonstrated through the long period of time that was spent on this phase, which was the longest. The regulatory coordination team was created to deal with these issues, and to promote and facilitate regulatory procedures in each center, since the study included some institutions with fully functioning clinical research units and others that had never participated in research. We proposed to have someone with expertise in regulatory processes always available to assist at all levels, from preparation of documents to submission of the project and its amendments to the Brazil Platform website. A similar strategy has been reported by others in multicenter studies.[17,23,28]

The data collection phase is essential. Good planning at this stage can prevent possible distortions and interviewer influence on the interviewee, which thus enables methodological and scientific rigor.[17,19,22,23] It is important to emphasize that the quality of the

data collected in surveys depends on the adequacy and quality of questionnaires, in order to guarantee validity and reproducibility through clear and simple questions.[16,17] Furthermore, to increase survey quality, it is essential to provide standardized training for data collectors.[17,23] In the ADHERE Brazil study, all professionals who collected data received training for this purpose. Another key feature was simultaneous data collection and storage through a computer platform connected to the internet, which minimized errors, since it allowed for immediate checking for missing or erroneously filled data. These errors could be reported back to the person responsible for data collection, so that prompt corrections could be requested. Systematic and consistent checking of quality during the ongoing data inclusion is a powerful measure for preventing errors and missing data.[17] In our study, each local center made adaptations and took appropriate steps to ensure that data were collected in a way that guaranteed satisfactory results.[17,23]

It is worth mentioning that it is the responsibility of statisticians to plan studies, interpret the data obtained through field research and present the results in a way that facilitates decision-making by researchers.[15,16,28] High-quality research is associated with formal early statistical planning.[29] Thus, it is essential to have statisticians' participation at all stages of a research project so that they can significantly contribute not only to data analysis, but also to the choice of method and analysis software, and to presentation and interpretation of results.[29] The availability and effective participation of the statistical coordination team at all levels of our study led to attainment of this quality threshold.

The limitation of this study was the scarcity of articles describing experiences relating to strategies for conducting multicenter epidemiological studies. Especially within the Brazilian scenario, we found reports only about obstacles to the ethics regulatory process.[26,27] This made it difficult to discuss the key points of the proposal from a local perspective. Therefore, we discussed the findings based on the available published reports. We think this aspect of our proposal highlights its originality and relevance.

Since the conceptualization of the ADHERE Brazil study, we have been making efforts to disseminate the proposal and the results. Our proposal was registered on the Clinical Trials and the Open Science Framework websites, and we have presented summaries of our results at international, national and regional meetings. In confirmation of the potential for applicability and reproducibility of our proposal, we have already identified two Brazilian multicenter studies that mirror our methodology: the DGF Brazil Study Group[30] and SARS-COV-2 Infection in Kidney Transplant Recipients: a Brazilian Multicenter Study (http://clinicaltrials.gov/: NCT04494776).

CONCLUSION

The experience acquired in conducting this study led us to conclude that through the project management actions described, it was possible to collect reliable data on adherence to immunosuppressive treatment after KT and to ensure that the ethical principles and safety measures involved in the research and data collection were adopted, since all work processes proposed for conducting the study were strictly followed. In addition, this article presents alternatives for conducting studies of similar nature to the ADHERE Brazil study.

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Incidence of multimorbidity and associated factors during the COVID-19 pandemic in Brazil: a cohort study

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Risk factors.
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COVID-19.

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Coronavirus disease 2019.

ABSTRACT

BACKGROUND: Due to the coronavirus disease 2019 (COVID-19) pandemic, access to healthcare services may have become difficult, which may have led to an increase in chronic diseases and multimorbidity.

OBJECTIVES: To assess the incidence of multimorbidity and its associated factors among adults living in the state of Rio Grande do Sul, Brazil.

DESIGN AND SETTING: Cohort study conducted in Rio Grande do Sul, Brazil.

METHODS: We included data from the two waves of the Prospective Study About Mental and Physical Health (PAMPA). Data were collected via online questionnaires between June and July 2020 (wave 1) and between December 2020 and January 2021 (wave 2). Multimorbidity was defined as the presence of two or more diagnosed medical conditions.

RESULTS: In total, 516 individuals were included, among whom 27.1% (95% confidence interval, CI: 23.5-31.1) developed multimorbidity from wave 1 to 2. In adjusted regression models, female sex (hazard ratio, HR: 1.97; 95% CI: 1.19-3.24), middle-aged adults (31-59 years) (HR: 1.78; 95% CI: 1.18-2.70) and older adults (60 or over) (HR: 2.41; 95% CI: 1.25-4.61) showed higher risk of multimorbidity. Back pain (19.4%), high cholesterol (13.3%) and depression (12.2%) were the medical conditions with the highest proportions reported by the participants during wave 2.

CONCLUSION: The incidence of multimorbidity during a six-month period during the COVID-19 pandemic was 27.1% in the state of Rio Grande do Sul, Brazil.

INTRODUCTION

Multimorbidity, defined as the presence of two or more chronic diseases, is associated with reduced quality of life.[1] Globally, multimorbidity affects one in three people, although this prevalence might be higher among women and older adults.[2] In Brazil, the prevalence of multimorbidity may reach up to 24% among adults.[3,4] However, the prevalence is even higher among older adults in Brazil, such that in 2015 it was found to be affecting at least half of this population.[5,6]

The elevated costs attributable to multimorbidity treatment are alarming. For example, the average medical economic cost of multimorbidity is 5.5 times greater than the treatment of only one chronic condition in Switzerland.[7] Each additional disease represents a 3.2-fold increase in requirement of healthcare services and roughly 33% greater treatment costs.[7] Multimorbidity is also associated with increased use of healthcare resources including medications, primary care visits, hospitalizations, elective procedures and emergency services.[8] In Brazil, a study carried out using data from the Brazilian National Health Survey (Pesquisa Nacional de Saúde, PNS) showed that patients with multimorbidity were more likely to use healthcare services.[9]

Nevertheless, treatments or diagnoses for multimorbidity may have become impaired due to the limitations on access to the healthcare system that occurred as an indirect consequence of the coronavirus disease 2019 (COVID-19) pandemic.[10] In December 2020, Brazil had the second-highest number of registered cases and deaths due to COVID-19.[11] This chaotic scenario limited the management of preexisting chronic disease, especially because of fear of viral contagion during medical appointments.[10] Furthermore, diagnosing of other morbidities may have been equally impaired during social distancing. Besides the higher risk of severe COVID-19 associated with the presence of chronic disease, co-occurrence of different morbidities might increase the risk of COVID-19 complications, including hospitalization. However, to the best of our knowledge, longitudinal studies that examine the incidence of multimorbidity during the COVID-19 pandemic remain warranted.

OBJECTIVE

The aim of this study was to evaluate the incidence of multimorbidity and its associated factors during the COVID-19 pandemic, among adults living in the state of Rio Grande do Sul, Brazil.

METHODS

We analyzed data from waves 1 and 2 of the Prospective Study about Mental and Physical Health (PAMPA) cohort, which was an ambispective study carried out in the state of Rio Grande do Sul, Brazil. In wave 1, the recruitment phase took place between June 22 and July 23, 2020, while wave 2 was carried out between December 1, 2020, and January 15, 2021. Wave 2 lasted longer (seven weeks) due to the holiday period. Full details of the study methodology can be found elsewhere.[12] The study protocol was approved (number: 4.093.170; date: August 27, 2019) by the institutional research ethics board of the Superior School of Physical Education of the Universidade Federal de Pelotas (UFPel), Brazil. All participants gave informed consent before answering any question from the questionnaire.

Recruitment phase

During the recruitment phase, we contacted participants through university professors, social media, local media and personal contacts in all macroregions in the state. Only adults aged 18 or older and living in Rio Grande do Sul were included in wave 1. We also contacted the local media in all macroregions to inform the local population about the present study. Moreover, all researchers involved shared the link to the study announcement with their personal contacts across the state. For wave 2, we contacted the previous participants who were still living in the state and had provided any contact information at the time of wave 1 (e.g. phone number or social media nickname). The questionnaires were constructed using the Google Forms application in wave 1 (Google, Mountain View, California, United States) and using the Redcap web application, version 9.0.3, in wave 2 (Vanderbilt University, Nashville, Tennessee, United States).

Sample size

We calculated the sample size based on three primary outcomes: low back pain; depressive and anxiety symptoms; and access to the healthcare system.[12] The total population of the state of Rio Grande do Sul was 10,693,929 in 2010, according to the 2010 Brazilian national census. We defined that a sample of 1,767 participants was required, under the assumptions of a 95% confidence interval, a margin of error of 1.8, and a possible loss-to-follow-up of 30%. Rio Grande do Sul is divided into seven macroregions named (in Portuguese): Serra, Norte, Nordeste, Centro-Oeste, Vales, Metropolitana and Sul. Based on the latest national census, the required sample size was divided proportionally to the number of people living in each region.

Outcome

In this study, we excluded participants who presented multimorbidity in wave 1. Multimorbidity was assessed through the same question previously used in the Brazilian Telephone-based Surveillance System for Noncommunicable Diseases:[13] "*Has any doctor ever told you that you have the following disease?*". A 12-item list was used, which included the following diseases: hypertension or high blood pressure, diabetes, high cholesterol, cancer, arthritis/arthrosis/fibromyalgia, asthma/bronchitis, back problem, heart disease, depression, memory problem, human immunodeficiency virus (HIV)/acquired immunodeficiency syndrome (AIDS) and other chronic diseases. Multimorbidity was considered to be present when a participant reported having two or more health issues.[14]

Exposures

Sociodemographic factors (age, sex, ethnicity, conjugal situation and educational level), behavioral information (physical activity) and nutritional data (body mass index, BMI) were used as possible confounders and as associated factors.

Weight and height were self-reported and were used to calculate BMI (kg/m^2). We considered subjects to be overweight if they had BMI > 25 kg/m^2 and obese if they had BMI ≥ 30 kg/m^2. To estimate what the subjects' physical activity level had been before social distancing, we used the following question: "*Before social distancing, were you engaged in physical activity regularly?*". If the participants answered "Yes", they were asked to declare the number of days with physical activity and its duration in minutes, in a regular pre-COVID-19 week. Participants who practiced for 150 minutes or more were classified as physically active, and those who practiced for less than 150 minutes weekly, as physically inactive, in accordance with the guidelines for physical activity from the World Health Organization.[15]

Decreased monthly income was assessed by the following question: "*Over the last six months, have your monthly earnings been affected by social distancing measures?*". There were three response options: "decreased", "unchanged" "increased". We classified this into a dichotomous variable for the analyses, with the option "yes" for decreased monthly income and "no" for unaffected or increased income.

Data analyses

All analyses were weighted according to the proportion of the participants in each macroregion, because of overrepresentation of respondents living in the Sul region (n = 436; 64.6%). The data were reported as the mean with 95% confidence interval (CI) or as proportions with 95% CI, as appropriate. Participants who provided some personal contact in wave 1 but did not respond to wave 2 were excluded (**Figure 1**). We used proportional-hazards global tests and visual examination of Schoenfeld residuals against time to assess proportional-hazards assumptions (data not shown). Crude and

adjusted Cox regression models were used to estimate the hazard ratio (HR) with a 95% CI.[16] The adjusted model (aHR) included sex, age, ethnicity, conjugal situation, education level, decreased monthly income, BMI and physical activity before the pandemic. All covariates were assessed at wave 1. Pearson's chi-square test was used to test the differences between included and excluded samples (**Table 1**). Analyses were performed using the Stata 15.1 software (Stata Corp, College Station, Texas, United States).

RESULTS

A flowchart describing the sampling process is presented in **Figure 1**. Out of 2,321 participants with valid responses in wave 1, 1,647 were lost during follow-up. Thus, a final sample of 674 participants was included in wave 2. However, 23% (95% CI: 19.9-28.3) were excluded from the present analysis because they reported having multimorbidity in wave 1. Consequently, 516 participants were eligible for this study.

The main characteristics of the sample are described in **Table 1**. Most of the participants were women (78.5%; 95% CI: 73.3-80.83), white (90.3%; 95% CI: 86.5-93.2) and lived with a partner (57.2%; 95% CI: 51.5-62.8). Almost half of the participants were aged between 31 and 59 years (49.9%; 95% CI: 44.2-55.6) and 23.5% (95% CI: 19.1-28.6) had at least one academic degree. Comparison of the excluded and lost-to-follow-up sample with the sample included from the second wave showed that none of the variables differed according to the chi-square test.

Overall, 27% (95% CI: 23.5-31.1) of the participants presented with new cases of multimorbidity. The risk of incident multimorbidity according to sociodemographic, behavioral and nutritional characteristics is presented in **Table 2**. Women (aHR: 1.97; 95% CI: 1.19-3.24) and subjects aged 31-59 years (aHR: 1.78; 95% CI: 1.18-2.70) and aged 60 years or over (aHR: 2.41; 95% CI: 1.25-4.61) were more likely to have incident multimorbidity. Even though BMI (obesity) and physical activity showed significant associations with multimorbidity in the crude analysis, no significant results were observed in the adjusted analyses (aHR: 1.54; 95% CI: 0.97-2.44; aHR: 0.68; 95% CI: 0.46-1.01, respectively).

The list of diseases and their frequencies are presented in **Figure 2**. The incidence of multimorbidity was 27.1% (95% CI: 23.5-31.1). The most common new diseases reported between waves 1 and 2 were among those in our initial 12-item list (28.5%). Back pain represented 19.4% of the new cases, followed by high cholesterol (13.3%) and depression (12.2%). There were no new occurrences of memory problems during the pandemic.

DISCUSSION

About one in four participants developed multimorbidity between waves 1 and 2 (six months). Women and adults aged 31 years or

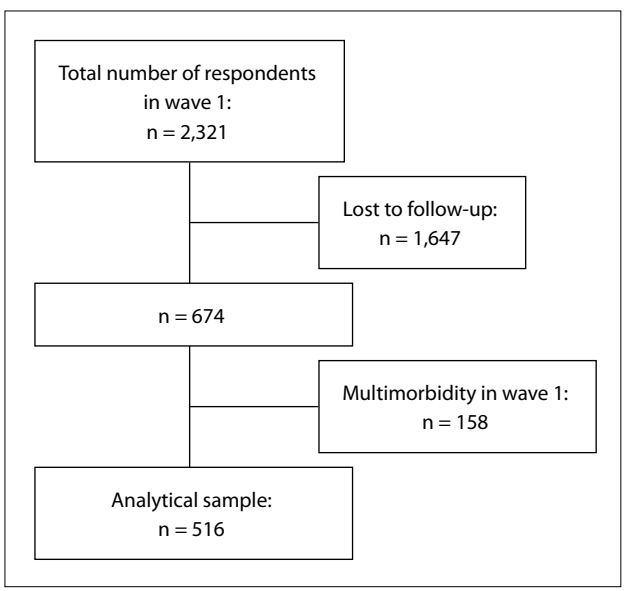
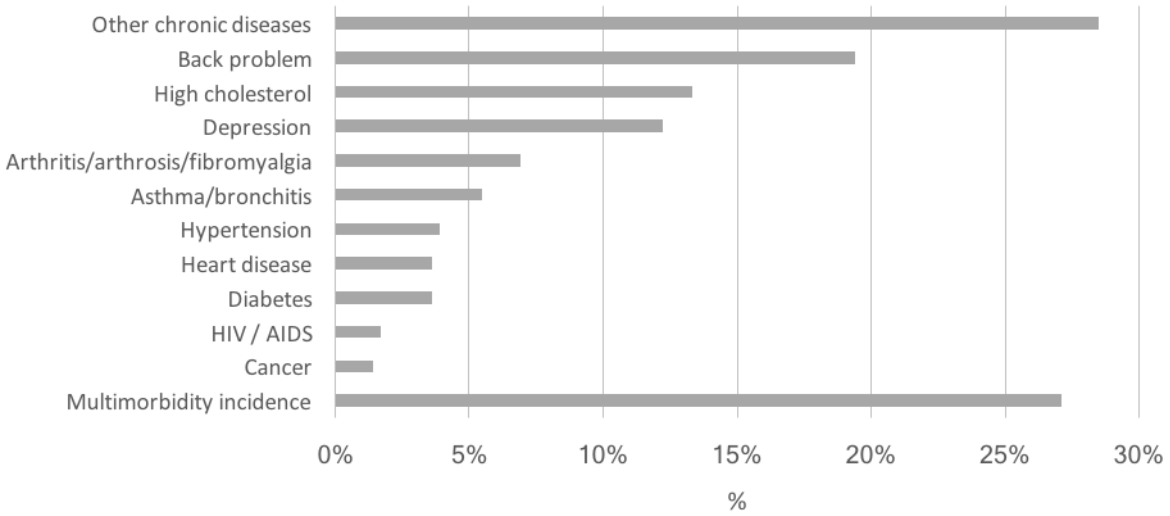


Figure 1. Flow chart describing sampling process.

Table 1. Sociodemographic and behavioral characteristics of the included and lost-to-follow-up participants. Rio Grande do Sul, Brazil (n = 516)

	Included sample %	Lost to follow-up %	P-value
Sex			0.149
Male	21.5	22.7	
Female	78.5	77.3	
Age (years)			0.370
18-30	42.7	28.0	
31-59	49.9	60.0	
60+	7.4	12.0	
Skin color			0.409
White	90.3	87.8	
Black	5.5	7.0	
Mixed	3.7	4.8	
Other	0.04	0.4	
Marital status			0.912
Living with a partner	57.2	64.3	
Living alone	42.8	35.7	
Education level			0.306
High school or lower	33.8	37.4	
University degree	23.5	25.1	
Specialization, Master's, PhD	42.7	37.5	
Decreased monthly income			0.475
No	66.0	53.7	
Yes	34.0	46.3	
Nutritional status			0.820
Normal	51.7	41.2	
Overweight	32.3	35.0	
Obese	16.0	23.7	
Physical activity before COVID-19 pandemic			0.262
Inactive	57.0	58.6	
Active	43.0	41.4	

Based on body mass index, calculated from self-reported height and body weight. COVID-19 = coronavirus disease 2019.



There were no new occurrences of memory problems. HIV = human immunodeficiency virus; AIDS = acquired immunodeficiency syndrome.

Figure 2. Multimorbidity incidence and list of the new diseases reported between the first and second waves.

Table 2. Hazard ratios for multimorbidity according to single lifestyle factors among participants in the Prospective Study About Mental and Physical Health (PAMPA) Cohort, Rio Grande do Sul, Brazil (n = 516)

	Crude hazard ratio (95% CI)	P-value	Adjusted hazard ratio[1] (95% CI)	P-value
Sex		< 0.001*		0.008*
Male	1.00		1.00	
Female	1.85 (1.14; 3.0)		1.97 (1.19; 3.24)	
Age (years)		< 0.001*		< 0.001*
18-30	1.00		1.00	
31-59	1.85 (1.27; 2.68)		1.78 (1.18; 2.70)	
60+	2.42 (1.32; 4.44)		2.41 (1.25; 4.61)	
Skin color		0.907		0.633
White	1.00		1.00	
Mixed	1.5 (0.83; 2.71)		1.82 (0.98; 3.37)	
Black	0.53 (0.17; 1.68)		0.53 (0.17; 1.70)	
Other	3.74 (0.52; 26.8)		4.11 (0.55; 30.7)	
Marital status		0.848		0.208
Living with a partner	1.00		1.00	
Living alone	1.03 (0.74; 1.45)		1.31 (0.92; 1.88)	
Education level		0.161		0.618
High school or lower	1.00		1.00	
University degree	1.11 (0.70; 1.76)		0.99 (0.62; 1.61)	
Specialization, Master's, PhD	1.31 (0.89; 1.95)		1.05 (0.68; 1.64)	
Decreased monthly income		0.736		0.489
No	1.00		1.00	
Yes	0.75 (0.53; 1.06)		0.83 (0.58; 1.19)	
Nutritional status		0.031*		0.081
Normal	1.00		1.00	
Overweight	1.37 (0.94; 1.98)		1.33 (0.90; 1.96)	
Obese	1.57 (1.01; 2.45)		1.54 (0.97; 2.44)	
Physical activity before COVID-19 pandemic		0.020*		0.066
Inactive	1.00		1.00	
Active	0.65 (0.45; 0.95)		0.68 (0.46; 1.01)	

*Statistically significant P-values; [1]Adjusted for sex, age, skin color, conjugal situation, education level, decreased monthly income, body mass index (BMI) and physical activity before coronavirus disease 2019 (COVID-19) pandemic, at baseline. CI = confidence interval.

over showed higher risk of incident multimorbidity. We observed a dose-response relationship between age and the risk of multimorbidity, in which the risk increased with increasing age.

Female sex was associated with higher risk of incident multimorbidity during the period of COVID-19 social distancing, compared with males. Previously, other studies had shown that women were twice as likely to report multimorbidity as were men, both in Brazil and in other countries.[2,4,17,18] Greater frequency of use of healthcare systems through medical appointments among women might explain this relationship. On the other hand, men might be more likely to be underdiagnosed with regard to chronic conditions and multimorbidity.[19] Furthermore, women experience more stressful events throughout their lives[20] and therefore are at higher risk of chronic diseases. Unfortunately, impairment of mental health during social distancing seems to be higher among women than among men.[21] Furthermore, we previously showed that access to the healthcare system during social distancing was more impaired among women.[10] Therefore, strategies to improve healthcare system access are required, in order to monitor the prevalence and incidence of chronic diseases, especially among women.

In Brazil, previous studies also showed that older adults, especially those over 60 years of age, had higher prevalence of multimorbidity than younger adults.[4,18] A systematic review from 2019 showed that individuals aged 65 or more were the most affected by multimorbidity, worldwide.[2] Although population aging might be associated with this increased prevalence, previous studies have suggested other factors, including impaired healthcare access and low social development.[22] For example, among older adults, multimorbidity is influenced by socioeconomic, demographic, lifestyle and family factors.[23] Also, older people became more isolated during the pandemic,[21] and the high number of new cases of depression may have been related to this.

The lack of statistically significant relationships with multimorbidity shown by BMI and physical activity in the adjusted analyses may have been due to the relatively short time between waves 1 and 2 (six months). For physical activity, a previous longitudinal study did not show any association with the development of multimorbidity in short-term follow-ups (two years).[24] However, for longer follow-ups (11 years), physical activity was shown to be protective against development of multimorbidity.[25] A previous study in Finland showed that physical inactivity and obesity were risk factors for incident multimorbidity. However, that study included only five chronic conditions in the multimorbidity list.[26] According to our results, being physically active before the pandemic did not protect against developing multimorbidity during the pandemic. Despite this, physical activity is important and needs to be encouraged. For instance, physical activity in the early ages of childhood plays a protective role against multimorbidity at older ages.[27]

The COVID-19 pandemic affected access to healthcare services, especially for chronic diseases like diabetes, chronic obstructive pulmonary disease and hypertension.[28] Especially in low and middle-income countries, COVID-19 affected healthcare services with consequences ranging from postponement of elective surgery and other medical procedures to delayed treatment of chronic diseases that might progress to severe conditions.[29] Our results reflected those findings, and we demonstrated that the less-reported problems before social distancing, such as back pain and depression, were the ones with higher frequency during wave 2. Moreover, a study conducted in India showed that individuals with multimorbidity experienced several care challenges, including interruption of treatment and routine check-ups during the pandemic.[30]

The increase in new cases of depression observed in our data was in agreement with the recent literature.[31] A sharp increase in the levels of psychological distress was evident during the pandemic, especially among women and adults aged younger than 60.[31] Moreover, a meta-analysis on community-based studies conducted during the COVID-19 pandemic showed that the prevalence of depression was seven times higher than the estimated prevalence in 2017.[32] Thus, strategies to attenuate the detrimental indirect effects of this pandemic on mental health, especially in higher-risk groups, are warranted.

Furthermore, spending more time at home with sedentary activities and less time exercising might explain the high number of spine problems. A large number of cases of back pain occurrences may have resulted from COVID-19, specifically related to the social distancing measures that made many people start working from home without adequate equipment. Previous studies also reported similar findings, thus showing that the social distancing caused by COVID-19 resulted in more occurrences of low back pain and more associated risk factors.[33]

Our study had some methodological limitations. First, we did not include an open question for "other diseases". Thus, it was not possible to identify which other diseases were reported by the participants. Second, our retention rate was lower than expected for face-to-face cohort studies.[12] However, our results are similar to those of other cohort studies that were conducted online.[34] Third, we assessed the list of diseases through self-reports, which is less accurate than use of medical records because it depends on the memory of each participant. However, most other studies also used self-reported data to assess multimorbidity, according to a systematic review conducted in 2019.[2] Fourth, our respondents included a high proportion of individuals with academic degrees. Given that data collection was online, and university professors shared the questionnaire through their academic contacts, a high number of participants with academic degrees was expected. Also, people with less education have less access to the internet. Furthermore, according to previous findings in the literature, from samples with less schooling, the incidence of multimorbidity could have been even higher.[35]

However, we can highlight the longitudinal design of our study evaluating multimorbidity incidence during the pandemic. This

may have generated important information about the development of chronic diseases in this context. Moreover, at the time when we conducted this study, we did not find any other cohort study assessing the incidence of multimorbidity during the COVID-19 pandemic. Our results may provide relevant information about the consequences of the COVID-19 pandemic on the health of the population of southern Brazil. Furthermore, our report provides important information about a new profile of people who will seek healthcare services during and after the pandemic.

CONCLUSION

The incidence of multimorbidity over a six-month period during the COVID-19 pandemic was 27.1% in the state of Rio Grande do Sul, in Brazil. Female sex and increasing age were risk factors for incident multimorbidity. Special attention to the risk that women and older people may develop multimorbidity is needed within public health policies, along with attention to the high numbers of new cases of depression and back pain during the COVID-19 pandemic.

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The impact of multimodality integrated positron emission tomography-computed tomography on improving the staging and management of head and neck malignancy: a cross- sectional study

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ABSTRACT

BACKGROUND: Clinical assessment of head and neck cancers is highly challenging owing to the complexity of regional anatomy and wide range of lesions. The diagnostic evaluation includes detailed physical examination, biopsy and imaging modalities for disease extent and staging. Appropriate imaging is done to enable determination of precise tumor extent and involvement of lymph nodes, and detection of distant metastases and second primary tumors.

OBJECTIVE: To evaluate the initial staging discrepancy between conventional contrasted computed tomography (CT) and 18F-fluorodeoxy-D-glucose positron emission tomography/computed tomography (^{18}F-FDG PET/CT) and its impact on management plans for head and neck malignancies.

DESIGN AND SETTING: Prospective cross-sectional study in two tertiary-level hospitals.

METHODS: This study included 30 patients with primary head and neck malignant tumors who underwent contrasted computed tomography and whole-body ^{18}F-FDG PET/CT assessments. The staging and treatment plans were compared with the incremental information obtained after ^{18}F-FDG PET/CT.

RESULTS: ^{18}F-FDG PET/CT was found to raise the stage in 33.3% of the cases and the treatment intent was altered in 43.3% of them, while there was no management change in the remaining 56.7%. ^{18}F-FDG PET/CT had higher sensitivity (96% versus 89.2%) and accuracy (93% versus 86.7%) than conventional contrast-enhanced computed tomography.

CONCLUSION: Our study demonstrated that ^{18}F-FDG PET/CT had higher sensitivity and accuracy for detecting head and neck malignancy, in comparison with conventional contrast-enhanced computed tomography. ^{18}F-FDG PET/CT improved the initial staging and substantially impacted the management strategy for head and neck malignancies.

INTRODUCTION

Head and neck cancers are the sixth most common type of cancer worldwide and the majority of them cause regional nodal metastases that decrease the chances of survival.[1,2] Head and neck cancer is characterized by high prevalence of nodal metastases at the time of initial presentation.[1-3] A large percentage of these cause regional nodal metastases that decrease the chances of survival by 50%.[3] Accurate timely staging will ensure proper treatment delivery.[4,5] Computed tomography (CT) and magnetic resonance imaging (MRI) are the standard imaging modalities used for the staging evaluation of head and neck cancer in routine clinical practice.[5] However, the limitations of these morphological imaging methods include difficulty in differentiating reactive enlargement and tumor-infiltrated lymph nodes and difficulty in detecting unsuspected distant metastases.[6]

OBJECTIVE

This study was conducted to evaluate the role of 18F-fluorodeoxy-D-glucose positron emission tomography/computed tomography (^{18}F-FDG-PET/CT), in comparison with contrast-enhanced computed tomography (CECT) for management of patients with head and neck cancer.

METHODS

This was a prospective cross-sectional study involving 30 patients who were attended at the otorhinolaryngology clinics of two tertiary-level hospitals in Malaysia after obtaining institutional

ethical approval (NMRR-09-1116-4585; dated July 13, 2010). Informed consent was obtained from all the enrolled patients.

The exclusion criteria were that the subjects should not be children, individuals with acute or chronic inflammatory disease, pregnant patients, lactating mothers, terminally ill patients or patients with any previous malignancy. All the patients selected (above 18 years old) were thoroughly examined by otorhinolaryngology surgeons, and biopsies were taken from suspicious regions.

All the patients underwent CECT and whole body ^{18}F-FDG PET/CT examinations at the hospital's center for diagnostic nuclear imaging, using a standard protocol for image acquisition. Staging of the disease was done based on the 7th edition of the tumor, node and metastasis (TNM) staging system of the American Joint Committee on Cancer (AJCC) after use of both imaging modalities. In addition, the oncologist was asked to outline the management intent for the patients, based on CECT; and to do this again after the positron emission tomography/computed tomography (PET/CT).

The change in management intent and the incremental information obtained after both imaging procedures had been done were compared and analyzed. The percentage of management changes implemented due to discrepancies between the imaging methods was recorded. The clinical impact of PET/CT was considered 'high' if it changed the treatment modality, and 'low' if there was no change in the treatment modality or intent.[7]

All the patients were monitored through regular follow-up at a specialist clinic. The cumulative survival rate among the patients was estimated from the date of diagnosis to the date of death due to any cause or the date of the last follow-up. It was noted whether any patients were lost to follow-up or were still alive at the end of the follow-up period. The five-year overall mean survival rate was calculated, and the mean survival time in months according to sociodemographic characteristics, tumor stages and treatment received was also analyzed.

RESULTS

Out of the 30 patients in this study, 60% (18/30) were male and 40% (12/30) were female. According to ethnicity, the majority were Chinese (56.7%; n = 17), followed by Malay (40%; n = 12) and Indian (3.3%; n = 1). The mean age (with standard deviation, SD) was 49.9 years (± 14.5) with first and second peak age incidences in the age ranges of 30-39 years and 60-69 years, respectively. Nasopharyngeal carcinoma was the commonest malignancy (56.7%), and the next commonest was carcinoma of the larynx and malignancy of the oropharynx (10%). A list of the primary sites of tumors is shown in **Table 1**, while the patients' characteristics, clinical and histopathological diagnosis are presented in **Table 2**.

All of the 30 patients underwent pre-treatment radiological assessment with CECT and ^{18}F-FDG PET/CT for the purpose of disease stratification through the AJCC 7th edition TNM staging, in order to determine the intended management plan. The CECT and ^{18}F-FDG PET/CT findings were classified as true positive (positive imaging study that was confirmed histopathologically), true negative (normal imaging study with no further evidence of cancer), false positive (positive imaging study with no histopathological evidence of cancer) or false negative (normal imaging study with further proven cancer).[8]

Through ^{18}F-FDG PET/CT, it was found that there were 27 true positive cases, one false positive, one false negative case and one true negative case. Three patients who were suspected of having benign lesions following a conventional clinical assessment were proven to be malignant cases after histopathological examination and ^{18}F-FDG PET/CT. Two of these patients were initially diagnosed through conventional staging as having a thyroglossal cyst, but malignancy was proven through ^{18}F-FDG PET/CT. This was subsequently confirmed to be papillary carcinoma, by means of histopathological examination. One of our patients initially presented with clinical and CECT features suggestive of mastoiditis and later developed widespread lesions over various sites that were positive through ^{18}F-FDG PET/CT. Histopathological examination confirmed this case as metastatic adenocarcinoma. The false positive case was a patient with a parotid lesion that was positive through ^{18}F-FDG PET/CT, but histopathological examination revealed this to be oncocytoma.

In our study, ^{18}F-FDG PET/CT imaging accurately identified the extent of primary tumors. Thus, the tumor (T) staging changed in five patients. PET/CT imaging also correctly detected the lymph nodes and changed the node (N) staging in three patients. In this manner, ^{18}F-FDG PET/CT raised the staging of 33.3% of the cases (n = 10), while 16.6% (5/30) showed changes in T-staging and metastasis (M) staging, and 10.0% (3/30) showed changes in N-staging.

The treatment plans were altered in the cases of 43.3% (13/30) of our study group patients, while there was no management change in

Table 1. List of primary sites of tumors

Primary site	Number of cases	Percentage
Nasopharyngeal carcinoma	17	56.6
Carcinoma of thyroid	2	6.7
Carcinoma of larynx	2	6.7
Carcinoma of unknown primary origin (CUP)	1	3.3
Lymphoma	1	3.3
Carcinoma of hard palate	1	3.3
Carcinoma of base of skull	1	3.3
Carcinoma of tonsils	1	3.3
Sarcoma of tonsils	1	3.3
Adenocarcinoma of base of skull	1	3.3
Others	2	6.7

the remaining 56.7% (17/30). 46.6% (14/30) of the patients showed stage migration, i.e., for 43.3% (13/30) the staging increased; and for 3.3% (1/30) the staging decreased. The management intent based on CECT and the changes after ^{18}F-FDG PET/CT are shown in **Table 3**.

Among the patients whose staging increased, 30% (3/10) of them benefited from addition of neoadjuvant chemotherapy. Distant metastases were identified in six patients and the management plans were changed from definitive to palliative intent. One of our patients with carcinoma oropharynx, for whom oncological surgery with radiotherapy had been planned, was found through ^{18}F-FDG/PET/CT to be developing lung metastases, which was confirmed through cytological tests. The management plan was therefore changed to palliative therapy. Another patient with metastatic adenocarcinoma of the skull also had a change in the treatment plan to palliative intent. Among the remaining four patients, who had nasopharyngeal carcinoma (NPC), two presented skeletal metastases and the other two were seen to have mediastinal nodal metastases.

^{18}F-FDG PET/CT decreased the staging of 6.6% of the patients (n = 2). One of these cases consisted of postoperative tonsillar sarcoma, in which there were low-activity lesions in distorted anatomy, which reflected the post-surgical change. The other case was incorrectly diagnosed as parotid lymphoma, and the final histopathological diagnosis was benign oncocytoma (**Figure 1**).

The influence of ^{18}F-FDG PET/CT on stage migration and its impact on management intent are shown in **Table 3**. The clinical accuracy of ^{18}F-FDG PET/CT for managing patients with head and neck cancers was derived from contingency tables.

The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of ^{18}F-FDG PET/CT were 96%, 50%, 96% and 50%, respectively. The accuracy of ^{18}F-FDG PET/CT for clinical evaluation of head and neck cancers was 93%. On the other hand, the sensitivity, specificity, PPV and NPV of CECT assessment were 89.2%, 50%, 96.1% and 25%. The accuracy of CECT assessment for detecting head and neck cancer was 86.7%. ^{18}F-FDG PET/CT improved the sensitivity and accuracy of detection of head and neck malignancy, in comparison with CECT

Table 2. Patient characteristics and clinical and histopathological diagnoses

Patient No.	Gender	Age	Clinical diagnosis/stage	Histopathological diagnosis	Follow-up
1	M	63	Thyroglossal cyst	Papillary carcinoma of thyroid	Alive
2	M	44	NPC/stage IV B	NPC	Lost
3	M	60	Carcinoma larynx/stage I	Carcinoma of larynx	Alive
4	F	60	Lymphoma of parotid	Oncocytoma of parotid	Alive
5	M	38	Lymphoma of tonsils	Sarcoma of tonsils	Alive
6	F	31	Benign cyst	Papillary carcinoma of thyroid	Alive
7	M	44	Benign nodule	Occult neck node carcinoma TxN1M0	Alive
8	F	73	Adnexal tumor/stage I	Adnexal carcinoma	Alive
9	F	62	Carcinoma of larynx/stage I	Carcinoma of larynx	Alive
10	F	56	NPC/stage III	NPC	Dead
11	M	50	NPC/stage II	NPC	Dead
12	M	70	NPC/stage I	NPC	Dead
13	M	39	NPC/stage III	NPC	Alive
14	M	37	Carcinoma of tonsils/stage IV B	Carcinoma of tonsils	Alive
15	M	63	Lymphoma/stage I	Lymphoma	Dead
16	M	22	NPC/stage II	NPC	Alive
17	F	68	Mastoiditis	Metastatic adenocarcinoma	Dead
18	F	63	Carcinoma of oropharynx/stage II	Carcinoma of oropharynx	Dead
19	M	65	NPC/stage III	NPC	Dead
20	M	46	NPC/stage III	NPC	Dead
21	M	42	NPC/stage IV B	NPC	Alive
22	F	55	NPC/stage III	NPC	Dead
23	M	54	NPC/stage III	NPC	Dead
24	F	33	NPC/stage II	NPC	Alive
25	M	39	NPC/stage II	NPC	Alive
26	M	41	NPC/stage III	NPC	Alive
27	F	33	NPC/stage II	NPC	Lost
28	F	34	NPC/stage IV B	NPC	Lost
29	M	50	Carcinoma of larynx T2N0M0/stage II	Carcinoma of larynx	Alive
30	F	26	NPC/stage III	NPC	Lost

NPC = nasopharyngeal carcinoma; CT = computed tomography; PET/CT = positron emission tomography/computed tomography; M = male; F = female.

Table 3. Management intent based on CT and after PET-CT, and impact of PET-CT findings on management intent

Patient No.	Diagnosis from CT staging (pre-PET/CT)	Management intent after CT	Diagnosis after PET/CT staging	Management intent after PET/CT	Impact of PET/CT on management intent
1	Thyroglossal cyst	Excision of cyst	Papillary carcinoma T1N0M0/stage II	Thyroidectomy and radioiodine therapy	High
2	NPC T1N3M0/stage IV B	3 cycles of neoadj Ct and CtRT	NPC T1N3bM0/stage IV B	3 cycles of neoadj CT then CtRT	Low
3	Carcinoma of larynx T1aN0M0/stage I	RT	T1aN0M0/stage I	RT	Low
4	Parotid tumor	Chemotherapy	Lymphoma of parotid	Surgery	High
5	Malignant tumor of tonsils T2N0M0/stage II	Surgery	Sarcoma of tonsils T2N0M0/stage II	Surgery	Low
6	Benign cyst	Excision of cyst	Papillary carcinoma T1N1bM0/stage I	Thyroidectomy and radioiodine therapy	High
7	Benign nodule	Excision	Occult neck node carcinoma TxN1M0	Neck dissection	Low
8	Adnexal tumor TIN0M0/ stage I	Excision	Adnexal carcinoma TIN0M0/stage I	Excision	Low
9	Carcinoma of larynx T1N0M0/stage I	RT	T1N0M0/stage I	RT	Low
10	NPC T1N2M0/stage III	CtRT	T1N3bM0/stage IV B	3 cycles of neoadj Ct and CtRT	High
11	NPC T2N1M0/stage II	CtRT	T4N1M0/stage IV A	3 cycles of neoadj Ct and CtRT	High
12	NPC T1N0M0/stage I	RT	T1N0M0/stage I	RT	Low
13	NPC T2N2M0/stage III	CtRT	T4N2M0/stage IV B	3 cycles of neoadj Ct and CtRT	High
14	Carcinoma of tonsils T2N3M0/stage IV B	Surgery and RT	T2N3M0/stage IV B	Surgery and RT	Low
15	Lymphoma/stage 1	Ct	Stage 3	Ct	Low
16	NPC T1N1M0/stage II	CtRT	T1N1M0/stage II	CtRT	Low
17	Mastoiditis	Surgery	Metastatic adenocarcinoma of base of skull T4N0M1	Palliative therapy	High
18	Carcinoma of oropharynx T2N1M0/stage II	Surgery and RT	T3N2M1/stage IV C	Palliative therapy	High
19	NPC T3N2M0/stage III	CtRT	T3N3M0/stage IV B	3 cycles of neoadj Ct then CtRT	High
20	NPC T3N0M0/stage III	CtRT	T4N0M1/stage IV C	Palliative therapy (6 cycles of Ct)	High
21	NPC T3N3M0/stage IV B	3 cycles of neoadj Ct then CtRT	T4N3M0/stage IV B	3 cycles of neoadj Ct then CtRT	Low
22	NPC T3N0M0/stage III	CtRT	T4N0M1/stage IV C	Palliative therapy (6 cycles of Ct)	High
23	NPC T2N2M0/stage III	CtRT	T2N2M1/stage IV C	Palliative therapy (6 cycles of Ct)	High
24	NPC T1N1M0/stage II	CtRT	T1N2M0/stage III	CtRT	Low
25	NPC T2N0M0/stage II	CtRT	T2N0M0/stage II	CtRT	Low
26	NPC T3N0M0/stage III	CtRT	T3N0M0/stage III	CtRT	Low
27	NPC T2N0M0/stage II	CtRT	T2N0M0/stage II	CtRT	Low
28	NPC T3N3M0/stage IV B	3 cycles of neoadj Ct then CtRT	T3N3M1/stage IV C	Palliative therapy (6 cycles of Ct)	High
29	Carcinoma of larynx T2N0M0/stage II	RT	T2N0M0/stage II	RT	Low
30	NPC T2N2M0/stage III	CtRT	T2N2M0/stage III	CtRT	Low

NPC = nasopharyngeal carcinoma; CT = computed tomography; PET/CT = positron emission tomography/computed tomography; RT = radiotherapy; Ct = chemotherapy; neoadj = neoadjuvant; CtRT = chemoradiotherapy.

assessment, to 96% and 93% from 89.2% and 86.7%, respectively. The negative predictive value from the CT assessment was lower than the NPV from PET/CT imaging.

After a period of five years, we performed a search in the patients' records at the specialist clinic. We found that 10 patients had died, 16 (53.3%) were survivors and four had been lost in the follow-up. The surviving patients had received radiotherapy alone and/or in combination with chemotherapy elsewhere and had returned. Nasopharyngeal carcinoma was the diagnosis for all the four lost patients. In the group of NPC patients (n = 17), seven patients died, while the remaining six survived. Among the dead patients, PET-CT raised the staging with regard to T-staging (n = 3), N-staging (n = 2) and M-staging (n = 3). Among the survivors, PET-CT also raised the T-staging (n = 1), N-staging (n = 3) and M-staging (n = 2). From the records of the dead patients (n = 10), seven had been diagnosed with NPC, one with lymphoma, one with metastatic adenocarcinoma at the base of the skull and one with carcinoma of the hard palate. ^{18}F-FDG-PET/CT increased the T-staging in five patients, the N-staging in three patients and the M-staging in five patients, while the staging of the patient with lymphoma was increased from stage I to stage III. Among the survivors (n = 16), PET-CT changed the TNM staging of nine patients through increasing the T-staging (n = 3), N-staging (n = 4) and M staging (n = 2). There was no change in the clinical staging of the remaining seven survivors. The estimated overall mean survival after diagnosis was 43.6 months (95% confidence interval, CI = 35.2-51.9). The survival rate diminished from 86.7% during the first six months to 66.7% by the 60th month of the study (**Table 4**).

Mean survival time in months according to sociodemographic characteristics, tumor stages and treatment received was analyzed and is presented in **Table 5**.

For all of our patients with head and neck malignancies, the Kaplan-Meier estimate for mean survival time (with standard error) for those age less than 65 years old was 46.6 (4.3) [95% CI = 38.1-55.1]; while for those aged 65 years and over, the estimate was 24.0 (11.0) [95% CI = 2.4-45.6]. The log-rank test revealed a statistically significant difference between the survival rates over time (P = 0.026). It was found that the mean survival time (with standard error) of the patients who received definitive treatment (surgery alone, surgery with radiotherapy, radiotherapy alone and radiotherapy with chemotherapy) was 50.3 (3.9) [95% CI = 42.6-57.9]. On the other hand, those who received palliative treatment had mean survival (with standard error) of 17.0 (7.9) [95% CI = 1.5-32.5]. This difference was statistically significant, with a P-value of 0.001.

DISCUSSION

Head and neck cancers encompass a heterogeneous group of tumors that are a biologically aggressive and therapeutically challenging category of disease.[7-9] The appropriate management decision for this complex form of cancer is based on the primary site, histological subtype, stage, resectability, patient's fitness and treatment preference.[10] Accurate staging is crucial for selection of the appropriate treatment modality in individual patients.

CT and MRI are widely used as the first-line imaging approach for staging of head and neck cancer. Both of these imaging modalities rely on morphological criteria like size and contrast enhancement patterns, which are not particularly specific for detection of metastases.[9,11] ^{18}F-FDG PET/CT has been shown to yield promising

Table 4. Estimated cumulative survival rate among the patients

Time (months)	Estimated cumulative survival rate
6	86.7
12	70.0
24	66.7
60	66.7

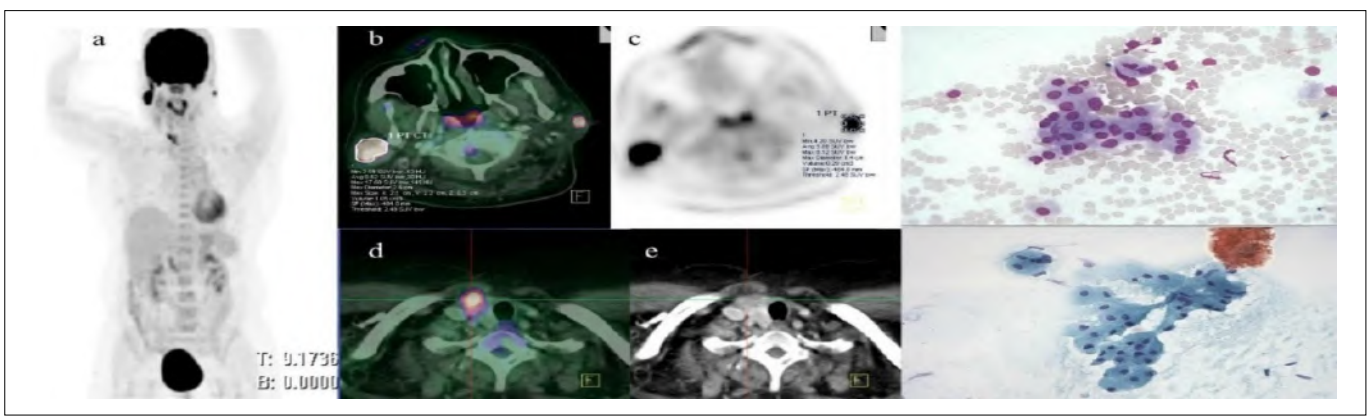

Figure 1. ^{18}F-fluorodeoxy-D-glucose positron emission tomography/computed tomography incorrectly diagnosed as malignancy of parotid in this 60-year-old woman who presented with progressively increasing parotid swelling for a duration of six months. The final diagnosis was Birt-Hogg-Dubé syndrome with benign oncocytoma of the parotid.

results for diagnosing and staging of head and neck squamous cell carcinoma, compared with standard imaging modalities.[12]

In our study, we sought to prospectively evaluate the influence of ^{18}F-FDG PET/CT on the initial staging and its impact on the treatment plan for head and neck cancer patients. The data for this study consisted of information from our own patients, in contrast with the data in other, multicenter studies, which relied on medical records. The majority of our study patients (56.7%) were diagnosed with nasopharyngeal carcinoma, which was in accordance with the epidemiological pattern of head and neck cancers in Malaysia. Nasopharyngeal carcinoma is one of the ten most common cancers in this multiracial Southeast Asian country.

Our results demonstrated that ^{18}F-FDG PET/CT significantly changed the overall multidisciplinary team decision regarding treatment intent, compared with clinical conventional CT staging. These changes to staging caused significant reclassification of patients' treatment decisions and their overall survival prognosis. The impact of ^{18}F-FDG PET/CT on the treatment decision was mainly due to the improvement in the accuracy of staging.

The data from our study demonstrated that ^{18}F-FDG PET/CT raised the staging in the cases of 33.3% (n = 10) of the patients. These data were in accordance with the findings from various published studies, which demonstrated management changes in the cases of 31-34% of head and neck cancer patients.[8,13–15] Meanwhile, the T-staging in our study cohort was changed in 16.6% (5/30) of the patients. In a study by Antoch et al., the T-staging was accurately determined in 82% of the cases, through use of fused PET/CT.[16] PET/CT can reveal the full tumor extent, even when a tumor is ill-defined with submucosal extent and diffuse infiltration. A study by Tantiwongkosi et al. showed that PET/CT could be helpful in identifying subtle but focally hypermetabolic NPC, when the CT and MRI findings are not obvious.[17]

Precise detection of cervical lymph node metastases is crucial for planning the surgical margins and radiotherapy.[18] According to our study, the N-staging was changed in 10% of the cases. A meta-analysis by Sun et al. showed that ^{18}F-FDG PET/CT had good diagnostic performance, compared with conventional imaging, for detection of regional nodal metastases.[19]

Our study supports the notion that ^{18}F-FDG PET/CT is effective in detecting distant metastases. Notably, 26.6% of the study patients had metastases and modification of M-staging. This modification was due to the higher sensitivity of ^{18}F-FDG PET/CT for detecting certain subtle lesions, which can be missed through conventional imaging or a single-stop modality with whole-body coverage.

PET/CT accurately detected skeletal metastases in two of our NPC patients. Three patients of our study group were found through PET/CT to be developing mediastinal nodal metastases. Another patient was seen to have widespread metastases in various organs. The treatment for all these patients with distant metastases was subsequently revised to palliative intent. Head and neck cancer patients with distant metastases are not considered curable, and most cases lead to palliative treatment strategies.[20] Therefore, detection of

Table 5. Mean survival time in months according to sociodemographic characteristics, tumor stages and treatment received

Factors relating to survival	Mean				P
	Estimate	Standard error	95% confidence interval		
			Lower bound	Upper bound	
Race					
Malay	48.500	5.851	37.033	51.455	
Chinese	42.000	5.927	30.383	59.937	0.09
Indian	12.000	0.000	12.000	12.000	0.327
Overall	43.600	4.270	35.230	51.970	
Gender					
Male	44.000	5.395	33.425	54.575	
Female	43.000	6.958	29.362	56.638	0.947
Overall	43.600	4.270	35.230	51.970	
Age group					
Less than 65 years	46.615	4.333	38.122	55.109	
Greater than or	11.023	11.023	2.396	45.604	0.026
equal to 65	43.000	4.270	35.230	51.970	
Tumor stage					
T0_T2	51.000	8.216	34.897	67.103	0.365
T3_T4	40.957	5.000	31.157	50.756	
Treatment					
Curative	50.250	3.918	42.571	57.929	
Palliative	17.000	7.927	1.464	32.536	0.001
Overall	43.600	4.270	35.230	51.970	

distant metastases is important because this avoids unnecessary or inappropriate treatment. ^{18}F-FDG PET/CT imaging may prevent unnecessary surgery in some patients, in whom this would have been associated with high morbidity and functional impairment, through identifying locoregional and distant metastases.[21]

The treatment plans were changed in 43.3% (13/30) of our patients, while no management change was made in the cases of the remaining 56.7%. Our study results showed changes that were similar to what was observed by Veit-Haibach et al.[22] In that study, the accuracies of TNM staging using PET/CT and CT were compared, and it was found that staging based on PET/CT imaging changed the therapy for 42% (13/31) of the patients, compared with therapy based only on CT.[22] In another study by El-Khodary et al., treatment changes were made in the cases of 41.7% of the patients.[9]

A variety of changes to treatment were made among our patients. These included addition of chemotherapy or radiotherapy and abandonment of localized surgery and radiotherapy with curative intent, which was replaced by treatment with palliative intent. The aim of chemotherapy was shifted from curative to palliative intent in 20% (6/30) of our patients. These patients were in a group at an advanced stage with presence of distant metastases. Our data were found to be consistent with the findings of previously published studies.[13,23-25]

In the present study, ^{18}F-FDG PET/CT was found to have improved sensitivity and accuracy for detecting head and neck malignancy, in comparison to conventional CECT. The sensitivity, specificity, PPV, NPV and accuracy of ^{18}F-FDG PET/CT were reported to be 96%, 50%, 96%, 50% and 93% respectively. This was comparable to the study published by Gordin et al in 2007.[26]

During the follow-up of our study group patients, we found that 10 patients had passed away. These patients' treatments were therefore reclassified from having curative to having palliative intent. This notably strengthens the argument that PET/CT has a major incremental impact with regard to identifying high-risk patients who do not benefit from aggressive curative treatment.

In interpreting ^{18}F-FDG PET/CT imaging, the challenges include physiological uptake of fluorodeoxy-D-glucose (FDG) by normal tissues, false positive results due to inflammation, limited resolution of small lesions and motion artefacts.[27,28] Cost-effectiveness is the major consideration in deciding whether to use of ^{18}F-FDG PET/CT as part of the initial imaging. Its cost needs to be weighed against the benefit of early detection of distant metastases, synchronous primary and resulting interventions.[29]

Our study had several limitations. The majority of our study cohort were NPC patients, which might have introduced a working bias. Moreover, our sample consisted of a small number of patients with head and neck cancers at different sites. Because of these limiting factors, the results from our study focused mainly on nasopharyngeal carcinoma and may not have reflected the situation regarding other head and neck cancers. Further prospective studies comprising larger patient cohorts are required in order to ascertain the impact of ^{18}F-FDG PET/CT on the management of various head and neck malignancies.

CONCLUSION

In conclusion, our study demonstrated that ^{18}F-FDG PET/CT had higher sensitivity and accuracy for detecting head and neck malignancy than those of conventional CECT. ^{18}F-FDG PET/CT provides additional information and accurate staging, which assist in planning for adequate treatment and in minimizing treatment-related toxicity and functional impairment. From our study findings, we would advocate for incorporation of ^{18}F-FDG PET/CT into the initial staging of clinically advanced head and neck malignancy.

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Effect of liberal or conservative oxygen therapy on the prognosis for mechanically ventilated intensive care unit patients: a meta-analysis

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AUTHORS' KEY WORDS:
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Critically ill.
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ABSTRACT

BACKGROUND: For critically ill patients, physicians tend to administer sufficient or even excessive oxygen to maintain oxygen saturation at a high level. However, the credibility of the evidence for this practice is unclear.

OBJECTIVE: To determine the effects of different oxygen therapy strategies on the outcomes of mechanically ventilated intensive care unit (ICU) patients.

DESIGN AND SETTING: Systematic review of the literature and meta-analysis conducted at Jiangxi Provincial People's Hospital, Affiliated to Nanchang University, Nanchang, China.

METHODS: We systematically searched electronic databases such as PubMed and Embase for relevant articles and performed meta-analyses on the effects of different oxygen therapy strategies on the outcomes of mechanically ventilated ICU patients.

RESULTS: A total of 1802 patients from five studies were included. There were equal numbers of patients in the conservative and liberal groups (n = 910 in each group). There was no significant difference between the conservative and liberal groups with regard to 28-day mortality (risk ratio, RR = 0.88; 95% confidence interval, CI = 0.59-1.32; P = 0.55; $I^2 = 63\%$). Ninety-day mortality, infection rates, ICU length of stay, mechanical ventilation-free days up to day 28 and vasopressor-free days up to day 28 were comparable between the two strategies.

CONCLUSIONS: It is not necessary to use liberal oxygen therapy strategies to pursue a higher level of peripheral oxygen saturation for mechanically ventilated ICU patients. Conservative oxygen therapy was not associated with any statistically significant reduction in mortality.

INTRODUCTION

Mechanical ventilation (MV) is a common support intervention in intensive care units (ICUs). More than half of ICU patients receive mechanical ventilation on admission.[1] It has been estimated that 2-3 million ICU patients receive MV annually around the world.[2,3] Respiratory failure is the main indication for MV among ICU patients.[4] Oxygen therapy is an important treatment for these patients.

Myocardial hypoxia was first identified as being responsible for angina in 1928.[5] Oxygen therapy, a harmless, potentially beneficial therapeutic modality, is becoming increasingly used in clinical practice. In traditionally liberal oxygen therapy, most patients are given oxygen exceeding the physiological level because of fear of tissue hypoxia.[6,7] Some patients, even without hypoxemia, are given oxygen therapy prophylactically for prevention of tissue hypoxia. A large population of mechanically ventilated ICU patients is exposed to hyperoxia.[8] When arterial oxygen partial pressure is on the flat part of the oxygen hemoglobin dissociation curve, high concentrations of oxygen do not increase oxygen delivery significantly, even if these can increase the partial pressure of oxygen markedly, according to the characteristics of oxygen hemoglobin dissociation.[9]

Hyperoxia can also cause potential harm to patients.[10] It can lead to lung interstitial fibrosis, tracheobronchitis, alveolar protein leakage, neutrophil infiltration,[11-13] impaired immune function,[14] increased vascular resistance, reduced cardiac output[15] and large quantities of free radicals.[16] In view of this, it has been proposed that conservative oxygen therapy strategy should be used[17] in order to avoid unnecessary hyperoxia while ensuring oxygen delivery. Several studies have indicated that conservative oxygen therapy improves the prognosis for ischemic stroke and myocardial infarction.[18,19]

Despite this, the guidelines available regarding oxygen therapy standards and targets are contradictory and inconsistent.[20-22] Studies on this topic have evaluated the effects of different oxygen therapy strategies on the prognosis for mechanically ventilated patients. However, the conclusions that they reached have not been completely coherent.[17,23-26] Therefore, we decided to conduct a secondary analysis.

OBJECTIVE

We performed a systematic review of the literature to determine the effects of different oxygen therapy strategies on the outcomes of mechanically ventilated intensive care unit (ICU) patients.

METHODS

We followed the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) statement[27] and the Cochrane Handbook[28] for the design, method and presentation of the results of this systematic review and meta-analysis.

Database search

In this systematic review and meta-analysis, we searched the PubMed, Embase, Cochrane Library and Web of Science databases. The following keywords were used for the search: "oxygen inhalation therapy", "liberal*", "conservative*", "conventional*", "respiration", "artificial" and "mechanical ventilation". We set the publication type to clinical trial only, and the publication language was limited to English. We searched for related literature from the time of database inception up to and including July 25, 2021. The search strategy is presented in **Appendix 1**.

Study selection

Two authors independently assessed all titles and abstracts for inclusion and then assessed the full texts of the studies considered.

The studies included had to satisfy the following criteria.

1. The trial needed to have been designed as a clinical control study.
2. The study subjects needed to be adult patients (aged > 18 years) requiring MV.
3. The studies needed to compare liberal and conservative oxygen therapies. We defined conservative oxygen therapy as having a target blood oxygen saturation of 90%-97%.[29,30] The treatment arm (liberal oxygen therapy) was defined as having a higher oxygen target, measured through any of the following: fraction of inspired oxygen (FiO_2), arterial partial pressure of oxygen (PaO_2), arterial oxygen saturation or peripheral oxygen saturation (SpO_2).
4. The all-cause mortality and number of deaths during the follow-up period needed to be reported in the results. We excluded studies on patients younger than 18 years or patients who were pregnant, along with studies limited to patients with chronic respiratory diseases or psychiatric diseases, patients on extracorporeal life support or patients treated with hyperbaric oxygen therapy or elective surgery. Observational and preclinical studies were also excluded.

Outcomes

The primary outcome of interest in the current analysis was 28-day mortality. The secondary outcomes analyzed included 90-day mortality, the rate of new infections, ICU length of stay, mechanical ventilation-free time within 28 days and vasopressor-free time within 28 days.

Data extraction and quality evaluation

Two authors independently screened the studies, extracted data and conducted quality assessments. When agreement could not be reached, the first two authors discussed the decision to include or exclude studies, until an agreement was reached. Two authors extracted and recorded the authors, publication year, study design, participants and population, demographic characteristics, baseline characteristics, details of intervention treatment (oxygen therapy), outcome measurements and results from each enrolled study. The risk of bias in the studies included was evaluated in accordance with the Cochrane risk of bias tool.[31] The following characteristics were assessed: random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessments, incomplete outcome data, selective reporting and other bias. For each characteristic, the risk of bias was rated as low, high or unclear (in cases in which there were insufficient details). Two authors independently assessed the study quality, and disagreements were resolved via discussion.

Statistical analyses

The statistical analysis was accomplished using the Cochrane systematic review software: Review Manager (RevMan) [Computer program], version 5.3 (The Nordic Cochrane Centre: Copenhagen, Denmark). Measurement data were expressed as means and standard deviations and 95% confidence intervals (95% CIs). Enumeration data were expressed as risk ratios (RRs) and 95% CIs. Assessment of heterogeneity was completed using the chi-square test. The I^2 statistic was used in order to determine the degree of heterogeneity. If the heterogeneity was determined to be low or moderate ($I^2 < 50\%$; $P < 0.1$), the fixed-effect model was applied. Otherwise, the random-effects model was used. In the presence of heterogeneity, to eliminate the influence of individual studies, especially small-sample and low-quality studies, leave-one-out sensitivity analysis was conducted.

RESULTS

Studies retrieved and included

We identified 200 studies from PubMed, Embase, Cochrane Library and Web of Science. After screening the titles and abstracts, 16 studies were included for full-text review. In three of the studies, some patients were not mechanically ventilated. In two studies, the number of deaths and mortality rate were not reported. In three other studies, oxygen therapy strategies could not be classified. In the end, four randomized controlled trials (RCTs) and one cohort study[17,23-26] were included in the meta-analysis (**Figure 1**).

Study characteristics and quality evaluation

The main characteristics of the eligible RCTs and cohort study are shown in **Table 1**. Five studies and 1806 mechanically ventilated ICU patients were included in the meta-analysis. The quality of the studies included in this meta-analysis was medium. The quality of the studies included, as assessed using the Cochrane risk-of-bias tool is shown in **Figure 2**. Because the interventions needed the cooperation of doctors, there was a lack of use of blinding methods. As such, there may have been some bias during implementation of the interventions.

Outcomes

Primary outcomes

Short-term mortality is shown in **Figure 3**. Three studies[17,25,26] provided data regarding 28-day mortality. Since there was high heterogeneity among the studies (P = 0.07; I^2 = 63%), the random-effects model was adopted. The result showed that there was no statistical significance in 28-day mortality between the conservative and liberal groups (RR = 0.88; 95% CI = 0.59-1.32, P = 0.55). Sensitivity analysis was performed to evaluate the effect of a single study on the overall estimate by sequentially excluding each study. The heterogeneity decreased significantly (I^2 = 24%; P = 0.25). After excluding one of the studies[25] and making adjustments, oxygen therapy strategy was found to be significantly associated with 28-day mortality, such that the conservative group performed better than the liberal group (RR = 0.78; 95% CI = 0.63-0.98; P = 0.03) (**Figure 4**).

Secondary outcomes

Medium-term mortality is shown in **Figure 5**. Four studies[23-26] provided data regarding 90-day mortality. As there was high heterogeneity among the studies (P = 0.1; I^2 = 53%), the random-effects model was adopted. The result showed that there was no

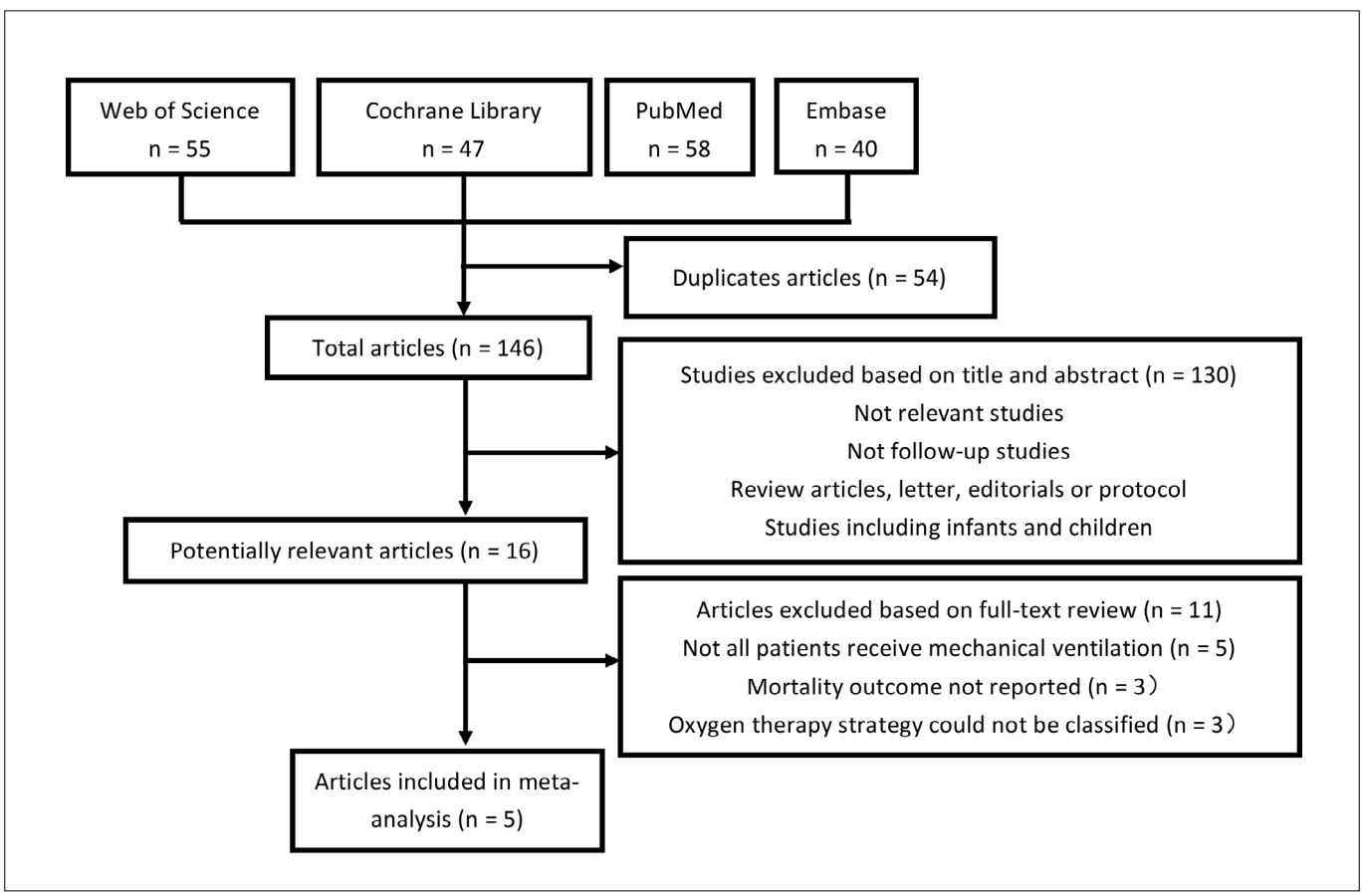


Figure 1. Flow diagram.

statistically significant difference in 90-day mortality between the conservative group and the liberal group (RR = 0.98; 95% CI = 0.85-1.44; P = 0.82).

New infections are shown in **Figure 6**. Three studies[17,25,26] provided data regarding the rate of new infections. Since there was no significant heterogeneity among the studies (P = 0.28; I^2 = 22%), the fixed-effect model was adopted. The result showed that there was no statistically significant difference in the rate of new infections between the conservative and liberal groups (RR = 0.91; 95% CI = 073-1.13; P = 0.73).

ICU length of stay is shown in **Figure 7**. Two studies[23,26] provided data regarding ICU length of stay. As there was no significant heterogeneity among the studies (P = 0.18; I^2 = 45%), the fixed-effect model was adopted. The result showed that there was no statistically significant difference in the ICU length of stay between the conservative and liberal groups (mean difference, MD = 0.15; 95% CI = -1.52-1.81; P = 0.86).

The mechanical ventilation-free time within 28 days is shown in **Figure 8**. Three studies[23,24,26] provided data regarding the mechanical ventilation-free time within 28 days. Since there was no significant heterogeneity among the studies (P = 0.18; I^2 = 42%), the fixed-effect model was adopted. The result showed that there was no statistically significant difference in mechanical ventilation-free

Table 1. Characteristics of the studies included

Study	Design	Characteristics									
		Conservative group					Liberal group				
		Sample size, n	Mean age, years	Men, n (%)	PaO_2/FiO_2, mean (SD)	APACHE III score mean (SD)	Sample size, n	Mean age, years	Men, n (%)	PaO_2/FiO_2, mean (SD)	APACHE III score mean (SD)
Asfar et al.[26]	Randomized controlled trial	217	66·3	140 (65%)	228 (103)	N/A	217	67·8	137 (63%)	220 (103)	N/A
Barrot et al.[25]	Randomized controlled trial	99	63.0	65 (65.7%)	116.8 (47.4)	66.9 (13.7)	102	63.5	64 (62.7%)	120.1 (53.6)	67.9 (14.4)
Mackle et al.[24]	Randomized controlled trial	479	58.1	306 (63.2%)	259 (146)	N/A	480	57.5	302 (62.8%)	245 (138)	N/A
Panwar et al.[23]	Randomized controlled trial	52	62.4	32 (62%)	248 (112)	77.5 (23.6)*	51	62.4	33 (65%)	247 (113)	67.9 (25.6)*
Suzuki et al.[17]	Cohort study	54	54	32 (59%)	302 (142)	68 (32)*	51	51	38 (75%)	278 (137)	68 (39)*

Study	Participants	Interventions	Changes in SpO_2, PaO_2 after Interventions			
			Conservative group		Liberal group	
			SpO_2 mean (SD), %	PaO_2 mean (SD), mmHG	SpO_2 mean (SD), %	PaO_2 mean (SD), mmHG
Asfar et al.[26]	Septic shock patients receiving mechanical ventilation in the ICU	Normoxia group: Target oxygen saturation 88%-95% Hyperoxia group: Mechanical ventilation with FiO_2 of 1.0 for 24 h after inclusion. Thereafter target as in the normoxia group	N/A	N/A	N/A	N/A
Barrot et al.[25]	ARDS patients receiving mechanical ventilation in the ICU	Conservative-oxygen group: Target oxygen saturation 88%-92% Liberal-oxygen group: The SpO_2 was maintained at a level of at least 96%	93.16 (0.45)*	71.5 (3.05)*	97.22 (0.45)*	101.9 (4.47)*
Mackle et al.[24]	Patients receiving mechanical ventilation in the ICU	Conservative-oxygen group: The SpO_2 was maintained between 90% and 97% Liberal-oxygen group: no restrictions	N/A	N/A	N/A	N/A
Panwar et al.[23]	Patients requiring invasive mechanical ventilation in the ICU	Conservative-oxygen group: Target SpO_2 of 88-92% Liberal-oxygen group: Target SpO_2 of ≥ 96%	93.5 (0.45)	70 (2.5)	96.8 (0.5)	92 (3.5)
Suzuki et al.[17]	Patients receiving mechanical ventilation in the ICU	Conservative oxygen therapy: The SpO_2 was maintained between 94% and 97% Conventional oxygen therapy: The SpO_2 was maintained between 97% and 99%	95.4 (0.48)*	82.5 (9.5)*	97.8 (0.31) *	106.8 (8.5)*

ARDS = acute respiratory distress syndrome; ICU = intensive care unit; SpO_2 = arterial saturation of peripheral oxygen; PaO_2 = arterial partial pressure of oxygen; SD = standard deviation; N/A = not applicable; *Estimated values

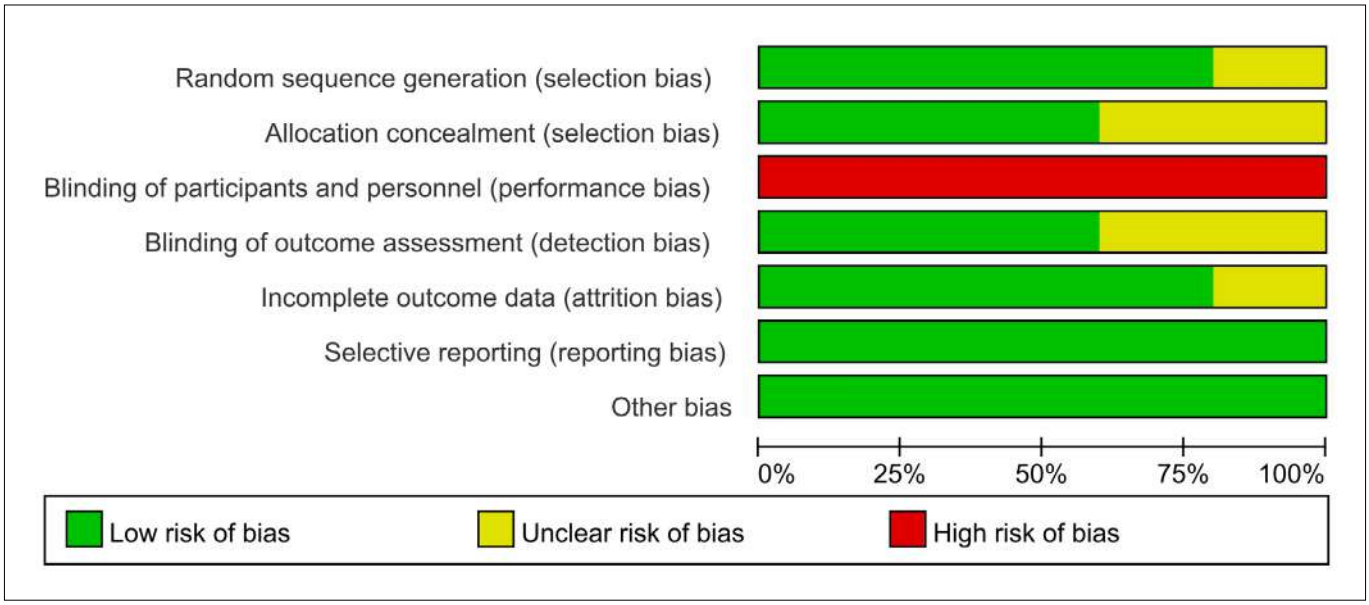
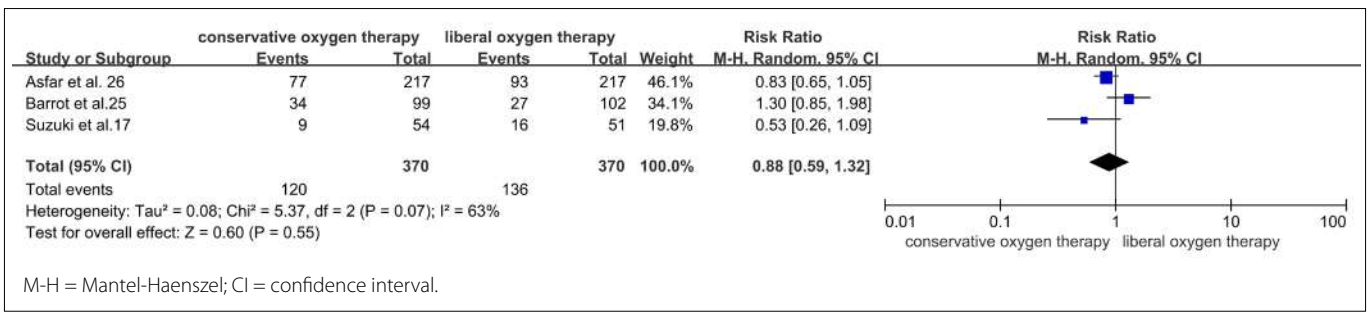
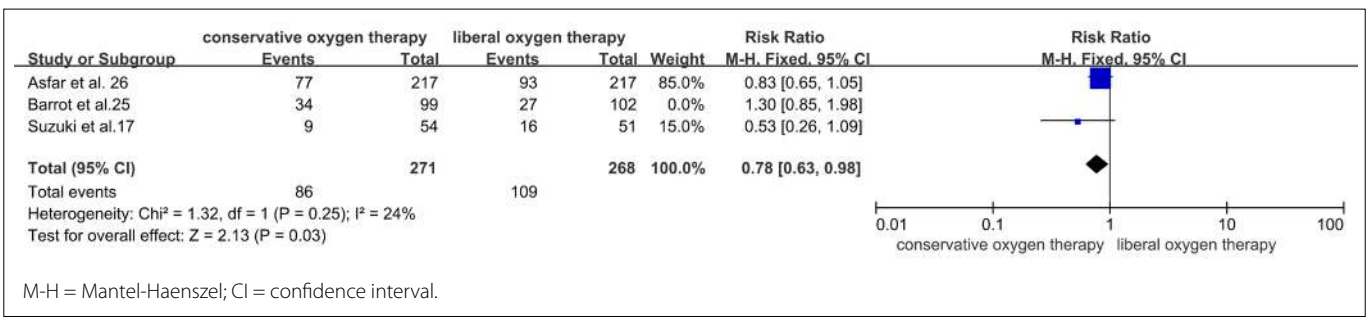


Figure 2. Risk-of-bias graph.



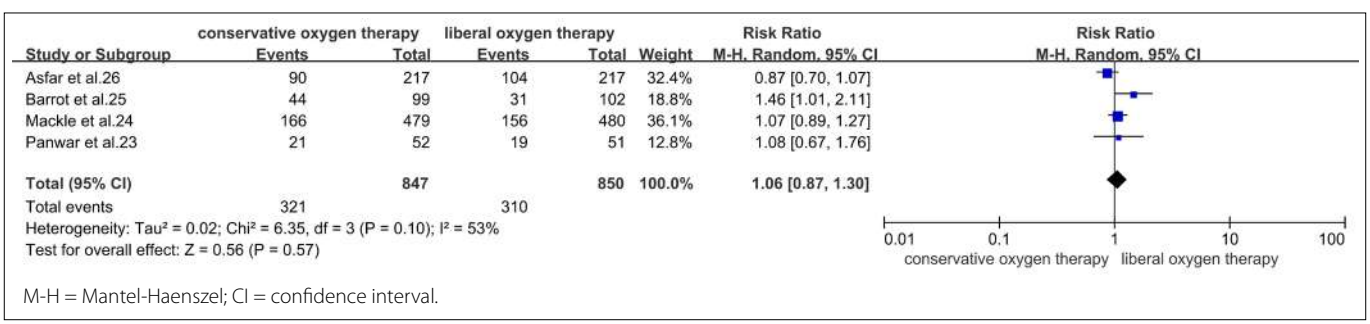
M-H = Mantel-Haenszel; CI = confidence interval.

Figure 3. 28-day mortality.



M-H = Mantel-Haenszel; CI = confidence interval.

Figure 4. Adjusted 28-day mortality.



M-H = Mantel-Haenszel; CI = confidence interval.

Figure 5. 90-day mortality**.**

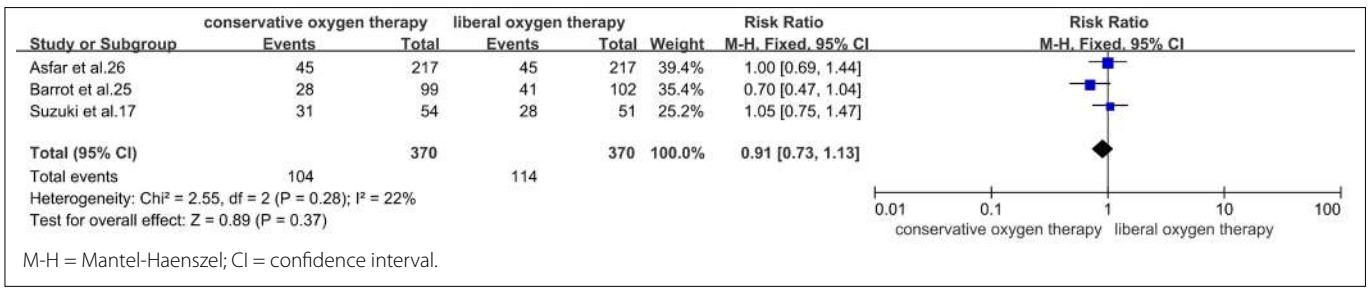
time within 28 days between the conservative and liberal groups (MD = 0.8; 95%; CI = -0.65-2.25; P = 0.28).

The vasopressor-free time within 28 days is shown in **Figure 9**. Three studies[23,24,26] provided data regarding the vasopressor-free time within 28 days. Since there was no significant heterogeneity among the studies (P = 0.15; I^2 = 48%), the fixed-effect model was adopted. The result showed that there was no statistically significant difference in vasopressor-free time within 28 days between the conservative and liberal groups (MD = 0.79; 95% CI = -0.71-2.30; P = 0.3).

The risk of bias in the studies included is shown in **Figure 10**. The funnel plot of the result showed that the primary outcome was symmetrical. Hence, there was no evidence of significant small-sample effects or publication bias.

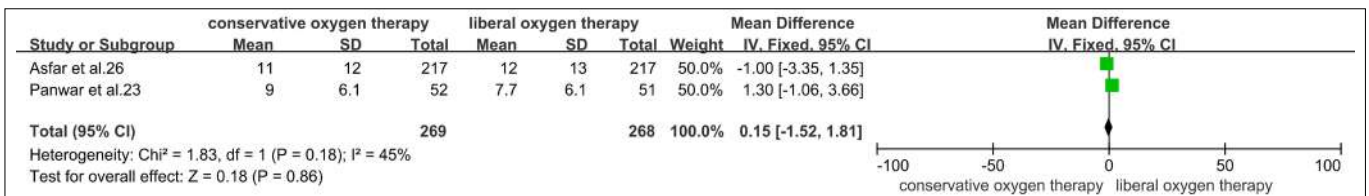
DISCUSSION

This systematic review and meta-analysis enrolled 1806 mechanically ventilated ICU patients. All the studies included were considered to be of high quality. Despite the high heterogeneity,



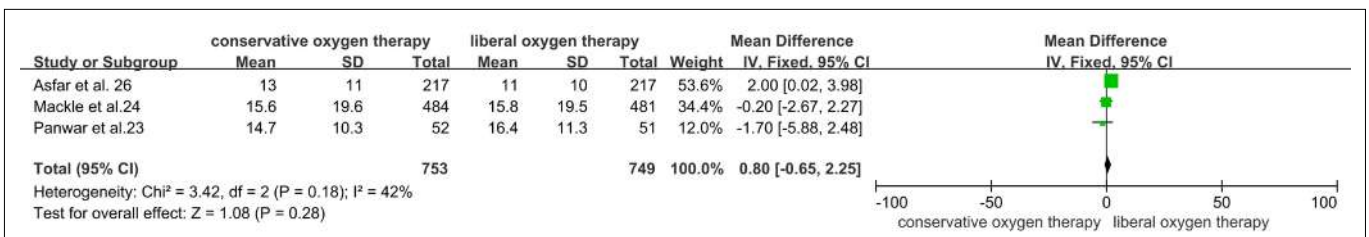
M-H = Mantel-Haenszel; CI = confidence interval.

Figure 6. New infection rate.



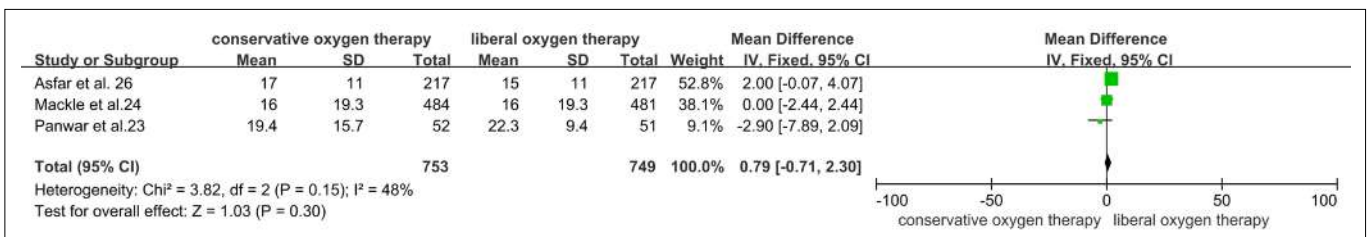
SD = standard deviation; IV = inverse-variance weighting; CI = confidence interval.

Figure 7. Intensive care unit length of stay.



SD = standard deviation; IV = inverse-variance weighting; CI = confidence interval.

Figure 8. Mechanical ventilation-free time within 28 days.



SD = standard deviation; IV = inverse-variance weighting; CI = confidence interval.

Figure 9. Vasopressor-free time within 28 days.

the results suggest that conservative oxygen therapy does not increase the risks of short-term mortality, medium-term mortality, new infections, longer ICU length of stay, shorter mechanical ventilation-free time within 28 days or shorter vasopressor-free time within 28 days, for mechanically ventilated ICU patients.

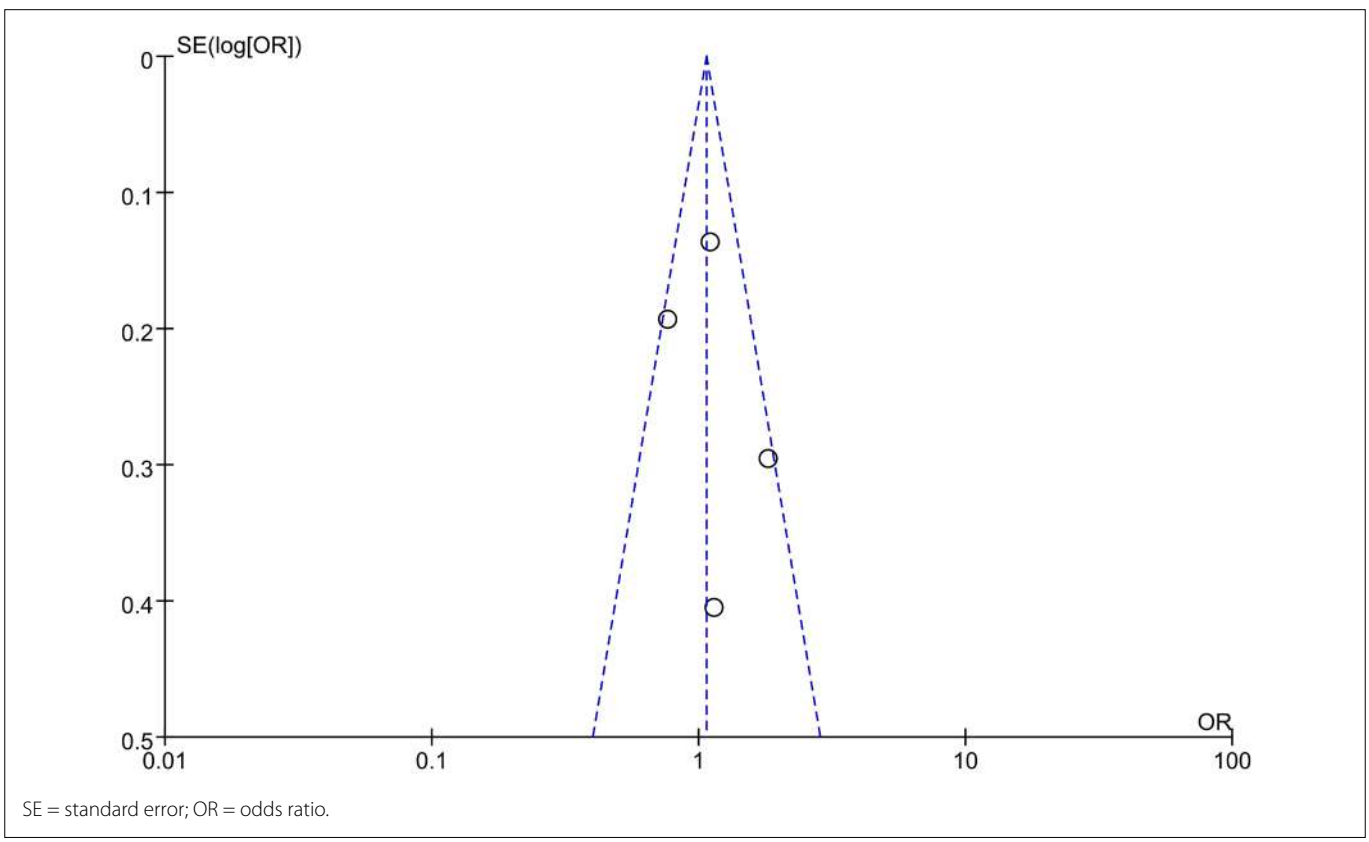
In clinical practice, oxygen therapy has been widely used to prevent or correct arterial hypoxemia for mechanically ventilated ICU patients. Due to concerns over the possible adverse outcomes of hypoxia exposure among critically ill patients, liberal oxygen therapy and hyperoxia are widely used for mechanically ventilated ICU patients. One study reported that 59% of patients have oxygen saturation greater than 98% most of the time.[32]

However, according to the formula of oxygen delivery (DO_2 = cardiac output × arterial oxygen content; arterial oxygen content = $(Hb \times 1.34 \times SaO_2) + (0.0031 \times PaO_2)$), oxygen delivery is governed by three key factors: arterial saturation (SO_2), cardiac output (CO) and hemoglobin (Hb). It is unreasonable to only use SaO_2 as the indicator for evaluating gas exchange in hypoxemic patients. Moreover, the oxygen dissociation curve of hemoglobin is "S-shaped": the upper part of the curve is very gradual, which means that it is very difficult to further increase SaO_2 by increasing blood oxygen content and PaO_2 in the upper part. For example, even when the patient's PaO_2 is increased, at the risk of hyperoxia exposure, from 100 mmHg to 150 mmHg, only an incremental increase (200 ml/l to 201.5 ml/l) in the blood oxygen content results from this.[9] It has also been reported that hyperoxia results in decreased heart rate, reduced CO and increased vascular resistance.[33]

Therefore, liberal oxygen therapy that only focuses on arterial oxygen saturation when increasing the oxygen delivery is unhelpful. Hyperoxia caused by liberal oxygen therapy may even be harmful. It can promote production of reactive oxygen species and expression of inflammatory cytokines, thus increasing the risk and severity of pneumonia,[11] epithelial and endothelial damage[13] and pulmonary interstitial edema.[12]

The results from the meta-analysis confirm that in acutely ill patients, liberal oxygen therapy is unhelpful and does not improve patient outcomes, but may increase mortality. When the range of SpO_2 is more than 94-96%, patients may be affected adversely.[4] Recent studies have shown that conservative oxygen therapy has no significant adverse effect on ICU patients with respiratory failure and hypoxic ischemic encephalopathy.[34,35] Conservative oxygen therapy is relatively safe for critically ill ICU patients.

Thus, oxygen therapy should be restricted. The goal of oxygen therapy should be to ensure adequate oxygen delivery while minimizing any unnecessary hyperoxia exposure. However, the question is how conservative it should be. The ideal situation is that supplemental oxygen administration should be guided through assessment of tissue oxygen delivery and consumption. However,



SE = standard error; OR = odds ratio.

Figure 10. Funnel plot for primary outcome.

these two parameters are difficult to obtain in clinical practice. In clinical trials, conservative oxygen therapy is usually carried out by keeping SpO_2 at the lower limit of normality.[24,25] To define conservative oxygen therapy solely on the basis of SpO_2 seems to ignore assessment of oxygen consumption.

As surrogate parameters for oxygen consumption, blood lactate concentration, central venous-to-arterial CO_2 difference and central venous or mixed venous oxygen saturation can also help in implementation of conservative oxygen therapy. Over recent years, there has been a conservative trend in oxygen therapy practice in some hospitals.[36]

However, for mechanically ventilated critically ill ICU patients, there is a lack of consensus and explicit guiding criteria regarding the use of conservative oxygen therapy. Clinicians who worry about hypoxemia will still increase the patient's oxygen saturation as much as possible, even at levels exceeding what they think is reasonable,[8] even though these clinicians are aware of the potential harm of liberal oxygen therapy.

The results from our study on mechanically ventilated ICU patients showed that there was no significant difference in clinical prognosis between use of liberal and use of conservative oxygen therapies. Conservative oxygen therapy did not result in additional risk; therefore, it is feasible and safe. It is worth mentioning that there was great heterogeneity regarding 28-day mortality among the studies reviewed here. By excluding each study one by one, we found that the heterogeneity arose from the study by Barrot et al.[25] Excluding Barrot's study decreased the heterogeneity ($I^2 = 24\%$).

After adjustments, the results from the meta-analysis revealed that conservative oxygen therapy reduced short-term mortality (RR = 0.78; 95% CI = 0.63-0.98; P = 0.03) (**Figure 4**). The reasons for this may have been related to the fact that the study population comprised acute respiratory distress syndrome (ARDS) patients. Such patients are characterized by difficult-to-correct hypoxemia. Hypoxemia arises from a diverse range of factors.[37] There may be little difference in clinical prognosis between liberal and conservative oxygen therapy use until the pathological basis of ARDS has been effectively improved. Moreover, the target for conservative oxygen therapy in the studies reviewed here was set at 88%-92% blood oxygen saturation, which is close to the lower limit recommended in ARDS guidelines.[38,39] In practice, there was some deviation between the actual and target oxygen saturation. This would undoubtedly have increased the risk of hypoxia exposure in the conservative group. The adverse events of mesenteric ischemia seen in the conservative group may indicate that conservative oxygen therapy close to the lower limit recommended may have been inappropriate.

Furthermore, apart from three studies[17,26] that only partially included patients with ARDS and the sample population in the study of Barrot et al.,[25] all the patients included were classified as presenting ARDS. The results suggest that there may have been a discrepancy between ARDS patients and non-ARDS patients regarding the prognosis from conservative oxygen therapy. In other words, this could imply that conservative oxygen therapy is beneficial for reducing short-term mortality among mechanically ventilated patients who do not present ARDS.

Limitations

The findings reported in this study must be interpreted with caution because of several limitations. Firstly, the definitions of conservative oxygen therapy and liberal oxygen therapy were not quite concordant in the studies that we enrolled, and this may have led to inaccuracies in the relative mortality rates between the conservative and liberal groups. Secondly, the number of studies included was relatively small and, therefore, subgroup analysis according to ARDS status was not possible. Thirdly, we assumed that the respiratory function of mechanically ventilated patients in ICUs would be severely impaired. However, some patients received MV for extrapulmonary reasons, and it was not possible to exclude these patients.

CONCLUSIONS

Liberal oxygen therapy and higher SpO_2 for mechanically ventilated ICU patients are not necessary. For partial MV patients, conservative oxygen therapy was not associated with a statistically significant reduction in mortality.

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Appendix 1. Search strategy.

Search strategy for PubMed

(("Oxygen Inhalation Therapy"[Mesh]) AND (((liberal*) OR (conservative*)) OR (conventional*))) AND (((Respiration, Artificial[MeSH Terms])) OR (mechanical ventilation)) AND (clinicaltrial[Filter])

Search strategy for Embase

('oxygen therapy'/exp OR 'oxygen therapy') AND (liberal* OR conservative* OR conventional*) AND (('respiration,'/exp OR respiration,) AND artificial OR (mechanical AND ('ventilation'/exp OR ventilation))) AND 'controlled clinical trial'/de

Search strategy for Cochrane library

((liberal*):ab OR (conservative*):ti,ab,kw OR (conventional*):ti,ab,kw) AND ((Respiration, Artificial):ab OR (mechanical ventilation):ti,ab,kw) AND (Oxygen Inhalation Therapy)

Search strategy for Web of Science

(TS= (Oxygen Inhalation Therapy) AND ((AB=(liberal*) OR AB=(conservative*)) OR AB=(conventional*))) AND ((TS=(Respiration, Artificial)) OR TS= (mechanical ventilation))

https://doi.org/10.1590/1516-3180.2021.0425.R1.07102021

Performance and reference intervals of thrombin generation test: results from the Brazilian longitudinal study of adult health (ELSA-Brasil). A cross-sectional study

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KEY WORDS (MeSH terms):
Clinical laboratory techniques.
Thrombin.
Reference values.

AUTHORS' KEY WORDS:
Calibrated automated thrombogram.
Thrombin generation assay.
Reference ranges.

ABSTRACT

BACKGROUND: The thrombin generation test (TGT) has shown promise for investigation of hemorrhagic and thrombotic diseases. However, despite its potential, it still needs standardization. Moreover, few studies have established reference values for TGT parameters. In Brazil, these values have not yet been established.

OBJECTIVE: To determine TGT performance and reference intervals for TGT parameters in healthy individuals.

DESIGN AND SETTING: Cross-sectional study conducted among participants in the Brazilian Longitudinal Study of Adult Health (Estudo Longitudinal de Saúde do Adulto, ELSA-Brasil).

METHODS: The reference sample consisted of 620 healthy individuals. The calibrated automated thrombogram (CAT) method, under low and high tissue factor (TF) conditions, was used to assess thrombin generation. Test performance was analyzed using intra and interassay coefficients of variation (CV) and reference intervals were calculated using the nonparametric method proposed by the International Federation of Clinical Chemistry and the Clinical and Laboratory Standards Institute.

RESULTS: The intraassay CV ranged from 1.4% to 2.2% and the interassay CV, 6.8% to 14.7%. The reference intervals for TGT parameters under low and high TF conditions were, respectively: lagtime: 3.0-10.3 and 1.4-3.7 min; endogenous thrombin potential (ETP): 1134.6-2517.9 and 1413.6-2658.0 nM.min; normalized ETP: 0.6-1.3 and 0.7-1.4; peak: 103.2-397.7 and 256.4-479.0 nM; normalized peak: 0.3-1.3 and 0.7-1.2; and time-to-peak: 5.6-16.0 and 3.4-6.7 min. These parameters were categorized relative to sex.

CONCLUSION: TGT performance was adequate and the proposed reference intervals were similar to those of other studies. Our findings may be useful for consolidating the TGT, through contributing to its standardization and validation.

INTRODUCTION

Thrombin has pro and anticoagulant properties and is considered to be the main protein involved in hemostasis regulation.[1] The thrombin generation test (TGT) is a method that evaluates the capacity of plasma for thrombin generation *ex vivo*.[2] Unlike the diagnostic and monitoring methods available routinely in laboratories for evaluating hemostasis, in which formation of a fibrin clot occurs when less than 5% of total thrombin has been generated, the TGT is capable of evaluating hemostasis in an overall manner and thus has greater sensitivity.[1,3] Hence, routine coagulometric methods measure only the initial phase of thrombin generation (TG) and are insensitive to prothrombotic states, while the TGT provides complete information on the phases of propagation and termination. The TGT therefore reflects the components of natural anticoagulation, such as proteins C and S and antithrombin, in addition to the tissue factor pathway inhibitor.[4]

The TGT was developed by Macfarlane and Biggs in the 1950s and later modified by Hemker.[2] After several further improvements, different methods for measuring thrombin generation were developed.[5,6]

However, the calibrated automated thrombogram (CAT) method is considered to be the reference method.[6] In this, the proteolytic activity of thrombin generated in plasma is

measured using a fluorogenic substrate.[2] The reaction is triggered through addition of either low or high picomolar concentration of tissue factor (TF), plus phospholipids and calcium ions. Fluorescence is measured continuously for 60 minutes and is proportional to the amount of thrombin generated. The measurements are obtained in a fluorimeter, and the Thrombinoscope software (Thrombinoscope BV, Maastricht, Netherlands) is used to convert the relative fluorescence units (RFU) into thrombin concentration (nM.min), in order to build a thrombin generation curve in real time and calculate its parameters. The main parameters are lagtime (corresponding to the period between addition of trigger reagents and the beginning of thrombin production), peak (maximum thrombin concentration produced in the amplification/propagation phase), time-to-peak (time necessary to reach the maximum thrombin production) and endogenous thrombin potential (ETP) (corresponding to the total amount of thrombin produced, i.e. reflecting the balance between procoagulant and anticoagulant forces).[3] Extended lagtime and time-to-peak and reduced ETP and peak indicate a state of hypocoagulability. On the other hand, a state of hypercoagulability is characterized by reductions of time-to-peak and lagtime and increased peak and ETP.[7]

From a clinical point of view, a laboratory test that has the capacity to accurately predict the clotting potential of an individual is needed. In this regard, the TGT can be used to understand coagulation mechanisms;[8] diagnose and monitor hemorrhagic diseases;[9,10] monitor the use of anticoagulants[4,11] and antiplatelet agents; and predict recurrence of venous thromboembolism.[12-14]

Despite its potential, the TGT still requires standardization and clinical validation studies.[3,6,15] In fact, some studies have shown that pre-analytical factors can significantly interfere in the TGT and limit its potential for clinical use.[16-19] Moreover, only a few studies[20-24] have determined reference ranges for TGT parameters. ELSA-Brasil was the first major Brazilian study to perform the TGT on a sample of its participants. Establishment of reference values can favor development of clinical studies and implementation of the TGT as a routine laboratory test.

OBJECTIVE

Therefore, the aim of this study was to evaluate TGT performance and propose reference intervals for TGT parameters, in a sample of healthy participants in the Brazilian Longitudinal Study of Adult Health (Estudo Longitudinal de Saúde do Adulto, ELSA-Brasil). This was done in accordance with the recommendations of the consensus document: "How to define and determine reference intervals in the clinical laboratory", proposed by the International Federation of Clinical Chemistry and the Clinical and Laboratory Standards Institute (CLSI).[25]

METHODS

Population

This study used data from the ELSA-Brasil multicenter cohort study, which involved 15,105 participants (aged 35 to 74 years) who were civil servants working in higher education or research organizations in six Brazilian cities. Detailed information on the baseline of the ELSA-Brasil study has been published elsewhere.[26,27] Approvals from ethics committees were granted (August 4, 2006; CAAE 0016.1.198.000-06), and all individuals provided their written informed consent.

Out of the 3,115 baseline participants of the state of Minas Gerais, this analysis was restricted to 2,970 individuals from whom TGT were obtained. Additionally, individuals who presented factors that might have affected hemostasis and, therefore, the TGT were excluded, in accordance with the exclusion criteria (**Table 1**). Out of these 2,970 participants, 2,350 were excluded. Thus, the study population was 620 participants. Moreover, TGT parameters values below the first percentile and/or above the 99th percentile were considered to be outliers and were excluded from the analysis.

Plasma samples

Venous blood sampling was performed in the mornings after fasting, in accordance with the CLSI Procedures for the Collection

Table 1. Exclusion criteria among participants and number of excluded individuals

Exclusion criteria	Number of patients (n = 2,970)	
	Excluded (n)	Kept (n)
Continuous use of any medication, including oral contraceptive use and hormone therapy use	1,829	1,141
Self-rating of health as fair, poor or very poor	95	1,046
Self-reported medical diagnosis of diabetes	7	1,039
Self-reported medical diagnosis of arterial hypertension	71	968
Self-reported medical diagnosis of cardiovascular disease[a]	16	952
Self-reported medical diagnosis of thrombosis or pulmonary embolism	19	933
Self-reported medical diagnosis of liver disease[b]	85	848
Self-reported medical diagnosis of cancer	17	831
Current smoker[c]	93	738
Body mass index (BMI) > 30 kg/m^2	108	630
Glomerular filtration rate (GFR) < 60 ml/min/1.72 m^3	10	620

[a]Acute myocardial infarction, angina, congestive heart failure, stroke and myocardial revascularization; [b]cirrhosis or hepatitis; [c]participants who declared that they had smoked at least one hundred cigarettes over the course of their lives and were still smoking.

of Diagnostic Blood Specimens by Venipuncture: Approved Standard.[28] Venipuncture was performed for vacuum collection into tubes containing one volume of trisodium citrate (0.105 M) to nine volumes of blood (BD Vacutainer System; Greiner tubes). These were identified using barcodes, to ensure confidentiality, security and traceability of the sample.[29] Platelet-poor plasma (PPP) was obtained by means of centrifugation at 2,500 g and 4 °C for 15 minutes, and was then stored at -80 °C until use. The time between blood collection and centrifugation was not more than 30 minutes.

Thrombin generation test

The TGT was performed in PPP using the CAT method (Thrombinoscope BV, Maastricht, Netherlands), using a 96-well plate, under two conditions for triggering the reaction: 1) low TF concentration; and 2) high TF concentration. The CAT method in PPP was carried out as described previously by Duarte et al.[15] The following TGT parameters were measured and analyzed: lagtime (min), peak (nM), time-to-peak (min) and ETP (nM.min). PPP-reagent low, PPP-reagent high, thrombin calibrator and calcium-containing fluorogenic substrate (FluCa) kit reagents were purchased from Diagnostica Stago (Reading, United Kingdom).

One control plasma pool (CPP) including male participants and another CPP including female participants were prepared in order to normalize the ETP of the other participants' samples, for internal quality control and determination of intra and interassay variabilities. Each CPP was obtained by mixing 170 samples from female and 170 from male participants in ELSA-Brasil in the state of Minas Gerais, who met the following criteria: C-reactive protein (CRP) ≤ 3 mg/dl (to exclude acute diseases); and not using female hormones or antithrombotic drugs that could potentially interfere with the hemostatic mechanism. The sample aliquots from the participants selected to compose each pool were mixed, aliquoted again and frozen for use during the experiments. CPP was added in duplicate to all plates.

The ETP of the female participants was normalized against the data generated in the female CPP, and the ETP of the male participants was normalized against the data generated in the male CPP, at low and high TF concentrations. According to Dargaud et al.,[16] normalization of the ETP values of samples, against an ETP value obtained using a CPP, guarantees lower interassay variability in the TGT.

Statistical analysis

The intra and interassay coefficients of variation (CV) were calculated for all experiments under both conditions (low and high TF), regarding all parameters of the TGT, which was carried out between March and December 2018. CPP was added in duplicate to all plates under the two conditions analyzed. The intraassay variability of the ETP, lagtime, peak and time-to-peak was determined using the CV of the duplicates for each experiment and then averaging all the CVs. The interassay variability of this parameter was determined by calculating the CV between the means of the duplicates of the CPP of all the 164 independent runs that were conducted over the ten-month period.

Skewness and kurtosis tests were applied to evaluate the distribution of TGT parameter values. A nonparametric method was used to determine the reference interval, calculated as the interval from percentile 2.5 to percentile 97.5 of the TGT parameter distribution. Student's t test was used to verify differences between subgroups defined according to sex and age. We assessed the need to recommend a specific age and sex reference range for the TGT using the Harris/Boyd statistical approach.[25] Following this approach, we calculated z scores from TGT parameter means and standard deviations (SDs) and compared these with a critical value (z*).

Separate reference intervals are recommended if at least one of the following conditions is met: 1) Calculated z exceeds critical value z*; 2) Statistical differences exist between TGT parameter SDs of each subgroup and the larger SD exceeds the smaller SD 1.5-fold, or if the proportion [larger SD/(larger SD – smaller SD)] is less than 3. It should be mentioned that the CLSI recommends that each subgroup of pre-analytical variables should be composed of at least 120 individuals.[25]

A P-value lower than 0.05 was considered statistically significant, and the analysis was performed using the STATA 9.0 statistical package (Stata; College Station, Texas, United States).

RESULTS

The population of this study consisted of 620 healthy participants for whom TGT data were available and who were not within the exclusion criteria (**Table 1**). The majority of the participants were men (56.6%), with ages between 35-54 years (81.6%). They self-declared as white (46.0%) and had reached full higher education (62.4%) (**Table 2**). In relation to age distribution, there was no statistical difference between men and women (mean age of the men = 47.7 years, SD = 8.0 years; and mean age of the women = 47.2 years, SD = 6.7 years).

The intra- and interassay CVs under low and high TF conditions from the 164 CPP runs are presented in **Table 3**.

Histograms showing the distribution of the TGT parameters are presented in **Figures 1** and **2**. It can be seen from these that the dispersion of all the parameter values was close to normal distribution.

All TGT parameters were statistically different under both low and high TF conditions and according to sex; and also, for some mean TGT parameters, according to age. Our results relating to sex showed that males had higher mean values for lagtime and time-to-peak and lower mean values for ETP, normalized ETP, peak and

Table 2. Sociodemographic characteristics of the 620 reference individuals

Characteristics	Frequency	
	n	%
Sex		
Male	351	56.6
Female	269	43.4
Age group (years)		
35-54	506	81.6
≥ 55	114	18.4
Self-rated race/skin color		
White	285	46.0
Brown[a]	231	37.2
Black	75	12.1
Others[b]	29	4.7
Education (years)		
< 11	41	6.6
11-14	192	31.0
≥ 15	387	62.4

[a]"Brown" or of mixed color; [b]includes native indigenous (n = 2), Asian descendent (n = 18) and missing information (n = 9).

Table 3. Intra and interassay coefficients of variation (CVs) of thrombin generation test (TGT) parameters under low and high tissue factor (TF) conditions

TGT parameters		Intraassay CV (%)	Interassay CV (%)
Lagtime	Low TF	1.9	12.4
	High TF	1.7	14.7
ETP	Low TF	1.7	9.3
	High TF	2.2	10.5
Peak	Low TF	2.1	10.4
	High TF	1.7	6.8
Time-to-peak	Low TF	1.9	10.3
	High TF	1.4	10.1

ETP = endogenous thrombin potential.

normalized peak, in comparison with female participants, under both TF conditions. Regarding age, the lagtime and time-to-peak values under the low TF condition were higher among individuals ≥ 55 years old than among those between 35 and 54 years old. Under the high TF condition, the lagtime and time-to-peak values were higher and peak and normalized peak values were lower among individuals ≥ 55 years old than among those between 35 and 54 years old (**Tables 4** and **5**).

Regarding sex, the calculated z for lagtime, ETP and time-to-peak under the low TF condition, and for lagtime, peak, normalized peak and time-to-peak under the high TF condition, was higher than z*. In addition, each category (male and female) had more than 120 participants. Regarding age, the calculated z for lagtime and time-to-peak under the high TF condition was higher than z*, but one category (age ≥ 55 years) had fewer than 120 individuals (n = 114), i.e. it did not meet the CLSI recommendations. Thus, we chose to present reference intervals categorized according to sex only for the parameters that met at least one criterion of the Harris/Boyd statistical approach and the CLSI recommendations.

Table 6 shows the reference intervals for TGT parameters under both low and high TF conditions. As expected, under the low TF condition, ETP and peak values were slightly lower than those obtained under the high TF condition. However, for the lagtime and time-to-peak parameters, the inverse was observed, i.e. higher values for low TF and lower values for high TF.

DISCUSSION

Our study, developed using a sample of healthy participants from a large cohort of Brazilian adults, showed adequate TGT performance, as measured through intra- and intertest variability. Furthermore, this study established reference intervals for TGT parameters, and showed that for some parameters, these intervals need to be stratified according to sex.

Use of a standardized procedure for the TGT resulted in acceptable validation criteria, with CVs for most TGT parameters of < 10%.[30] In our study, the intra and interassay variability of the ETP, lagtime, peak and time-to-peak ranged from 1.4% to 2.2% and from 6.8% to 14.7%, respectively. These findings are in agreement with those of Duarte et al.,[15] in which the intra-assay CV for all parameters was below 10%; and with those of Ten Cate-Hoek et al.,[23] in which none of the TGT parameters, under either condition, presented CV greater than 5%. In a study by Bloemen et al.,[20] the CVs of all parameters were shown to range from 10% to 27%. One explanation for this higher CV could particularly be that individualized reagents of different origins may have been used, which would involve more steps in carrying out the technique; whereas in our study and in the others mentioned, single-manufacturer kits were used.

There is evidence that the TGT is a more sensitive method for assessing hemostasis than routine coagulometric assays, such as prothrombin time, activated partial thromboplastin time or individual coagulation factor assays.[31,32] The TGT has basically been performed under two experimental conditions: low and high concentrations of TF. Bagot et al.[33] reported that most researchers now consider that use of a low TF concentration provides greater sensitivity due to its higher capacity for evaluating the intrinsic pathway, natural coagulation inhibitors and fibrinogen. However, the TGT using high TF concentration may be useful for evaluating more hypercoagulable states, i.e. when patients are using anticoagulants,[6] and for analyses to investigate the natural anticoagulation mechanism, through addition of activated protein C[34] or thrombomodulin.[35-37]

In accordance with most of the studies summarized in **Table 7**, we also proposed and performed determination of TGT reference intervals under both experimental conditions, with low and high

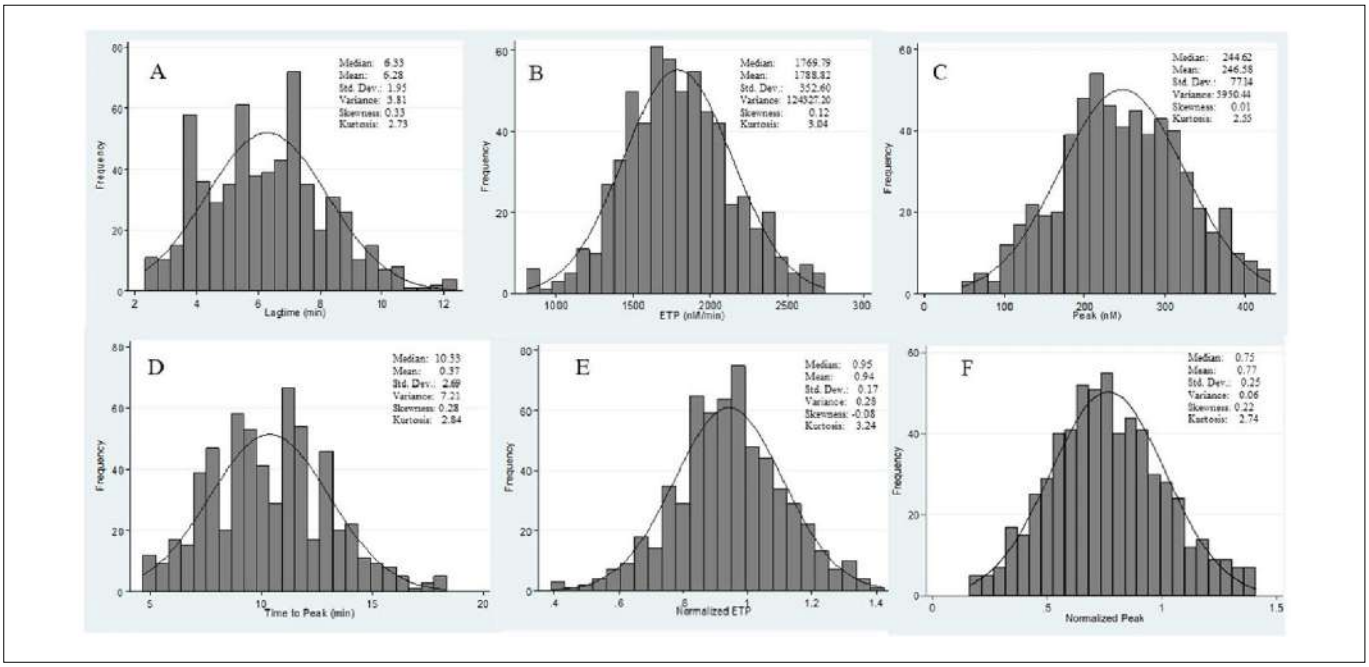

Figure 1. Distribution curves for (A) Lagtime; (B) Endogenous thrombin potential (ETP); (C) Peak; (D) Time-to-peak; (E) Normalized ETP; (F) Normalized peak under the low tissue factor (TF) condition.

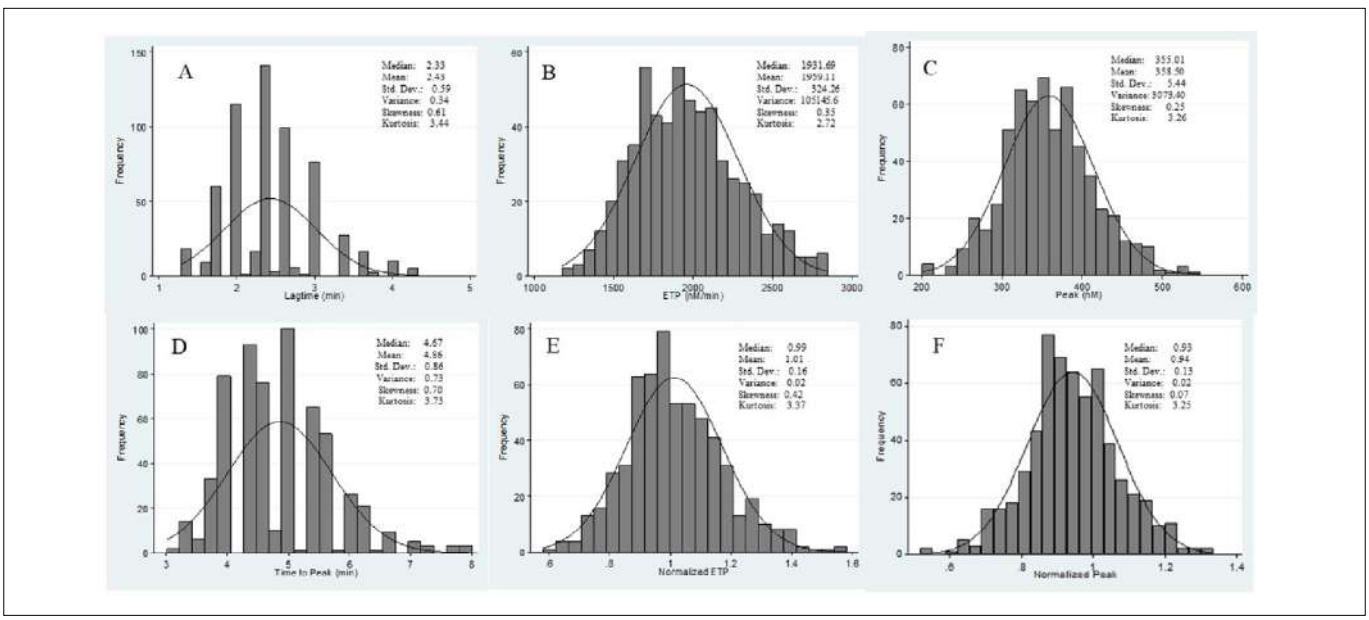

Figure 2. Distribution curves for (A) Lagtime; (B) Endogenous thrombin potential (ETP); (C) Peak; (D) Time-to-peak; (E) Normalized ETP; (F) Normalized peak under the high tissue factor (TF) condition.

TF, and our findings were similar to those of these previous studies. Only Lundbech et al.[21] and Haidl et al.[24] conducted evaluations only with low or high TF, respectively. The five studies presented were carried out in Austria, Germany, Denmark and Holland, and used the CAT method to evaluate thrombin generation. In four of them, Diagnostica Stago or Thrombinoscope BV kits were acquired. Only one study, Bloemen et al.,[20] used individualized reagents from different sources. The studies by van Paridon et al.[22] and Lundbech et al.[21] stratified the reference intervals according to sex, while Bloemen et al.,[20] Ten Cate-Hoek et al.[23] and Haidl et al.[24] did not stratify.

Table 4. Mean, standard deviation and 95% confidence intervals of thrombin generation test (TGT) parameters under the low tissue factor (TF) condition, according to sex and age, among reference individuals (n = 620)

Demographic variable	Parameter	Lagtime (min) (n = 607)	ETP (nM.min) (n = 607)	Peak (nM) (n = 607)	Time to peak (min) (n = 608)	Normalized ETP (n = 597)	Normalized peak (n = 607)
Sex							
Male (n = 351)	n	343	345	345	345	333	346
	Mean (SD)	6.8 (1.98)	1724.2 (332.1)	239.3 (72.5)	10.9 (2.6)	0.92 (0.16)	0.72 (0.23)
	95% CI	6.5-7.0	1689.0-1759.3	231.6-246.9	10.6-11.1	0.90-0.93	0.69-0.74
Female (n = 269)	n	264	262	262	263	264	261
	Mean (SD)	5.7 (1.8)	1874.0 (361.3)	256.2 (82.0)	9.7 (2.7)	0.97 (0.17)	0.83 (0.26)
	95% CI	5.5-5.9	1830.0-1917.9	246.3-266.2	9.4-10.1	0.95-0.99	0.80-0.86
	[a]P-value	0.000	0.000	0.007	0.000	0.000	0.000
Age group (years)							
35 - 54 (n = 506)	n	499	495	496	499	486	495
	Mean (SD)	6.1 (2.0)	1795.5 (356.6)	247.4 (77.2)	10.2 (2.7)	0.95 (0.17)	0.77 (0.25)
	95% CI	6.0-6.3	1764.0-1827.0	240.6-254.2	10.0-10.5	0.93-0.96	0.75-0.79
≥ 55 (n = 114)	n	108	112	111	109	111	112
	Mean (SD)	7.0 (1.7)	1759.5 (334.4)	243.1 (77.3)	11.1 (2.5)	0.92 (0.16)	0.75 (0.25)
	95% CI	6.7-7.3	1696.8-1822.1	228.5-257.6	10.6-11.5	0.89-0.95	0.71-0.80
	[a]P-value	0.000	0.330	0.595	0.004	0.196	0.591

The numbers of participants for each parameter differ due to outliers and missing information for each of them (outliers + missing: lagtime male 3 + 5, female 3 + 2; 35-54 years 2 + 5, ≥ 55 years 4 + 2; ETP male 1 + 5, female 5 + 2; 35-54 years 6 + 5, ≥ 55 years 0 + 2; peak male 1 + 5, female 5 + 2; 35-54 years 5 + 5, ≥ 55 years 1 + 2; time to peak male 1 + 5, female 4 + 2; 35-54 years 2 + 5, ≥ 55 years 3 + 2; normalized ETP male 3 + 15, female 3 + 2; 35-54 years 5+15, ≥ 55 years 1 + 2; normalized peak male 0 + 5, female 6 + 2; 35-54 years 6 + 5, ≥ 55 years 0 + 2). [a]P-value obtained through Student's t test.
ETP = endogenous thrombin potential; SD = standard deviation; CI = confidence interval.

Table 5. Mean, standard deviation and 95% confidence intervals of thrombin generation test (TGT) parameters under the high tissue factor (TF) condition, according to sex and age group, among reference individuals

Demographic variable	Parameter	Lagtime (min) (n = 605)	ETP (nM.min) (n = 599)	Peak (nM) (n = 604)	Time to peak (min) (n = 604)	Normalized ETP (n = 593)	Normalized peak (n = 595)
Sex							
Male (n = 351)	n	341	341	344	343	333	341
	Mean (SD)	2.6 (0.6)	1905.6 (311.6)	338.6 (47.4)	5.1 (0.8)	1.0 (0.2)	0.92 (0.1)
	95% CI	2.5-2.6	1872.4-1938.8	333.6-343.6	5.0-5.2	0.99-1.02	0.91-0.93
Female (n = 269)	n	264	258	260	261	260	254
	Mean (SD)	2.2 (0.5)	2029.8 (327.7)	384.8 (54.4)	4.6 (0.8)	1.0 (0.2)	0.97 (0.12)
	95% CI	2.2-2.3	1989.6-2070.0	378.2-391.5	4.5-4.7	1.01-1.05	0.96-0.99
	[a]P-value	0.000	0.000	0.000	0.000	0.051	0.000
Age group (years)							
35-54 (n = 506)	n	493	488	492	492	481	484
	Mean (SD)	2.4 (0.6)	1967.7 (355.7)	362.6 (55.7)	4.8 (0.8)	1.0 (0.2)	0.95 (0.12)
	95% CI	2.3-2.4	1937.8-1997.6	357.7-367.6	4.7-4.8	1.00-1.03	0.94-0.96
≥ 55 (n = 114)	n	112	111	112	112	112	111
	Mean (SD)	2.7 (0.6)	1921.4 (266.3)	340.4 (50.6)	5.3 (0.8)	1.0 (0.2)	0.91 (0.13)
	95% CI	2.6-2.8	1871.3-1971.5	330.9-349.9	5.1-5.4	0.97-1.03	0.88-0.93
	[a]P-value	0.000	0.175	0.000	0.000	0.243	0.001

The numbers of participants for each parameter differ due to outliers and missing information for each of them (outliers + missing: lagtime male 5 + 5, female 1 + 4; 35-54 years 6 + 7, ≥ 55 years 0 + 2; ETP male 5 + 5, female 7 + 4; 35-54 years 11 + 7, ≥ 55 years 1 + 2; peak male 2 + 5, female 4 + 5; 35-54 years 6 + 8, ≥ 55 years 0 + 2; time to peak male 2 + 6, female 3 + 5; 35-54 years 5 + 9, ≥ 55 0 + 2; normalized ETP male 3 + 15, female 2 + 7; 35-54 years 5 + 20, ≥ 55 years 0 + 2; normalized peak male 0 + 8, female 6 + 5; 35-54 years 6 + 11, ≥ 55 years 0 + 2). [a]P-value obtained through Student's t test.
ETP = endogenous thrombin potential; SD = standard deviation; CI = confidence interval.

Table 6. Reference intervals for the thrombin generation test (TGT) parameters under low and high tissue factor (TF) conditions

TGT parameters		Median	Reference interval	
			Percentile 2.5	Percentile 97.5
Low TF condition				
Lagtime (min)	All	6.3	3.0	10.3
	Male	6.7	3.3	10.7
	Female	5.7	2.7	9.7
ETP (nM.min)	All	1769.8	1134.6	2517.9
	Male	1711.2	1063.0	2393.2
	Female	1860.4	1202.8	2619.0
Peak (nM)	All	244.6	103.2	397.7
Time-to-peak (min)	All	10.3	5.6	16.0
	Male	10.9	6.3	16.1
	Female	9.33	4.7	15.7
Normalized ETP	All	0.9	0.6	1.3
Normalized peak	All	0.7	0.3	1.3
	Male	0.7	0.3	1.2
	Female	0.8	0.4	1.3
High TF condition				
Lagtime (min)	All	2.3	1.4	3.7
	Male	2.7	1.6	4.0
	Female	2.3	1.3	3.4
ETP (nM.min)	All	1931.7	1413.6	2658.0
Peak (nM)	All	355.1	256.4	479.0
	Male	335.8	248.9	437.6
	Female	381.8	279.7	499.6
Time-to-peak (min)	All	4.7	3.4	6.7
	Male	5.0	3.6	7.0
	Female	4.3	3.4	6.3
Normalized ETP	All	1.0	0.7	1.4
Normalized peak	All	0.9	0.7	1.2

ETP = endogenous thrombin potential; min = minutes.

In our study, significant differences according to sex were found in the means of all TGT parameters, under both conditions. Women had lower lagtime and time-to-peak averages, and higher ETP, peak and normalized peak, i.e. they had higher thrombin generation than men. These findings are similar to those of the study by van Paridon et al.,[22] who suggested that female endogenous sex hormones had an influence on hemostasis, such that this would increase the fibrinogen levels and reduce the natural anticoagulant levels. This would explain the differences in TGT parameter values between the sexes.[38]

Regarding age, the mean lagtime and time-to-peak under low TF and high TF conditions were higher and the mean peak and normalized peak were lower only with high TF, among individuals aged 55 years or over, in comparison with those aged between 35 and 54 years. Some other studies have described the effect of age on the TGT.[22,24,39,40] Van Paridon et al.[22] observed that there was a positive association between lagtime and age among both men and women, which corroborates our findings. They also observed that ETP and the peak increased with age among both men and women, and that the potential for thrombin generation tended to decrease with age. Other studies have generally suggested that a positive association exists between thrombin generation and increasing age.[24,39,40] It should be noted that these studies had small samples. We therefore believe that both the previous findings and our findings relating to age still need to be better clarified.

It is known that the plasma levels of hemostatic factors can present racial differences. In general, blacks have higher levels of factors VII and VIII and lower levels of proteins C and S than those of whites.[41-44] However, the data on possible racial differences relating to the potential for thrombin generation in a healthy population remain inconclusive.[45] In our study, no significant differences

Table 7. Reference intervals for thrombin generation test (TGT) parameters from different studies

Study	Subject population	Country of origin	Method/ equipment/ reagents	Reference intervals to PPP	
				Low condition (TF ~1 pM)	High condition (TF ~5 pM)
ELSA-Brasil	620 healthy adult individuals aged 35-74 years	Brazil	– CAT/ Fluoroskan Ascent™ microplate fluorometer – Kits were obtained from Diagnostica Stago	2.5%-97.5% • Lagtime (min): 3.0-10.3 Male: 3.3-10.7 Female: 2.7-9.7 • ETP (nM.min): 1134.6-2517.9 Male: 1063.0-2393.2 Female: 1202.8-2619.0 • Peak (nM): 103.2-397.7 • Time-to-peak (min): 5.6-16.0 Male: 6.3-16.1 Female: 4.7-15.7 • Normalized ETP: 0.9-1.3	2.5%-97.5% • Lagtime (min): 1.4-3.7 Male: 1.6-4.0 Female: 1.3-3.4 • ETP (nM.min): 1413.6-2658.0 • Peak (nM): 256.4-479.0 Male: 248.9-437.6 Female: 276.7-499.6 • Time-to-peak (min): 3.4-6.7 Male: 3.6-7.0 Female: 3.4-6.3 • Normalized ETP: 0.7-1.4

Coninue...

Table 7. Continuation

Study	Subject population	Country of origin	Method/ equipment/ reagents	Reference intervals to PPP	
				Low condition (TF ~1 pM)	High condition (TF ~5 pM)
Lundbech et al., 2020[21]	124 blood donors aged 21-66 years	Denmark	– CAT/ Fluoroskan Ascent™ microplate fluorometer – Kits were obtained from Thrombinoscope BV	X ± 1.96*SD Subgroups: 95% CI • Lagtime (min): 4.4-9.4 Male: 7.1-7.6 Female: 6.4-6.7 • ETP (nM.min): 554.0-1952.0 Male: 1093.0-1258.0 Female: 1249.0-1440.0 • Peak (nM): 46.0-288.0 Male: 139.0-164.0 Female: 167.0-203.0 • Time-to-peak (min): 8.0-15.0 Male: 11.7-12.5 Female: 10.3-11.2	NA
Van Paridon et al., 2019[22]	1,210 apparently cardiovascularly healthy subjects without history of CVD (myocardial infarction, congestive heart failure, coronary artery disease, venous thromboembolism, atrial fibrillation or peripheral artery disease), presence of CVRFs (obesity, dyslipidemia, arterial hypertension or diabetes mellitus) or use of antithrombotic agents, oral contraceptives or hormonal replacement therapy. The median age was 47 years (IQR 42-55) among males and 48 years (IQR 45-55) among females.	Germany	– CAT/ Fluoroskan Ascent™ microplate fluorometer – Kits were obtained from Thrombinoscope BV	Lagtime: median (IQR) ETP and Peak: mean (SD) • Lagtime (min): Male: 5.07 (4.67-5.67) Female: 4.67 (4.33-5.33) • ETP (nM.min): Male: 1047 (216) Female: 1099 (203) • Peak (nM): Male: 108 (51) Female: 115 (48.7) • Time-to-peak (min): NA	Lagtime: median (IQR) ETP and Peak: mean (SD) • Lagtime (min): Male: 2.67 (2.33-3.00) Female: 2.39 (2.33-2.67) • ETP (nM.min): Male: 1322 (196) Female: 1318 (212) • Peak (nM): Male: 236 (52.2) Female: 259 (53.3) • Time-to-peak (min): NA
Bloemen et al., 2017[20]	129 healthy adult individuals (did not have any predisposition to/history of thrombosis or bleeding or they had not taken any oral anticoagulant or antiplatelet drugs for at least 2 weeks before testing). The median age was 32.0 years (IQR 27.0-43.5).	Netherlands	– CAT/ Fluoroskan Ascent™ microplate fluorometer – Individualized reagents from different sources	2.5%-97.5% • Lagtime (min): 3.3-5.8 • ETP (%): 72.3-141.5 • Peak (%): 30.5-97.2 • Time-to-peak (min): 6.1-10.9	2.5%-97.5% • Lagtime (min): 1.7-2.9 • ETP (%): 77.7-142.9 • Peak (%): 73.3-126.9 • Time-to-peak (min): 3.1-5.0
Ten Cate-Hoek et al., 2008[23]	137 healthy individuals (without anticoagulant, anti-platelet or oral contraceptives drugs, and no pregnant women) recruited from the community. Mean age was 53.7 years.	Netherlands	– CAT/ Fluoroskan Ascent™ microplate fluorometer – Kits were obtained from Thrombinoscope BV	95% CI • Lagtime (min): 3.9-4.1 • ETP (%): 99.0-106.3 • Peak (%): 92.7-105.5	95% CI • Lagtime (min): 2.4-2.8 • ETP (%): 96.6-109.2 • Peak (%): 89.7-97.5
Haidl et al., 2006[24]	35 healthy adult volunteers consisting of students and medical staff who were not taking any medication that would influence coagulation aged < 35 years.	Austria	– CAT/ Fluoroskan Ascent™ microplate 9 fluorometer – Individualized reagents from different sources	NA	Mean ± 2-fold SD • Lagtime (min): 1.35-2.39 • ETP (nM.min): 1745.0-2737.0 • Peak (nM): 433.0-669.0 • Time-to-peak (min): 2.75-4.31

TF = tissue factor; ETP = endogenous thrombin potential; CAT= calibrated automated thrombogram; CVD = cardiovascular disease; CVRFs = cardiovascular risk factors; SD = standard deviation; CI = confidence interval; PPP = platelet-poor plasma; ELSA = Longitudinal Study of Adult Health (Estudo Longitudinal de Saúde do Adulto); IQR = interquartile range; NA = not applicable; min = minutes.

in TGT parameters were observed in relation to the race/color of the participants (data not presented). However, it should be noted that color/race was obtained through self-reporting. Pena et al.[46] showed that in Brazil, skin color, phenotypically evaluated, has a very weak correlation with the degree of ancestry.

The reference ranges in this study were determined in accordance with the CLSI recommendations. We presented general and sex-stratified reference intervals for the parameters of lagtime, ETP, time-to-peak and normalized peak under the low TF condition and lagtime, peak and time-to-peak under the high TF condition. For these parameters, the results from the Harris and Boyd test showed that the calculated z of these parameters exceeded the z*, thus indicating that reference intervals categorized according to sex would be clinically useful.[25] However, we did not present reference intervals according to age category, because only two parameters (lagtime and time-to-peak under the high TF condition) showed calculated z greater than z*. Moreover, one category (age ≥ 55 years) had fewer than 120 individuals (n = 114) and thus did not meet the CLSI recommendations.

One of the greatest challenges in studies in which the proposal is to determine reference intervals for biological analytes is how to select of healthy individuals. Most studies use convenience sampling, consisting of medical students, blood donors, etc. This is questionable because these groups are not representative of the population in which these parameters are evaluated.[25,47,48] Nonetheless, most of the studies that have put forward reference ranges for TGT parameters used samples that were precisely from blood donors,[21] students and medical staff[24] or young and healthy adult individuals,[20] as can be observed in **Table 7**.

Thus, our study innovated and aggregated information on this topic, since the completeness and quality of the ELSA-Brasil data ensured that a healthy reference sample was selected. This study was developed using a sample from the general population that did not include any individuals with medical diagnoses of diabetes, hypertension, CVD, venous thromboembolism, cancer or liver diseases, or any obese individuals, smokers, individuals with altered glomerular filtration rate (GFR) and individuals making regular use of medications (including female hormones). Hence, conditions that may affect hemostasis were excluded.

Although our reference ranges can be generalized to other populations in order to identify patients with risks of bleeding and thrombosis, it is important to emphasize that these ranges should be used with caution. These values may differ according to the ethnic origin of the population, geographical location or living habits, among other factors.[25] In addition, pre-analytical variables, including individual preparation; devices and collection tubes (with or without corn trypsin inhibitor, CTI); sample storage time and processing; and the analysis method itself, such as the use of different sources, batches and concentrations of TF and different mixtures of phospholipids (PL), along with proper interpretation of results, may also have an impact on laboratory test values.[16,20,21] Thus, we emphasize that each laboratory should validate these reference ranges according to the protocols, reagents and equipment used. It should also be noted that internal calibration is necessary to correct intraassay variation, while normalization of ETP and peak against a normal control plasma, which was performed in our study, is important for correcting significant temporal variations and for comparing plasma populations within and between institutions.[40]

One limitation of our study was that the TGT was done on PPP that had been prepared by means of centrifugation on whole blood collected in citrate, in a single stage (15 minutes at 2,500 g). This contrasts with the recommendations, i.e. centrifugation in two stages: 2000 g for 5 minutes and 10,000 g for 10 minutes. Loeffen et al.[30] showed that double centrifugation is more appropriate for eliminating the interference of platelets and microparticles, which may contribute to the variability in the results from the TGT. Therefore, it cannot be ruled out that this interference could have occurred in our TGT evaluations. Nonetheless, it is worthwhile quoting the words of Tripodi,[49] regarding the PPP to be tested using the TGT: "Double centrifugation has been advocated, but cannot be used on a regular basis, as it is not the standard practice in general laboratories that work by automated procedures. Furthermore, many samples prepared for general purpose via the standard centrifugation cannot be later used for TGT. An acceptable compromise would be the blood centrifugation at 3,000 g for 15 minutes (controlled room temperature). This procedure would allow getting plasma with minimal residual platelets". On the basis of Tripodi's statement, and given that our samples were centrifuged at 2,500 g for 15 minutes, it is likely that they did not contain many residual platelet fragments. Another limitation is that the intraassay CV was derived from only one duplicate per plate, although the most appropriate method would have been use of triplicates or greater numbers of replicates.

On the other hand, this study presents the strong point of being the first to perform the TGT on a large cohort of Brazilian adults, with selection of a good-quality reference sample. It represents additional progress towards standardization and validation of the TGT via the CAT method. Furthermore, a few laboratories in Brazil have worked with and validated protocols for this technique, using internal controls and standardization of parameters, especially ETP.

The implication for practice from our findings is that they may motivate other similar studies in other parts of the world aimed at investigating potential interference in the TGT from pre-analytical factors and consolidating the TGT reference intervals. Such studies will contribute towards standardization and validation of the TGT and, therefore, facilitate its clinical use.

Lastly, it should be noted that our measurement of thrombin generation among the baseline sample of ELSA-Brasil may stimulate studies with the aim of assessing whether TGT parameters, especially ETP, are associated with clinical conditions, especially chronic non-communicable diseases in the Brazilian population, or whether the TGT can be a predictor of mortality when the thrombin level is above the maximum value of the reference interval.

CONCLUSION

TGT performance was adequate and the proposed reference intervals were similar to those of other studies. Our findings may be useful for consolidating the TGT, through contributing to its standardization and validation.

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Mental health interventions for suicide prevention among indigenous adolescents: a systematic review

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ABSTRACT

BACKGROUND: The legacies of colonization and of policies of forced assimilation continue to be a cause of intergenerational trauma, manifested through feelings of marginality, depression, anxiety and confusion, which place indigenous peoples at increased risk of suicide.

OBJECTIVES: To assess the quality, content, delivery and effectiveness of interventions for preventing suicides among indigenous adolescents.

DESIGN AND SETTING: Systematic review conducted with Cochrane methodology, Campo Grande, Mato Grosso do Sul, Brazil.

METHODS: The Cochrane library, MEDLINE, EMBASE, CINAHL, LILACS and PsycINFO databases were searched for studies published up to February 2021. The following inclusion criteria were used: published in any language; interventions that aimed to prevent suicides among indigenous adolescents; randomized or non-randomized study with a control or comparative group; and validated measurements of mental health problems.

RESULTS: Two studies were identified: one on adolescents in the remote Yup'ik community in south-western Alaska, and the other on Zuni adolescents in New Mexico. Both studies showed evidence of effectiveness in interventions for reducing some of the risk factors and increasing some of the protective factors associated with suicide. High levels of community engagement and culture-centeredness were key anchors of both studies, which ensured that the intervention content, delivery and outcome measurements aligned with the beliefs and practices of the communities. Both studies were judged to have a moderate risk of bias, with biases in sample selection, attrition and inadequate reporting of results.

CONCLUSIONS: The current evidence base is small but signaled the value of culturally appropriate interventions for prevention of suicide among indigenous adolescents.

REGISTRATION DETAILS: The study protocol is registered in the international prospective register of systematic reviews (PROSPERO); no. CRD42019141754.

INTRODUCTION

There are more than 476 million indigenous people in 5,000 cultures living in 90 countries worldwide. Despite composing 5% of the global population, they account for 15% of the extremely poor population.[1] A comprehensive review of 28 indigenous and tribal peoples' health in 23 countries published in 2016 gave nuanced insights into the heterogeneity of their health and wellbeing. There was evidence of poorer health and social outcomes for many indigenous populations, compared with their benchmark populations.[2]

A gap of more than five years in life expectancy at birth (i.e. lower in indigenous than in non-indigenous populations in the same country) was recorded for indigenous populations in Australia, Cameroon, Canada (First Nation and Inuit), Greenland, Kenya, New Zealand and Panama. Infant mortality rates among indigenous infants were more than twice those observed for non-indigenous or national populations in Brazil, Colombia, Greenland, Peru, Russia and Venezuela. Poverty and poor education levels, employment status and access to healthcare services are all important contributors to health disparities.

Despite representing a rich diversity of cultures, indigenous peoples continue to be among the world's most disadvantaged groups, regardless of whether they live in high-income countries (e.g. the Inuit in Canada) or lower-middle income countries (e.g. the Baka in Cameroon). The legacies of colonization and of policies of forced assimilation continue to be a cause of

intergenerational trauma, manifested through feelings of marginality, depression, anxiety and confusion, which place indigenous peoples at increased risk of suicide.[3,4]

Youth suicide is the second leading cause of mortality among individuals aged 15-29 years[5] and it disproportionately affects indigenous youth.[6,7] Indigenous children (5-17 years old) in Australia die from suicide at five times the rate of their non-indigenous peers (10.1 per 100,000 versus 2 per 100,000 in 2013-2017). Similarly, in New Zealand, the suicide rate among Maori youth aged 15-24 years is more than twice that of non-Maori peers (40.7 per 100,000 versus 15.6 per 100,000 among non-Maori youths), and in Canada the rate among Inuit youth is 11 times that of non-indigenous youths on average.[8]

Most interpretations of this gap highlight the persistent social and economic disadvantage experienced by indigenous youth relative to their non-indigenous peers.[9] The epidemic of youth suicide is relatively recent in some cultures, with an increase over time, more so in the latter half of the 20th century. Men account for the majority of suicides, and the 15 to 24-year age group has the highest suicide rate of any age group.[10,11] Furthermore, suicide among indigenous young people may be unreported due to misclassification.

The risk factors include mental health disorders, stressful life events, substance abuse and poor physical health, all of which occur at disproportionately higher rates in indigenous populations.[12,13] Suicide among youth is also known to occur in clusters, and suicidal behaviors (i.e. ideation or attempts) are one of the strongest risk factor for death due to suicide. These behaviors relate to depression, conduct disorders and substance and alcohol abuse.[6] Protective factors include high social support, cultural connectedness, personality factors such as high self-esteem or internal locus of control, and increasing age.[6]

Over the last 20 years, indigenous people's rights have been increasingly recognized by international organizations such as the United Nations Permanent Forum on Indigenous Issues, which also has a permanent forum for youth.[2] The 2030 Agenda for Sustainable Development refers to indigenous people six times: three times in the political declaration, twice in the target under Goal 2 on Zero Hunger (target 2.3) and once in Goal 4 on education (target 4.5). However, many others among the Sustainable Development Goals (SDGs) are relevant for indigenous peoples, particularly those with the focus on reducing inequalities and reducing mortality due to non-communicable diseases (including suicide) by 33% by 2030. Given the vulnerability of indigenous communities, implementation of the SDGs provides opportunities for policy actors to promote initiatives that improve outcomes among indigenous communities.

With such high rates of suicide among indigenous youth,[8] culturally appropriate suicide interventions are urgently needed. Many indigenous populations hold a holistic view of health and wellbeing and interventions need to align with these perspectives and also engage with the economic, socioenvironmental and historical issues that contribute to youth suicide in indigenous cultures.

Only two reviews of indigenous suicide prevention programs have been published so far, which only captured studies published up to 2012.[9,10] In the first review, Clifford, Doran and Tsey[10] reported on nine programs: two among Aboriginal Australians and seven among Native Americans. These programs targeted all ages, and there was a general lack of rigorous evaluation designs, considering that only one study evaluated outcomes using a comparator group. In the second review, Harlow and Clough[9] reported on nine programs targeting youths; five targeted Native Americans; three targeted Aboriginal Australians; and one targeted First Nation Canadians. As in the previous review, poor evaluation designs were noted.

We therefore recognized that there was a general lack of methodologically rigorous study designs across geographically and culturally diverse indigenous populations. Moreover, it was clear that an updated review with a broad eligibility criterion was needed in order to maximize the possibility of capturing any study that attempted to evaluate suicide prevention programs using a comparator group among indigenous adolescents. This review forms part of a larger study that is developing a culturally appropriate intervention for indigenous adolescent mental health in Brazil. In the current review, the aim was to assess the quality, content, delivery and evidence of effectiveness of interventions designed to prevent suicides among indigenous adolescents (aged 10-19 years), so as to inform intervention development and implementation of future prevention initiatives.

OBJECTIVE

The objective of this study was to synthetize the scientific evidence on suicide prevention programs targeting indigenous youths. Our principal research question was: what interventions, including single or multi-component interventions, prevented suicides (or not); and why did they work (or not)?

METHODS

This review adhered to the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) guidelines.[14] The study protocol was registered with the international prospective register of systematic reviews (PROSPERO), under registration number CRD42019141754.

Types of studies

We searched for any randomized or non-randomized study that had a control or comparative group.

Types of participants

The participants searched for were adolescents aged 10-19 years who self-identified as indigenous peoples and were accepted as

such by their community.[2] We were guided by the policy definition developed by the International Labor Organization in 1989 and adopted by the United Nations. This characterizes indigenous peoples as tribal peoples in independent countries whose social, cultural and economic conditions distinguish them from other sections of the national community and whose status is regulated wholly or partly by their own customs or traditions or by special laws or regulations; and peoples in independent countries who are regarded as indigenous because of their descent from the populations who inhabited the country, or a geographical region to which the country belongs, at the time of conquest or colonization or the establishment of present state boundaries and who, irrespective of their legal status, retain some or all of their own social, economic, cultural and political institutions.[15,16]

Types of interventions

We searched for in-person or e-health interventions that targeted young indigenous people anywhere in the world. We considered a wide range of delivery channels (e.g. in person, online or phone), different practitioners (healthcare practitioners, teachers or lay healthcare providers) and sectors (i.e. primary, secondary and tertiary-level healthcare, education or guardianship councils).

Types of outcome measurements

We searched for the following primary outcomes: self-injury acts, suicidal ideation, suicide attempts and death due to suicide. We also searched for the following secondary outcomes: wellbeing/quality of life; and social functioning including educational outcomes.

Electronic searches

We searched for experimental studies with a comparator group that were designed to prevent suicide among indigenous adolescents. The following electronic databases were searched up to February 10, 2020: Cochrane Library (up to February 10, 2020), MEDLINE (Medical Literature Analysis and Retrieval System Online) (1966 to February 10, 2020), EMBASE (Excerpta Medica Database) (1974 to February 10, 2020), CINAHL (Cumulative Index to Nursing and Allied Health Literature) (1981 to February 10, 2020), LILACS (Literatura Latino-Americana e do Caribe em Ciências da Saúde) (1982 to February 10, 2020) and PsycINFO (1887 to February 10, 2020).

The organization of the search strategy followed the Cochrane recommended strategy of PICO (Population, Intervention, Context and Outcomes). We included indigenous AND interventions AND mental health factors/problems. The full search strategy shown in **Table 1** was adapted for each electronic database. An additional search using the same terms was carried out in Google Scholar. The search was limited to human studies and had no language restrictions. Reference lists of all systematic reviews were reviewed to identify additional relevant citations.

We did not use any language restrictions. If articles were not in English, Italian, Arabic or Portuguese (the native languages of the present authors), we used academic networks (e.g. Cochrane) to translate the critical parts (methods and results) to enable screening of abstracts.

Searching other resources

We cross-checked references from other systematic reviews and searched for references suggested by specialists in the area.

Inclusion criteria

The inclusion criteria were as follows: randomized or non-randomized studies that had a control or comparative group; participants who were adolescents aged 10-19 years and self-identified as indigenous; presence of mental health problems in this population as defined in the Diagnostic and Statistical Manual of Mental Disorders 5th edition (DSM-V); use of in-person/e-health interventions that were delivered to indigenous adolescents anywhere globally. Consideration was given to all delivery channels (e.g. in person, online or phone), different practitioners (healthcare practitioners, teachers, lay healthcare providers) and different intervention sectors (i.e. primary, secondary and tertiary-level healthcare, education or guardianship councils).

Exclusion criteria

The exclusion criteria were as follows: the aims or methodology of the study did not fit the inclusion criteria; the study included populations that were not indigenous or did not make any distinction between indigenous and non-indigenous populations; or the study excluded adolescents, or no data was provided on adolescents who were included in the study.

Table 1. Search terms used and adaptation to each database

(Indigenous or Indigenous or native* or Native* or Māori or Maori or Aborigin* or aboriginal or "Torres Strait Island*" or "torres strait island*" or "first nation*" or "first people*" or Inuit or Metis or Métis or ethnic* or "population groups") AND (intervention* or program* or treatment* or treat* or therap* or service* or prevent* or diversion* or initiative*) AND (wellbeing or "well being" or mental or depress* or anx* or suicide* or trauma* or alcohol* or drinking or cannabis or cocaine or methamphet* or amphet* or substance* or addict* or heal* or empower* or grief or loss* or stress* or psychosis or psychoses or psychotic or resilien* or recovery or "mental health" OR schizophrenia or mania or mood or internalizing or externalizing or affective or behavioural or drugs or "crack cocaine" or addiction or "mental illness" or happiness or emotion* or psych* or psychology) adapted to every other database.

Data collection and analysis

Two review authors (AJG, CE) independently assessed all studies identified from the database searches, by screening titles and abstracts using the EndNote Web tool (www.myendnoteweb.com). A third review author (SH) resolved any disagreements. The reasons for including or excluding trials were recorded. Next, AJG and CE independently assessed the full-text reports for inclusion, against the selection criteria. Afterwards, these two authors discussed the results from the selection process and made a consensual decision on which articles were to be included/excluded.

Data extraction and management

Two review authors (AJG, CE) independently extracted data from the studies included, using a standard data extraction form. We aimed to include qualitative data using a narrative synthesis of barriers and facilitators.

A standardized, pre-piloted form was used to extract data from the studies included, for assessment of study quality and evidence synthesis. The format included the following features:

- Study details: aim, study design including whether a feasibility study was conducted in collaboration with the community in order to co-develop the design, design details (including evaluation), country in which study was conducted, details on location of intervention delivery (i.e. city or community), target condition/risk factor (i.e. subthreshold symptoms and experience of child maltreatment).
- Participants: sample size (intervention and control groups at baseline and follow-up), sociodemographic characteristics (e.g. age, gender, ethnicity and socioeconomic status) and attrition from the study.
- Intervention details: description of intervention including frequency and duration of treatments/sessions, mode of delivery (face to face or internet), format (one to one or group), culturally appropriate content and cost of intervention.
- Delivery of the intervention: setting in which intervention was delivered (school, home or healthcare practice), who delivered the intervention (i.e. medical doctor, nurse, psychologist, teacher, lay health worker, peer promotion, etc.), whether the intervention was delivered by one practitioner or a team of individuals or online, the fidelity of implementers to the protocol, culturally appropriate modes of delivery and whether there was intersectoral collaboration (i.e. between the health and education sectors or guardianship councils).

The RE-AIM framework was used to enhance the assessment of program elements that could improve sustainable adoption and implementation of effective, generalized/localized, evidence-based interventions.[17] RE-AIM targets the *'Reach'* of the target population; *'Effectiveness or Efficacy'* of the intervention (impact of an intervention on important outcomes, including potential negative effects, quality of life and economic outcomes); *'Adoption'* by target staff, settings or institutions; *'Implementation'* in terms of consistency, costs and adaptions made during delivery; and *'Maintenance'* of intervention effects among individuals and settings over time.

Assessment of risk of bias in studies included

In the light of the well-documented limitations of the use of 'western' methods in an indigenous context, our critical appraisal included identification of culturally appropriate methodologies such as Storytelling and Community-Based Participatory Research, with the inclusion of indigenous peoples in the research process in a way that was respectful and reciprocal. We included comparator group designs, as well as randomized study designs, in recognition that the former may be more appropriate for the indigenous context.[18,19]

Two review authors (AJG, CE) independently assessed the risk of bias of the studies included using the Risk Of Bias In Non-randomized Studies - of Interventions (ROBINS I) tool for non-randomized studies and the Risk of Bias tool 2.0 for randomized studies, which are available in the Cochrane Handbook for Systematic Reviews of Interventions, version 6.3 (Cochrane Handbook, Oxford, United Kingdom, and Melbourne, Australia).[20]

Measurements of treatment effect

As reported in our published protocol, we planned to synthetize dichotomous or continuous data. However, the two studies included did not report the same outcome. Hence, no measurements of treatment effect were calculated.

Unit of analysis, missing data, assessment of reporting biases and heterogeneity

We took the individual to be the unit of analysis. We planned to do the following: email the corresponding authors of each study regarding missing data; conduct a meta-analysis; assess inconsistencies between studies using the I^2 statistic (percentage of total variation across studies that was due to heterogeneity rather than chance); contact the trial authors to clarify the information if mismatches between study protocols and reports were identified; and explore the impact of including such studies by conducting a sensitivity analysis. However, these actions were unnecessary because of the small number of studies.

Data synthesis

We had planned to present the data separately for randomized and non-randomized studies, and to do a meta-analysis on the trials if combination of data on the outcomes was possible. Given that only two studies were included, we present a narrative analysis on the individual studies. We had planned to produce a

'Summary of findings' table using the five GRADE assumptions (study limitations, consistency of effect, imprecision, indirectness and publication bias).[21,22] However, subgroup analysis, investigation of heterogeneity and sensitivity analyses were not possible given the small number of studies included.

RESULTS

The search identified a total of 1,498 studies and three systematic reviews; 579 studies were duplicates. A total of 922 studies were screened for titles and abstracts. Of these, a total of 41 studies were read in full. Of these, 39 studies were excluded because they did not meet the criteria regarding study design or population studied; or because they were ongoing studies. The authors of the three ongoing studies were contacted through email, and they confirmed that the studies were either at data collection or analysis stage and that we would be informed about their publication. Thus, a total of two studies were included, and data were extracted and critically appraised. **Figure 1** shows the results from the screening process.

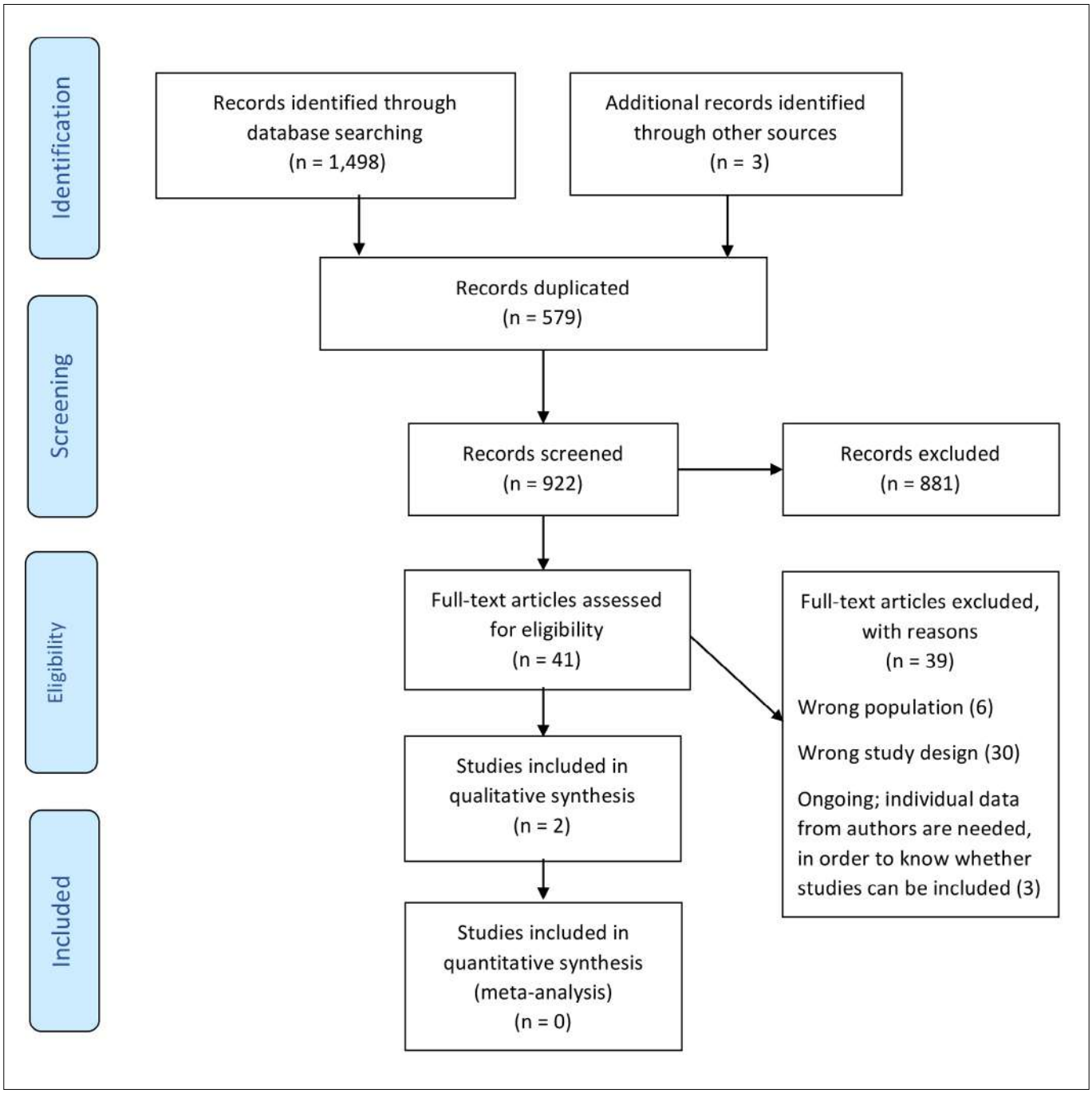

Figure 1. Study flow diagram.

The two studies included[23,24] involved 364 adolescents aged 12-18 years, were published in English between 1995 and 2018 and met all the inclusion criteria. The proportions of females were 64% in the study by LaFramboise et al.[24] and 40% in the study by Allen et al.[23] LaFromboise et al. conducted a quasi-experimental study on adolescents of the Zuni population of New Mexico, United States.[24] Allen et al. conducted a randomized controlled study on adolescents in the remote Yup'ik community in south-western Alaska, United States.[23] Both studies used a group of indigenous adolescents as controls and aimed to assess the effectiveness of suicide prevention programs among indigenous adolescents. The intervention by LaFramboise et al. was based on Bandura's cognitive social theory[24] and used a wait-list control group to assess effectiveness. Allen et al. used a community-based framework, delivered multiple modules and assessed the effectiveness of each of them regarding suicide prevention.[23]

Content and delivery

The *'Zuni Life Skills Curriculum*', which was the name adopted for the program by LaFramboise et al.,[24] consisted of developing life skills to address cognitive and behavioral factors relating to suicidal behavior. The Zuni life skills curriculum was structured around seven main units: building self-esteem; identifying emotions and stress; increasing communication and problem-solving skills; recognizing and eliminating self-destructive behaviors; information about suicide; suicide intervention training; and setting of personal and community goals. The program was adapted to align with the values, beliefs and attitudes of the Zuni people. It was delivered to students three times a week for 30 weeks by two non- Zuni female teachers and two trained Zuni male teachers. Fidelity to the protocol for delivery of the program was observed through periodic observations by a project coordinator. Students were assessed by a research team member before and after the intervention. The indigeneity of the research team member was not reported.

The program by Allen et al.[23] was called '*Qungasvik*', which is a Yup'ik word for toolbox. It was conceived as a multi-level community-strengthening and culturally appropriate intervention for rural Yup'ik adolescents. The intervention was based on local practices of Yup'ik communities with the aim of developing motives for life and sobriety. The modules addressed issues at different levels (individual, family and community) and were delivered in one or more sessions of 1-3 hours. Each module promoted two to four protective factors for protection that had been identified in a culture-specific model of protection: individual characteristics (mastery-friends and mastery-family); family characteristics (cohesion, expressiveness and conflict subscales); community characteristics (support and opportunities, as two protective community subscales); peer influences (two scales from the American Drug and Alcohol Survey); reflective processes; and reasons for life. The same program was delivered in two Yup'ik communities. The Qungasvik intervention manual was not prescriptive. It outlined 26 modules, along with a process for community adaptation to local customs and circumstances, taking into account the current season and advice from community members. The authors observed that adaptations were greater with community ownership, with ecological alignment to the context of remote communities in the region.

The characteristics of the studies included can be seen in **Table 2**.[23,24]

Methodological quality assessment - Risk of bias for non-randomized studies

Overall bias

Both studies were judged to have an overall moderate risk of bias due to the following factors. *Bias due to confounding:* The study by LaFramboise et al. was classified as presenting moderate risk of bias, since they matched students before the intervention, in order to reduce the bias from confounding, and used a wait-list.[24] The study by Allen et al.[23] was classified as presenting low risk of bias, since the intervention was adapted to individual, family and community levels, therefore reducing the risk of bias. *Bias in selection of participants into the study:* Both studies were classified as presenting serious risk of bias. LaFramboise et al. lost 24% of the students and Allen et al. lost 30% of students during the follow-up.[23,24] *Bias in classification of intervention:* The interventions were well described in both studies and hence classified as presenting low risk of bias. *Bias due to deviations from intended interventions:* Both studies were classified as presenting low risk of bias. LaFramboise et al. reported that important co-interventions were balanced across intervention groups, and that there were no deviations from the intended interventions that were likely to impact on the outcome.[24] Allen et al. provided outlines of the 26 modules, which were adapted to the community that received them. *Bias due to missing data:* Both studies were at serious risk of bias due to loss to follow-up. *Bias in measurement of outcomes:* Both studies were at moderate risk of bias. Outcome assessments were comparable across the intervention and comparator arms, but the outcome measurement was influenced by knowledge of the intervention received by study participants. *Bias in selection of the reported result***:** Both studies were at moderate risk of bias. Although free from bias regarding selective reporting outcomes, neither study took account of missing data from participants. **Table 3** presents a risk of bias summary, in which the present authors' judgements about each risk of bias item are given.

Effectiveness

Both studies observed a positive effect with regard to reducing suicide risk. LaFromboise et al. found that a cognitive social

Table 2. Characteristics of studies included

LaFramboise and Howard-Pitney[23]	
Country	United States - New Mexico
Community	Zuni community
Objective	To evaluate the effectiveness of a life-skills-focused suicide prevention program in reducing behavioral and cognitive factors identified as correlates of suicidal behavior (Zuni life skills curriculum).
Design	Quasi-randomized study with a control and intervention arm. Students were not randomized to each group
Population	**Students:** 128 students in the Zuni Public High School. The sample was 64% female and 36% male (83 girls, 45 boys), and ages ranged from 14 to 19 years (mean 15.9 years). Scores on the Suicide Probability Scale before intervention suggested that 81% of these students were in the moderate to severe ranges of suicide risk. 40% of students reported that a relative or friend had committed suicide. With regard to their own suicidal behavior, 18% reported having attempted suicide. Of those who had attempted, 79% had attempted two or more times, 70% had tried within six months before the intervention, 17% needed a medical visit and 22% had told no one about the attempted suicide.
Instruments	Suicide Probability Scale (SPS);[25] Hopelessness Scale (HS);[26] Indian Adolescent Health Survey (IAHS) seven-point Likert scale for self-efficacy evaluation (7PL);[27] six-point Likert scale for judgment of behavioral observation (6PL-BO); and six-point Likert scale for peer ratings (6PL-PR).
Theoretical framework	Bandura's cognitive social theory using roleplay. Students were asked to enact four roleplays with a confederative client, while being videotaped. Following two warm-up roleplays, each student roleplayed two scenarios concerning adolescent suicide with the same confederative client. Both scenarios involved a situation in which suicide intervention was appropriate; however, the second roleplay presented a more serious and imminent suicide threat. Roleplays were presented in counterbalanced order, and each lasted approximately 10 min. All suicide scenarios were rated independently by two judges (American Indian), blind to group assignment, who were trained as a team for 18 hours to apply the rating criteria uniformly. Peers were asked to rate their classmates on the extent to which they were able to intervene in a suicidal situation.
Content and delivery	The *Zuni Life Skills Curriculum* consists of a program focused on life skills and cognitive and behavioral factors related to suicidal behavior. This program was structured around seven major units: building self-esteem; identification of emotions and stress; increasing communication and problem-solving skills; recognition and elimination of self-destructive behaviors; receiving suicide information; receiving suicide intervention; and setting personal and community goals. The curriculum was developed to align with Zuni values, beliefs and attitudes. This program was presented to students three times a week for 30 weeks by teachers and trained cultural resource persons. **Teachers:** Two non-Zuni female teachers were chosen to deliver the curriculum. They were aided by two Zuni male community members (a curriculum specialist and a mental health technician). **Confederative clients:** Two female university students from the Menominee and Choctaw tribes participated in the behavioral assessment of the roleplay of a person with suicidal intent. **Judges:** Two American Indian postgraduate students served as trained judges to evaluate the problem-solving and suicide intervention skills in a behavioral assessment using ten six-point Likert scale items ranging from 1 (strongly disagree) to 6 (strongly agree). **Peer ratings:** After the evaluation, a subsample of 62 students (28 male and 34 female students), evenly divided between intervention and non-intervention groups, was randomly selected from the total sample for participation in a 30-minute behavioral evaluation. Also, after the evaluation, peer ratings of classmates' suicide intervention and problem-solving skills were obtained. In the behavioral observation, students were asked to enact four roleplays with a confederative client, while being videotaped. Following two warm-up roleplays, each student roleplayed two scenarios concerning adolescent suicide with the same confederative client. Both scenarios involved a situation in which suicide intervention was appropriate; however, the second roleplay presented a more serious and imminent suicide threat. Roleplays were presented in counterbalanced order, and each lasted approximately 10 minutes.
Outcomes	Psychological outcomes: hopelessness, suicide probability and depression; self-efficacy skills: suicide prevention, problem solving, active listening, anger management and stress management; behavioral observation; and peer ratings. Fidelity to the curriculum was monitored through random classroom observations by an on-site intervention coordinator, which took place on a bimonthly basis in each intervention class.

Continue...

Table 2. Continuation.

LaFramboise and Howard-Pitney[23]	
Results	Out of the 128 students assessed before the intervention, 98 (76%) were evaluated after the intervention. A between-groups comparison of the descriptive variables assessed before the evaluation (gender, age, grade, suicide probability, suicide attempt and other suicide history variables) was conducted. These evaluations indicated that the 30 students lost to follow-up were not significantly different from the 98 students who completed both the pre and the post-intervention evaluation. To create equivalent groups pre-intervention, the students were paired according to hopelessness and probability of suicide. Thirty-one pairs were formed and analyzed pre and post-intervention. The means and standard deviations for each post-intervention outcome were: • Hopelessness (the lower the score, the better): $\bar{x}$ = 3.5, SD = 2.6 for intervention; versus $\bar{x}$ = 4.6, SD = 2.9 for no intervention (P = 0.05); • Suicide probability scale (the lower the score, the better): $\bar{x}$ = 54.3, SD = 11.6 for intervention; versus $\bar{x}$ = 58.9, SD = 13.0 for no intervention (P = 0.07); • Depression (the lower the score, the better): $\bar{x}$ = 3.3, SD = 0.9 for intervention; versus $\bar{x}$ = 3.4, 1.1 for no intervention (*ns*); • Suicide prevention (the lower the score, the better): $\bar{x}$ = 4.7, SD = 0.8 for intervention; versus $\bar{x}$ = 4.7, SD = 1.2 for no intervention (*ns*); • Active listening (the higher the score, the better): $\bar{x}$ = 4.6, SD = 0.9 for intervention; versus $\bar{x}$ = 4.5, SD = 1.0 for no intervention (*ns*); • Anger management (the lower the score, the better): $\bar{x}$ = 5.1, SD = 1.1 for intervention; versus $\bar{x}$ = 4.5, SD = 1.5 for no intervention (P = 0.03); • Stress management (the lower the score, the better): $\bar{x}$ = 4.5, SD = 0.9 for intervention; versus $\bar{x}$ = 4.5, SD = 1.6 for no intervention (*ns*). Roleplays by 28 of the 62 paired students (14 in the intervention group and 14 in the non-intervention group) were evaluated by the judges. Significant improvements in suicide intervention and problem-solving were reported for the intervention group. In the peer evaluation, there were no perceived significant differences for these skills.
Main findings	This study found that merging a socially cognitive life-skills approach with peer support was effective for reducing some of the risk factors and increasing some of the protective factors associated with suicide.
RE-AIM framework	**Reach:** 128 students measured pre-intervention and 98 measured post-intervention, after eight months of intervention. The sample was 64% female and 36% male (83 girls and 45 boys), and ages ranged from 14 to 19 years, with a mean age of 15.9 years. **Effectiveness:** Theory-based intervention improved suicide intervention and problem-solving skills. **Adoption:** Extensive community input during the development of the curriculum. Trained Zuni members helped to deliver program. **Implementation:** Fidelity to the curriculum was observed bi-monthly by on-site intervention coordinator. Costs of the intervention were not reported. **Maintenance:** Intervention was maintained over 30 weeks of the school year. No evaluation post-project was reported.
Allen et al.[22]	
Country	United States - Alaska
Community	Yup'ik community
Objective	To compare the effectiveness of high-intensity *Qungasvik* intervention in one community (treatment), with a lower-intensity intervention in a second community (comparison) that implemented fewer modules of the same intervention.
Design	Dynamic wait-list design
Population	**Community 1:** fifty-four Yup'ik youths (23 males and 31 females) aged 12-17 years (mean 14.24 years; SD = 1.72) who were community residents. **Community 2:** seventy-four Yup'ik youths (54 males and 20 females) aged 12-17 years (mean 14.62; SD = 1.82) who were community residents.
Instruments	Multi-level theory-of-change measurement model (MTCM), based on the assumption that change in intermediate variables at the community, family, peer and individual levels leads to change in two ultimate outcome variables measuring protection from suicide and alcohol-use disorder risk.
Theoretical framework	Using a community-based participatory framework, Yup'ik communities over the past 25 years have guided a melding of these two worlds, by generating the *Qungasvik* "toolbox", a cultural intervention based on a local indigenous theory of personal and community change, and then collaborating with university researchers to describe it using the methods of western science. In contrast to most American Indian and Alaskan Native preventive interventions that are problem-focused and individual-level, this intervention is strengths-based and multi-level.

Continue...

Table 2. Continuation.

Allen et al.[22]	
Content and delivery	'*Qungasvik*', a Yup'ik word for toolbox, is a strengths-based, multi-level, community/cultural intervention for rural Yup'ik youths. The intervention is grounded in local practices that are distinctive to rural Yup'ik communities, in order to promote modules on reasons for life and sobriety. The modules focused on issues relating to the individual, family or community level, and were delivered in one or more one to three-hour sessions. Each module promoted two to four out of 13 protective factors that had been identified in a culture-specific model of protection: individual characteristics (mastery-friends and mastery-family); family characteristics (cohesion, expressiveness and conflict subscales); community characteristics (support and opportunities, as two protective community subscales); peer influences (two scales from the American Drug and Alcohol Survey); reflective processes; and reasons for life. The same program was delivered in two Yup'ik communities. The *Qungasvik* intervention manual is not prescriptive. It outlines 26 modules, along with a process for community adaptation to local customs and circumstances, taking into account the current season and advice from community members. The co-researchers observed that the adaptive process resulted in greater community ownership and intervention that was more ecologically valid for the distinctive characteristics of each remote community in the region.
Outcomes	**Intermediate outcomes:** individual characteristics (mastery-friends and mastery-family); family characteristics (cohesion, expressiveness and conflict subscales); community characteristics (support and opportunities, as two protective community subscales); and peer influences (two scales from the American Drug and Alcohol Survey). **Ultimate outcomes:** Reflective Process (RP), consisting of the youths' reflections on the potentially negative consequences from drinking alcohol that have elements of culture-specific meaning; and Reasons for Life (RL), which is a cultural adaptation and strengths-based extension of the Brief Reasons for Living Inventory for Adolescents.
Results	In community one, the youths attended a mean of 6.78 modules (SD 6.76), while in community two, the youths attended a mean of 2.31 modules (SD 3.24). Mixed-effects regression models contrasted the treatment and comparison arms, and identified that the treatment had a significant effect on Reasons for Life ($d = 0.27$; $P < 0.05$) but not on Reflective Processes, thus favoring the greater intervention that was delivered in community one. *Qungasvik* aimed to promote protection from co-occurring suicide and alcohol risk, but no significant findings were observed regarding alcohol protection, and there were no differences in intermediate outcomes between the communities. The more intensive intervention (compared with the less intensive intervention) resulted in a positive impact on RL ($d = 0.28$; $P < 0.05$), but not on RP or intermediate variables. This was interpreted as a finding that the intensive intervention produced significantly greater growth in protection from suicide, but not for alcohol risk. The analyses found that there was significant growth over time within the intensive group, but not in the less intensive intervention group, regarding RL ($d = 0.43$; $P < 0.05$) but not in relation to RP. There was also significant growth within the intensive group regarding individual characteristics ($d = 0.34$; $P < 0.05$), but not in relation to family or community characteristics. Peer effects grew in the less intensive group, but not in the intensive intervention group ($d = 0.50$; $P < 0.01$).
Main findings	The *Qungasvik* intervention had a protective effect on suicide risk among rural Yup'ik Alaskan Native youths. A high-intensity version of the *Qungasvik* intervention produced significantly greater intervention impact than did the low-intensity intervention. A protective effect was found against the risk of suicide among rural young native Yup'ik with improved mean scores for individual characteristics, family characteristics, community characteristics, reasons for life and reflective process.
RE-AIM framework	**Reach:** 54 participants (23 males and 31 females) in community 1; and 74 participants (54 males and 20 females) in community 2. Twelve percent of the target population was lost to follow-up. The mean age and SD in community 1 were 14.24 (1.72); the mean age and SD in community 2 were 14.62 (1.82). **Effectiveness:** The theory-based *Qungasvik* intervention had a protective effect against suicide risk among rural Yup'ik Alaskan Native youths. **Adoption:** Community adaptation to local customs and environment, with advice from people with cultural knowledge and leadership (e.g. community elders). **Implementation:** Systematic process for ensuring adherence to protocols, including planning of activities as a group, identifying people with expertise to carry out the activity and debriefing on where the activity succeeded in its goals and what has been learned. **Maintenance:** Modules focused on individual, family, and community level factors and were delivered over a one-year period. No evaluation post-project was done.

Legend: Suicide Probability Scale **(SPS)**[25]: an instrument used to measure hopelessness, hostility, negative self-evaluation, and suicidal ideation; Hopelessness Scale **(HS)**[26]: an inventory used to assess negative expectations about the future; Indian Adolescent Health Survey **(IAHS)**[27]: a standardized instrument for evaluation of depression among North American indigenous adolescents; seven-point Likert scale for self-efficacy evaluation **(7PL)**: a seven-point Likert scale for evaluation of self-efficacy for a number of skills taught in the curriculum (suicide prevention skills; active listening; problem-solving; anger management; and stress management); six-point Likert scale for judgment of behavioral observations **(6PL-BO)**: an observational evaluation instrument used to judge the extent to which students were able to demonstrate suicide intervention skills and engage in problem-solving; six-point Likert scale for peer ratings **(6PL-PR)**: an observational evaluation instrument used by peers to judge the extent to which students were able to demonstrate suicide intervention skills and engage in problem-solving. $\bar{x}$: mean; SD: standard deviation.

approach to life skills delivered by teachers was effective for reducing some of the risk factors (e.g. hopelessness, suicide likelihood or depression) and for increasing some of the protective factors (e.g. stress and anger management) in relation to suicide.[23] In Allen's study, a mixed model of comparative effectiveness for each outcome was used, comprising the following: individual characteristics, family characteristics, community characteristics, peer influences, reasons for life and reflective Processes. These combined four variables called time, community, protection and time x community.[23] Thus, the authors found that there were important effect sizes for individual characteristics (Cohen's d = 0.59; P < 0.01); family characteristics (Cohen's d = 0.67; P < 0.01); community characteristics (Cohen's d = -0.67; P < 0.01); and community protection (Cohen's d = 0.93; P < 0.01). For peer influences, there was no change across the results: reasons for life in community (Cohen's d = 0.36; P < 0.05); community protection (Cohen's d = 0.88; P < 0.01); time x community (Cohen's d = 0.27; P < 0.05); reflective processes in community (Cohen's d = -0.49; P < 0.01); and community protection (Cohen's d = 0.41; P < 0.05).

DISCUSSION

Summary of evidence

The current review aimed to assess the quality, content, delivery and evidence of effectiveness of interventions that were designed to prevent suicides among indigenous adolescents (aged 10-19 years). We only identified two studies that included impact evaluation using a comparator arm. Both of these studies were theoretically underpinned and aligned with the cultural beliefs and practices of the communities. They showed promising results for the prevention of suicide and provided detailed descriptions of the content and delivery of the interventions. The follow-up period was more than six months, but there was limited reporting of the long-term impact or of cost-effectiveness of the interventions.

Several strategies were used in the studies included, comprising intervention co-development with communities, intervention delivery by cultural resource individuals and external workers, capacity building among teachers and culturally adapted roleplay for problem-solving with peer evaluation. These strategies have also been used in studies that did not report impact evaluations but had positive assessments.[28,29] Community engagement, empowerment of communities via capacity development, and alignment of programs to histories and sociocultural contexts have been key learnings from numerous studies on indigenous healthcare.[9] Culturally secure mental health school and/community programs are particularly important for indigenous young people, given the general lack of accessible primary healthcare services in indigenous communities, which has been a key driver for migration to urban areas, where discrimination can lead to a chain of stressful life events, such as loss of freedom, rejection, stigmatization and violence.[30-33]

The studies included strongly signaled the possibility of developing effective interventions that align with the cultural contexts of indigenous communities, in order to reduce suicide mortality. The *Zuni Life Skills Curriculum* used the values and knowledge of the Zuni community in constructing the intervention, which was attributed by the authors of that study to be responsible for the success of the intervention. Not only were young people receiving an intervention but also positive mental health was being promoted in their community through identification of emotions, development of problem-solving skills and building of self-esteem. The *Qungasvik* intervention was modeled on the Yup'ik culture, to promote culturally specific protective practices at the individual, family and community levels, which would align with indigenous perspectives on holism and wellbeing that had been passed down through the generations, including beliefs in the unity of mind, body and spirit.[34]

Valuing local indigenous perspectives in interventions is essential since the processes of illness and hopelessness in indigenous populations are often attributed to cultural disengagement from traditions, with the consequent loss of strong cultural identity. Cultural identity can promote resilience, protect against mental health symptoms and buffer against distress prompted by discrimination. Another relevant point to emphasize is that both studies successfully incorporated a strong participatory element whilst maintaining scientific rigor for impact evaluation, using a comparator group/wait list comparison group.

Randomized controlled trials can be seen to be incongruous within the indigenous context, which is strongly centered

Table 3. Risk-of-bias summary: the present authors' judgements about each risk-of-bias item

	Bias due to confounding	Selection of participants	Classification of intervention	Deviations from intended interventions	Bias due to missing data	Bias in measurement of outcomes	Bias in selection of the reported result	Overall bias
LaFramboise and Howard-Pitney[23]	Moderate	Serious	Low	Low	Serious	Moderate	Moderate	Moderate
Allen et al.[22]	Low	Serious	Low	Moderate	Serious	Moderate	Serious	Moderate

on communities and not individuals. The value of participatory research in indigenous contexts has been central to decolonizing research methodologies and has the potential for sustained changes. Decolonization is an ongoing process of becoming, unlearning and relearning, regarding who we are as researchers and educators, and taking responsibility for participants.[35]

The Yup'ik community has a subsistence economy augmented by a limited number of tribal, state and federal jobs, primarily in government, healthcare and schools, in contrast with the rest of the United States national population. This community has greater commuting difficulties, due to the region's characteristics and lower government investment. Additionally, it has lower per capita income and higher alcohol abuse and suicide rates than the general population of the United States.[36]

Official data indicate that approximately 10% of the New Mexico population is indigenous and that 34% live in extreme poverty. Historical prejudice against the indigenous population and lack of control over its lands, livelihoods and future have been highlighted in other studies as a key contributor to poor mental health.[30]

Limitations of the review and the field of knowledge

This review found that the evidence base for rigorous evaluation of the impact of interventions for preventing suicide among indigenous adolescents is sparse. Most of the studies that were ineligible for the present review provided rich detail regarding potentially valuable intervention processes but did not conduct evaluations. These findings highlight the need for more evaluation, in order to build a basket of effective strategies with cultural appeal for indigenous populations.

Despite the overall inadequacy of the data, there is little doubt about the marked mental health disparities experienced by indigenous peoples globally.[37] For example, among indigenous Australians, the rates of anxiety, substance use and any mental disorder were found to be 3.8-fold, 6.9-fold and 4.2-fold higher, respectively, than those of the general Australian population.[38] It is also important to note that the rates were lower among those living on traditional lands in indigenous reserves and in remote areas than among those living in mainstream communities.

Poor mental health among indigenous peoples has been correlated with the historical trauma from colonization and the loss of traditional lands due to climate change and/or misappropriation of their lands.[39] This has exposed them to multiple risk factors for poor mental health, including dislocation of kinship networks, discrimination, poverty and isolation, which have led to high rates of substance abuse and family violence.[40]

However, cultural heterogeneity among indigenous peoples cautions against the generalizability of the strategies reported from the two studies in this review. For example, there are around 300 different ethnic groups in Latin America and the Caribbean that speak around 274 languages. An understanding of the different socioeconomic, cultural and political contexts and processes that affect mental health disorders among indigenous peoples is critical to informing culturally responsive interventions.[37-40]

CONCLUSION

The evidence organized in this review is descriptive and comes from two studies. The risk of bias of each study was considered to be moderate, in that there was insufficient reporting of how the intervention engaged with some key structural determinants (e.g. poverty and gender) and the pathways towards achieving an impact were insufficiently evaluated. High levels of community engagement and culture-centeredness were key anchors of both studies, and these elements provide valuable lessons for future studies on suicide prevention among indigenous adolescents.

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Reactions of physicians in the state of São Paulo to the use of telemedicine during the SARS-CoV-2 pandemic: cross-sectional study

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ABSTRACT

BACKGROUND: Telemedicine can be a component of integrated healthcare practices and its use is not a recent phenomenon around the world. In Brazil, its more widespread use began during the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic, through extraordinary authorization from the Brazilian Ministry of Health.

OBJECTIVES: To describe some aspects of use of teleconsultation among a sample of physicians in the state of São Paulo during the SARS-CoV-2 pandemic.

DESIGN AND SETTING: Cross-sectional study based on a survey conducted by the São Paulo Medical Association (Associação Paulista de Medicina, APM) on medical practice during the SARS-CoV-2 pandemic between December 18, 2020, and January 18, 2021.

RESULTS: This survey generated responses from 2,052 physicians. Of these, 981 (47.8%) reported not practicing any form of telemedicine. Among those who reported practicing telemedicine, 274 (28.4%) reported not receiving remuneration directly for the attendance provided and 225 (23.3%) reported receiving remuneration equal to what they would have received from face-to-face consultations. Regarding the professional linkage of the physicians who undertook telemedicine attendance, the majority (499; 51.8%) only attended private patients. Regarding the resources used to provide telemedicine attendance, most of the respondents used specialized digital platforms (594; 61.6%), electronic health records (592; 61.4%) and electronic prescriptions (700; 72.6%).

CONCLUSION: This study demonstrates that important issues such as professional remuneration, use of electronic platforms and medical records, ensuring data protection and relationships between physicians and other stakeholders still need to be better defined, in order to achieve the desired scale and reach the outcomes defined.

INTRODUCTION

Telemedicine can be defined as provision of healthcare in which the participants are separated in time and/or space, while telehealth is of broader nature, involving all health-related telecommunications applications.[1] Telemedicine thus involves use of interactive information and telecommunications technologies, combined with computer systems, telemetry and biosensors to provide quality healthcare services that are not physically face-to-face and are outside the clinical-hospital space. It thus enhances the relationship between healthcare professionals and their patients, through eliminating geographical and time barriers.[2]

On the other hand, remote consultation can be defined as care provision mediated by technologies in which professionals and patients are in different physical spaces. It covers the same characteristic steps and responsibilities as in face-to-face attendance, including subjective, objective and diagnostic assessments, therapeutic proposals, requests for complementary tests, guidance and planning of care.[3]

The use of telemedicine is not a recent phenomenon: there have been reports of its use since the 1960s, but its more widespread utilization began with the development of the internet in the 1990s.[4] For example, the American health plan and health provider Kaiser Permanente reported that in 2018, 47 million of the medical consultations they provided and 31 million prescriptions were issued online.[5] The company Willis Towers Watson assessed cost effectiveness indicators in a study with the title "Current telemedicine technology can mean big savings," published in 2014.[6]

The study suggested that telehealth had the potential to save more than $6 billion a year for companies in the United States. It constitutes an important tool within healthcare and there is evidence that it has an economic impact on national healthcare systems.[7,8]

Use of telemedicine as a component of integrated healthcare practices, such as preventive and chronic condition management programs, can be effective for clinical and administrative outcomes. Its attributes include greater availability of attendance, access to electronic medical records, online requesting of diagnostic tests, electronic prescription, availability of scientific material to support clinical decisions and reduction of the average duration of consultations. Moreover, as healthcare services become organized increasingly through integrated logic models, telemedicine ceases to be a support service and starts to have a cross-cutting role in all care.[9]

The Declaration of the 58th General Assembly of the World Medical Association (WMA) in Copenhagen, Denmark, published in 2007 and amended by the 69th WMA General Assembly, held in Iceland in 2018, defines telemedicine as remote practicing of medicine. Its interventions, diagnoses, treatment decisions and recommendations are based on data, documents and other information transmitted through telecommunication systems.[10]

With this definition as a reference point, remote practicing of medicine involving elaboration of diagnoses and treatments has generated intense debates within the medical profession. The promulgation of Resolution 2227/2019 of the Brazilian Federal Medical Medicine (Conselho Federal de Medicina, CFM), which extended and regulated the practices of telemedicine, can be highlighted.[11] However, soon thereafter, this resolution was revoked at the request of regional medical councils, after complaints by both professionals and entities through various arguments (lack of debate and in-depth assessment of the subject, risks to patients, potential loss of jobs and/or precariousness of medical activity).

Although telemedicine has been discussed and used in healthcare systems for many years, including the adoption of technological innovations such as artificial intelligence, in Brazil it is still not fully used by healthcare professionals. However, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic has stimulated its use, particularly since the Ministry of Health published its Ordinance 467 on March 20, 2020, authorizing the use of teleconsultation.[12] This enabled the continuation of a direct relationship between doctors and their patients, including for making diagnoses and defining treatments, during the healthcare crisis. Following this, law 13,989 was published by the Federal Congress,[13] ratifying the Ministry of Health's ordinance and also creating an environment for exchanges of medical documents such as prescriptions for medicines and legal attestations.

Use of telemedicine in the healthcare system involves adoption of technological resources, education of healthcare professionals and patients and integration into the healthcare system. Telemedicine attendance is considered to be a medical act, with all the technical and ethical implications involved in face-to-face consultation. In addition, telemedicine involves issues of data protection and patient privacy. In Brazil, the General Law on the Protection of Personal Data (Lei Geral de Proteção de Dados Pessoais, LGPD), published as Federal Law 13,709 in 2018, regulates how data can be collected and processed.[14]

On the other hand, there are some challenges, particularly in developing countries. A survey among Pakistani physicians concluded that evidence of effectiveness of telemedicine across different fields was inconsistent and lacked technical, legal, cultural and ethical considerations. Inadequate training, low levels of technological literacy and lack of infrastructure are the main barriers in implementing telehealth.[15]

A narrative review of factors influencing telehealth use across different medical specialties indicated that, while professional societies for specialties with lower telehealth use have played a limited role in providing guidance on telehealth use, their counterparts for the specialties with higher telehealth use have played a proactive role in advocating for consistent payment policies, developing guidelines for telehealth use, educating providers on getting started with telemedicine, advocating for telehealth training in medicine residency and developing resources for engaging patients in telehealth use. The review revealed that lack of reimbursement, lack of technology training and a 'gatekeeper' mindset could all serve as barriers to adoption of telehealth at the individual provider level. Hospitals and specialty societies could play an organized and proactive role in addressing each of these barriers through campaigning for better payment, promulgating guidelines for telehealth use, educating providers on how to get started with telehealth, promoting telehealth training in medical residency and engaging patients in telehealth services.[16]

On the other hand, a systematic review of outpatient telehealth implementation in the United States during the COVID-19 global pandemic identified three barriers impacting the implementation and use of telehealth resources: patient telehealth limitations, lack of telehealth guidelines for clinical care and issues relating to training, technology and finance.[17]

In the existing literature on telehealth, there has been consistent emphasis on the importance of recognizing the complexity of implementing telehealth services for successful and sustainable use. There are also multiple interdependent dimensions of telehealth to consider, including processes, user-experience and sustainability. Correspondingly, the design and implementation of telehealth services often involves engagement of stakeholders from a variety of disciplines, both within and outside the setting of the organization, including healthcare providers, managers, administrators, patients, information and communication technologists, economists and policymakers.[18]

With the rise of the COVID-19 pandemic, the course of telemedicine underwent a major upswing. As shelter-in-place became the norm around the world, patients and clinicians had to adapt to a new, yet not novel, way to provide medical care. Use of telemedicine will continue to grow in the post-pandemic world, but its development will depend on several factors. Some of those factors are related to patients, some to the physician and their practices and some to reimbursement.[15]

This article tries to fill the gap in the literature regarding the short-term reaction of physicians in the state of São Paulo, Brazil, to the use of telemedicine/teleconsultations after this procedure became officially approved in this country, due to the SARS-CoV-2 pandemic.

OBJECTIVE

To describe some aspects of use of teleconsultation among a sample of physicians in the state of Sao Paulo during the SARS-CoV-2 pandemic.

METHODS

This was a cross-sectional study based on a survey among doctors. It consisted of an analysis on responses to questions that formed part of a periodic survey conducted by the São Paulo Medical Association (Associação Paulista de Medicina, APM) on medical practice during the SARS-CoV-2 pandemic. Invitations to participate were sent out via electronic means (e-mails, social networks and websites) using the APM's register of physicians, between December 18, 2020, and January 18, 2021.

In order to participate, potential respondents needed to firstly agree to the terms of a free and informed consent statement that they received. The present study was approved by the Ethics Compliance Committee on Research Involving Human Beings of the Getulio Vargas Foundation (Fundação Getulio Vargas, FGV), through opinion report no. 265/2020.

The variables analyzed in the present study were the following:

a. Adoption of telemedicine (and its modalities)
b. Remuneration of telemedicine attendance
c. Linking of telemedicine attendance
d. Technological resources used:
   d.1. Digital platform
   d.2. Electronic health record
   d.3. Electronic prescription
e. Telemedicine training
f. Patients perceptions of telemedicine

The data collected were compiled in the Microsoft Excel software, version 15.0, 2015 (Microsoft, Redmond, Washington, United States). These data were then tabulated for descriptive analysis, considering absolute and relative frequencies.

RESULTS

This survey, which was available via electronic means from December 18, 2020, to January 18, 2021, generated responses from 2,052 physicians. It was sent out to a general mailing list of physicians containing 89,486 email addresses. The survey management system found that 4789 emails were opened and, from these, responses were received from 2,052 physicians. Among these physicians, 981 (47.8%) reported not practicing any form of telemedicine.

Among the physicians who reported practicing telemedicine, 274 (28.4%) reported that they had not received any remuneration directly for the attendance provided and 225 (23.3%) reported that they had received remuneration equal to what they would have received from face-to-face consultations. A further 149 physicians (15.5%) reported that they had received payment that was lower through the virtual procedure than what they would have received from face-to-face consultations. Lastly, 164 (17.0%) directly set the price of the consultation with the patient (**Table 1**). It is likely that many of these professionals started to provide care remotely, on a temporary basis, and thus did not develop a definitive remuneration model.

Regarding the public attended through telemedicine, most of the respondents who used this model (630 physicians; 65.4%) attended both new and old patients. Most of the patients whom they attended did not have any complaints or evidence of SARS-CoV-2 infection (**Table 2**).

Regarding the professional linkage of the physicians who undertook telemedicine attendance, the majority (499; 51.8%) only attended private patients. Other physicians did this while working in medical-hospital institutions (188; 19.5%); or through participation in private healthcare insurance plans (231; 24.0%); or through

Table 1. Remuneration of physicians who attended telemedicine consultations

Type of remuneration	n	%	95% CI	
I did not receive remuneration for telemedicine attendance	274	28.4	25.6%	31.3%
I was paid per consultation, at the same rate as established for face-to-face consultations	225	23.3	20.7%	26.0%
I was paid per consultation, with an amount agreed jointly with the patient	164	17.0	14.6%	19.4%
I was paid per consultation, at a rate lower than that established for face-to-face consultations	149	15.5	13.2%	17.7%
I w I was paid per hour of work	147	15.2	13.0%	17.5%
I was paid per consultation, at a rate higher than that established for face-to-face consultations	5	0.5	0.1%	1.0%
Total	**964**	**100.0**		

CI = confidence interval.

an employment relationship with the organization in which they worked (46; 4.8%), as shown in **Table 3**. These findings highlight that many professionals started to remotely care for their former patients, on a temporary basis. With reorganization of care, after the emergency situation, this scenario will probably tend to change.

Regarding the resources used to provide telemedicine attendance, most of the respondents used specialized digital platforms (594; 61.6%), electronic health records (592; 61.4%) and electronic prescriptions (700; 72.6%) (**Table 4**).

Regarding the experiences of patients who used the attendance provided through telemedicine, most of the physicians reported that the users accepted and liked the experience (788; 51.5%). However, 678 (44.3%) said that their patients accept this form of attendance only because of the SARS-CoV-2 pandemic but did not really like it. Another 64 (5.9%) said that their patients did not agree to use this resource.

Telehealth does not consist merely of transposition of face-to-face care to a virtual environment. It is permeated by actions of education, care, diagnosis and procedures.[11] It requires training for proper and efficient use of the tools available. The present survey revealed that, out of the total number of respondents, 1,607 (88.33%) had not participated in any educational activities relating to telemedicine and that 27.97% (574) did not have any interest in participating in this in the future. Among the physicians who had participated in training activities, the majority had attended programs of duration less than four hours (238; 11.6%). In this context, the need for training professionals to provide care using the resources of telemedicine becomes relevant. Recently, a Brazilian guidebook for remote consultation was published and, certainly, other resources will be made available to Brazilian professionals.[3]

DISCUSSION

Telemedicine has been widely used in several countries. Legal and regulatory issues still prevent it from advancing in Brazil. Its emergency use due to the SARS-CoV-2 pandemic has stimulated the entry of new service providers into the market and the use of information and communication technology (ICT) resources in a somewhat improvised way by professionals.

Telemedicine has the potential to increase the capacity for case resolution and facilitate coordination of care and therapeutic adherence. It can consequently reduce hospitalizations and unnecessary searches for emergency services. In terms of patient safety issues, its use during the pandemic can be considered to have constituted an appropriate use of resources, thereby reducing the misuse of face-to face consultations.

Although this use of telemedicine resources was an innovative experience for many of the physicians surveyed, the results from this study revealed that almost half of the respondents did not use telemedicine. In addition, more than a quarter of the participants who used it (28.4%) did not receive payment for the care they provided.

Telehealth needs to be part of an integrated care model, with action in a network. Telehealth should contribute to facilitating access to services, while maintaining coordination of care that is

Table 2. Types of patients who received telemedicine attendance

Types of patients attended	Without SARS-CoV-2				With SARS-CoV-2			
	n	%	95% CI		n	%	95% CI	
Only old patients	281	29.1	26.3%	32.0%	53	5.5	4.1%	6.9%
Both new and old patients	452	46.9	43.7%	50.0%	178	18.5	16.0%	20.9%

SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2; CI = confidence interval.

Table 3. Professional linkage of the physicians who undertook telemedicine attendance, according to the types of patients attended

Types of patients attended	n	%	95% CI	
Only private patients	499	51.8	48.6%	54.9%
Healthcare insurance plan beneficiaries	231	24.0	21.3%	26.7%
Patients at a medical-hospital care institution	188	19.5	17.0%	22.0%
Patients in organizations in which the physician was employed	46	4.8	3.4%	6.1%
Total	**964**	**100**		

CI = confidence interval.

Table 4. Resources used by physicians for telemedicine attendance

Resources used	Yes, n (%)	95% CI		No, n (%)	95% CI	
Access to a specific digital platform for conducting teleconsultation	594 (61.6%)	58.5%	64.7%	370 (38.4%)	35.3%	41.5%
Remote attendance done with support from electronic medical records	592 (61.4%)	58.3%	64.5%	372 (38.6%)	35.5%	41.7%
Electronic prescriptions used	700 (72.6%)	69.8%	75.4%	264 (27.4%)	24.6%	30.2%

CI = confidence interval.

integrated with other dimensions and which gives due regard to the social determinants of health. In a recent study, the correlation between socioeconomic determinants and use of telemedicine services was measured and it was concluded that adoption of these services was significantly impacted by the social determinant factors of health, such as income, education level, race and insurance type.[18]

Lastly, incorporation of new technology within care, for example through use of remote consultations, brings the need to seek training for professionals regarding its use and its integration with secondary and tertiary-level healthcare services; and regarding adoption of safe processes, both for doctors and for patients. Training physicians to deliver high-quality, secure and personable healthcare through telemedicine can alleviate concerns and promote population-wide adoption of the technology. This is a key strategy that needs to be included in medical education and it is important to create opportunities for practitioners to learn more about this approach.[8]

It is important to build strategies and policies to enhance the use of telemedicine through deployment of appropriate infrastructure, continuous training and use of advanced technologies, with the aim of overcoming some pre-existing barriers and thereby ensuring high quality for professional medical actions.[18]

In a recent discussion about the telemedicine and current clinical practice trends during the COVID-19 pandemic, Wahezi et al. pointed out that adoption of telemedicine among physicians depends on reimbursement and on education to improve telemedicine consultations.[15]

Many physicians used telemedicine as support for their existing patients. It is known that there are more effective results when remote consultations are integrated into comprehensive healthcare.[2,8,9] However, in the present study, many physicians who undertook telemedicine as a source of care did not use dedicated platforms (38.4%); nor did they use electronic health records (38.6%) or electronic prescriptions (27.4%). These resources are important for care to be provided in a more professional manner, so as to ensure the conditions for increased patient and provider safety, information integration and patients' adherence to treatment.

Limitations

The unquestionable limitation of this analysis relates to the sample analyzed: out of the total number of questionnaires distributed, only just over 2,000 were answered, which represents about 4% of the possible sample size. Moreover, even in this sample, only about 50% of the respondents said that they had been using telemedicine. This shows that although there was unclear risk of bias among the respondents, existence of this risk has to be recognized. In addition, the survey was applied only to physicians working in the state of São Paulo and is therefore not representative of the universe of Brazilian professionals.

CONCLUSION

Currently, there is an international consensus that telemedicine is an important tool for medical practice that facilitates access to care, based on incorporation of new technologies and integration of the dimensions of prevention, diagnosis, treatment and monitoring. The SARS-CoV-2 pandemic has led to rapid adoption of telemedicine in various parts of the Brazilian healthcare system. However, as demonstrated in this study, important issues such as professional remuneration, use of electronic platforms and medical records, ensuring data protection and relationships between physicians and other stakeholders (healthcare insurance plans, hospitals and diagnostic centers) still need to be better defined, in order to achieve the desired scale and reach the outcomes defined.

Further research will be necessary with regard to the Brazilian scenario. There is a need to longitudinally assess different indicators relating to the efficiency of remote consultations and the perceptions of professionals and citizens.

This study has provided real-life knowledge of the general impressions and reactions of Brazilian physicians regarding telemedicine. This can improve the teaching of soft skills to medical and continuing education students, so as to impact their behavior as providers using the new technology. Managers may learn that physicians have concerns regarding adequate payment for use of these processes. Lastly, through telemedicine, the Brazilian population will increasingly be brought into the contemporary 21st century way of practicing part of medical care, which is less time and effort-consuming.

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Early reduction of pulmonary arterial hypertension in patients using a long-term mechanical ventricular assistance device: a cross-sectional study

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ABSTRACT

BACKGROUND: Severe pulmonary arterial hypertension (PAH) is a contraindication for heart transplantation (HT). It has been correlated with increased early and late mortality, mainly associated with right ventricular failure. Ventricular assistance devices (VADs) can promote reduction of intracardiac pressures and consequent reduction of PAH over the medium and long terms, thus enabling future candidature for HT. The diminution of early pulmonary pressure within this scenario remains unclear.

OBJECTIVE: To evaluate the reduction of PAH and correlate data from right catheterization with the earliness of this reduction.

DESIGN AND SETTING: Cross-sectional study in a general hospital in São Paulo, Brazil.

METHODS: This was a retrospective analysis on the medical records of patients undergoing VAD implantation in a single hospital. Patients for whom VAD had been indicated as a bridge to candidature for HT due to their condition of constant PAH were selected.

RESULTS: Four patients with VADs had constantly severe PAH. Their mean pulmonary artery systolic pressure (PASP) before VAD implantation was 66 mmHg. Over the 30-day period after the procedure, all the patients evolved with a drop in PASP to below 60 mmHg. Their new average was 36 mmHg, which was a drop of close to 50% from baseline values. The one-year survival of this sample was 100%.

CONCLUSION: VAD implantation can reduce PAH levels. Early reduction occurred in all patients. Thus, use of VAD is an important bridge tool for enabling candidature for HT among patients with constantly severe PAH.

INTRODUCTION

Despite advances in the therapeutic arsenal for treating heart failure, comprising lifestyle changes, pharmacological therapy and cardiac stimulation devices, a considerable portion of the population progresses to refractoriness. In such situations, more advanced treatments such as heart transplantation or use of a ventricular assistance device (VAD) need to be considered. Heart transplantation remains the therapy of choice and provides greater life expectancy for patients with advanced heart failure.[1] However, technological advances involving use of VADs, with reduction of adverse events and greater management experience among specialists, have allowed greater availability, use and tolerability of this method worldwide, including increasing use of this therapy in Brazil. VADs can be used as a bridge to transplantation or as the destination therapy.[2]

Constant pulmonary arterial hypertension (PAH) is one of the main contraindications for heart transplantation.[1,3] In this context, it has been observed, after VAD implantation, that patients with contraindications for heart transplantation due to pulmonary arterial hypertension presented decompression of the left cardiac cavities and reverse remodeling of the pulmonary vessels, with reversal of PAH. This gave rise to the possibility of candidature for heart transplantation. Data in the current literature show that this change occurs over the first three to six months after VAD implantation,[1] with some reports of earlier reduction.

OBJECTIVE

The objectives of the present study were to evaluate the reduction of PAH and correlate data from right catheterization with the earliness of this reduction.

METHODS

This was a retrospective evaluation on patients with advanced heart failure who underwent VAD implantation between 2013 and 2019, through data collection from the electronic medical records. Patients for whom VAD had been indicated as a bridge to candidature for heart transplantation due to their condition of constant PAH were selected. Constantly severe PAH was defined as pulmonary artery systolic pressure (PASP) > 60 mmHg or pulmonary vascular resistance (PVR) > 3.0 Woods units, after a vasodilation test during right catheterization.[12]

The outcomes analyzed were the reduction of pulmonary artery pressures within 30 days of VAD implantation, analyzed by means of transthoracic echocardiogram, and one-year survival. This present study was approved by our institution's ethics committee on July 13, 2020 (#1799; CAAE 33484320.5.0000.5461).

RESULTS

Out of the total of 23 patients who received VAD during the period from 2013 to 2019, four had had an indication for VAD implantation due to constantly severe PAH, as a bridge to candidature for heart transplantation. The devices implanted were Heart Mate II and Heart Mate III, with half of the sample in each group. The patients' clinical characteristics are demonstrated in **Table 1**. Their mean age was 52 years, 50% were male, 75% had ischemic etiology and the mean ejection fraction of the left ventricle was 28%.

Table 2 presents the hemodynamic variables of the four patients before implantation of the VAD. These variables were obtained through right catheterization. All the patients were classified as INTERMACS 3, with a mean HeartMate II risk score of 1.1. The mean PASP in the preoperative period before VAD implantation in these patients was 65 mmHg ± 4 mmHg.

Over the 30-day period after the procedure, all the patients evolved with a fall in PASP to below 60 mmHg, with an average value of 36 mmHg ± 10 mmHg (**Figure 1**). One of the patients underwent heart transplantation: this outcome can be ascribed to having achieved a reduction in PAH levels. The other patients continued to use the device, while presenting significant improvements in their functional class. None of the patients were using oral drugs for PAH management (such as phosphodiesterase-5 inhibitors). The one-year survival of this sample was 100%.

Table 1. Characteristics of the patients who underwent ventricular assistance devices (VAD) implantation

Characteristics	Number of patients = 4
Average age (years)	51 ± 13
Male, n (%)	2 (50)
Ischemic cardiomyopathy, n (%)	3 (75)
INTERMACS 3, n (%)	4 (100)
Creatinine, mg/dl	1.28 ± 0.26
Sodium, mEq/l	134.3 ± 4.1
Hemoglobin, g/dl	12.0 ± 2.8
Albumin, g/dl	3.8 ± 0.4
Mean ejection fraction of LV, n (%)	28 ± 1
LV diastolic diameter (mm)	69 ± 4
PASP (mmHg)	65 ± 4
HeartMate mean risk score	1.1 ± 0.8
Implanted device, n (%)	
HeartMate II™	2 (50)
HeartMate III™	2 (50)

Continuous variables expressed in ± standard deviation, SD; LV = left ventricular; PASP = pulmonary artery systolic pressure.

DISCUSSION

This is the first Brazilian report on early effective reduction of constant PAH through long-term VAD use. This case series showed an average reduction of PASP over 30 days, from 65 mmHg ± 4 mmHg to 36 mmHg ± 10 mmHg (P = 0.256). The results show that decompression of the left ventricle by the VAD acted to reduce the pressure of left ventricle filling and that, retrogradely though the principle of communicating vessels, it acted to reduce pulmonary pressure, which is a determining factor for eligibility for heart transplantation.

Pulmonary hemodynamic evaluation remains an essential examination for inclusion of patients in the heart transplantation waiting list, through enabling evaluation of the degree of pulmonary hypertension and its reversibility with vasodilators. The main data obtained from this evaluation are the PASP, mean pulmonary artery pressure (mPAP), pulmonary artery occlusion pressure (PAOP) or pulmonary capillary pressure (PCP), transpulmonary gradient (TPG) and PVR.[4] High levels of pre-transplantation pulmonary pressure, resistance and gradient have been correlated with increased post- heart transplantation mortality rates. These are therefore considered to be contraindications for this procedure, given the high risk of failure of the right ventricle of the graft.

Use of VAD as a tool capable of reducing PAH was first demonstrated in pulsatile flow devices.[5,6] It is now known to also be efficacious with continuous flow devices.[3-9] Zimpfer et al. demonstrated, in a cohort of 35 patients with a six-week follow-up, that there was a drop in mPAP levels by about 18 mmHg,[4] which is compatible with the results demonstrated in our study. In another cohort of 50 patients, Ranjit et al found that there was a reduction in mean PASP of 20 mmHg, in an evaluation conducted three months after implantation of the device.[10] These results allow us to infer that the reduction in PASP occurs mainly in the first 30 days and is maintained over the passage of the months.

We noticed that results similar to those described here had been found earlier, i.e. with favorable results within less than the 30-day period of our analysis, even without use of drugs to aid in this process, such as phosphodiesterase-5 inhibitors.

Table 2. Hemodynamic variables obtained by means of right cardiac catheterization prior to ventricular assistance device (VAD) implantation

	PASP (mmHg)	mPAP (mmHg)	SBP (mmHg)	mBP (mmHg)	PAOP (mmHg)	CO (l/min)	PVR (Wood units)	Post-PASP* (mmHg)	LVDD (pre)	LVDD (post)
Patient 1	67	43	87	53	40	5.1	0.6	33	67	67
Patient 2	60	32	84	68	23	1.6	5.6	51	75	65
Patient 3	64	38	80	53	22	5.2	3.1	31	66	66
Patient 4	70	48	100	80	20	2.6	10.8	28	68	62

*Value obtained by means of echocardiogram within 30 days after VAD implantation; hemodynamic measurements obtained after pulmonary reactivity test (patient 1: milrinone + dobutamine; patient 2: dobutamine + nitric oxide; patient 3: dobutamine + nitroprusside; patient 4: dobutamine + milrinone + nitric oxide); PASP = pulmonary artery systolic pressure; mPAP = mean pulmonary artery pressure; SBP = systolic blood pressure; mBP = mean blood pressure; PAOP = pulmonary artery occlusion pressure; CO = cardiac output; PVR: pulmonary vascular resistance; LVDD = left ventricular diastolic diameter.

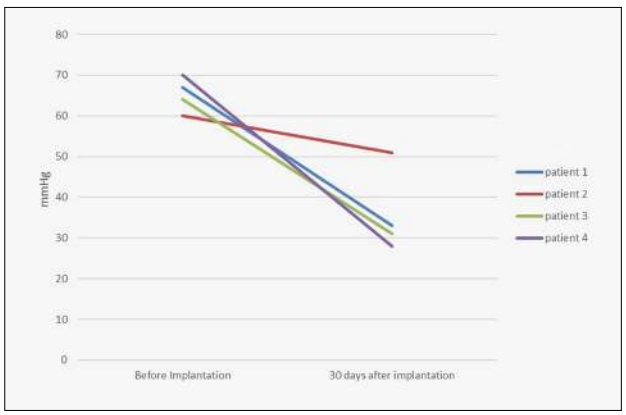

Figure 1. Pulmonary artery systolic pressure (PASP) levels in mmHg before ventricular assistance device (VAD) implantation (hemodynamic evaluation) and 30 days after VAD implantation (noninvasive echocardiogram evaluation).

Limitations

The main limitation that impeded provision of additional data from this observational study was the absence of pulmonary hemodynamic evaluation by means of a pulmonary artery catheter after VAD implantation. Evaluation of PASP was performed by means of echocardiography. However, the data obtained from this cohort of patients in Brazil allowed us to confirm the findings from studies conducted elsewhere in the world, thus emphasizing the importance of VAD devices as a bridge to candidature for heart transplantation, among patients for whom this is initially contraindicated due to constant PAH.

CONCLUSION

Considering that all patients who underwent VAD implantation achieved early reduction of pulmonary pressures, thus enabling candidature for heart transplantation, this study opens up the possibility of new approaches with protocols for use of VAD as a bridge for candidature among patients with constant PAH, such that these patients can subsequently be offered heart transplantation.

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Application of telesimulation in a medical undergraduate course during the SARS-CoV-2 pandemic: a quantitative and retrospective study

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AUTHORS' KEY WORDS:
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Coronavirus disease 19.

ABSTRACT

BACKGROUND: Because of the social isolation and distancing measures that were imposed to stop the spread of coronavirus disease 19 (COVID-19), new ways of teaching were implemented.

OBJECTIVES: To describe the implementation of telesimulation and seek to assess students' perceptions regarding telesimulation.

DESIGN AND SETTING: Retrospective quantitative study conducted within the hospital simulation at a private medical school in São Paulo, Brazil.

METHODS: After telesimulation training, students answered a questionnaire that provided an overall assessment of this activity, self-assessment and assessments of the facilitators and infrastructure provided by the University.

RESULTS: Among the students, 50% reported that the activity was below expectations and 45% reported that it was in line with their expectations. The strong points of the activity were the clinical cases, workload and teachers. The main challenge was students' difficulty in reflecting on their learning and the infrastructure.

CONCLUSIONS: Since students have less experience and fewer clinical encounters than residents or professionals, they also face more difficulty. Although telesimulation may have provided a valid alternative to replace simulation training during the COVID-19 pandemic, more face-to-face activities should be offered to students, when possible.

INTRODUCTION

Over recent years, the use of clinical simulation within healthcare education has grown, since it offers the opportunity to integrate skills and clinical reasoning with motor and behavioral skills simultaneously. Moreover, clinical simulation allows students to learn from their mistakes in a safe environment while replicating a real-life environment.[1,2]

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic has brought major disruption to all academic institutions, particularly medical courses. Within a short time, face-to-face activities were transformed into online activities mediated through technologies.[3] Most activities focused on the theoretical part of the curriculum or, at most, clinical reasoning, since all in-service training was suspended, potentially bringing a significant loss to students' learning, especially among those at the end of the course.

To reduce students' losses, we implemented telesimulation, which is a model that has been used to provide education, training and evaluation within healthcare. Telesimulation is defined as a process that combines telecommunication and simulation, to allow most students to attend simulations online.[4,5] Telesimulation has also been described as a useful resource in other fields such as robotic surgery and ophthalmological surgery,[4-8] which do not particularly belong to emergency medicine. However, these are super-specialized fields and may not be applicable to undergraduates.

OBJECTIVES

The aim of our study was to describe the implementation of telesimulation and seek to investigate medical students' satisfaction with telesimulation.

METHODS

The simulation took place every week between August and December 2020 since it is integrated into the institution's competency-based curriculum. Students were divided into two groups, with an interval of one hour for sanitization procedures. Each week, there was a new opportunity for other students to participate as volunteers, which promoted the opportunity for everyone to participate.

The students in the face-to-face activity were volunteers, with a maximum of six students per activity. Before the start of every session, the students were guided through the biosecurity norms and signed an imaging rights statement, since the rest of the students were following the activity remotely and synchronously. A variety of scenarios were played out, around the following themes: chronic obstructive pulmonary disease, pulmonary thromboembolism, foreign body airway obstruction, pancreatitis, septic shock and diabetic ketoacidosis.

Our hospital simulation consisted of a large room that had been adapted to follow all sanitary protocols. The simulation included cameras and microphones that allowed us to film and record the simulated patient (who could be either an actor or a simulator) and undertake multiparametric monitoring through the institution's communication platform (**Figure 1**), which in this case was Microsoft Teams. In this model, one teacher stayed in the face-to-face simulation and another in the online environment attending to students who were participating remotely.

The technique used for these simulations consisted of a rapid cycle of deliberate practice, since those students lacked the necessary practice, which consequently would make it unlikely that there would be any reflection process. A rapid cycle of deliberate practice splits simulation scenarios into small segments with feedback, in the form of pause, feedback and try again. Students only move forward when no mistake is made in that segment.[9] In addition, the input from the facilitator made the simulated experience more motivating for the students, especially those at home. A second facilitator was responsible for engaging and interacting with students at home. This form of telebriefing was performed considering that the students already were aware of the feedback structure that would be adopted in the rapid cycle of deliberate practice. Students could access the simulation session on their smartphones or other device. Another facilitator was responsible for the online students in case of doubts.

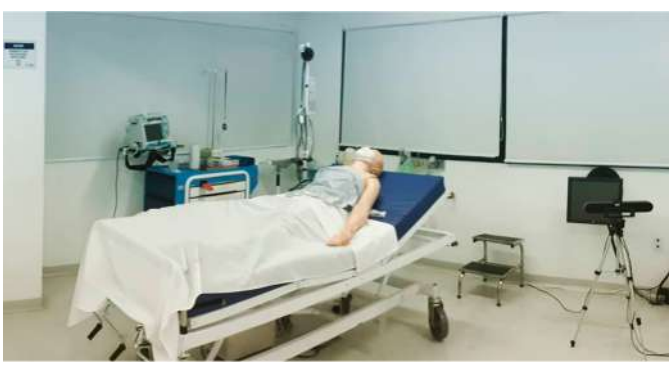

Figure 1. Example of a room for telesimulation.

After the activities of that semester had been completed, all students received an online questionnaire, which it was not mandatory to respond to. This asked the students to give their overall assessment of this activity, self-assessment and assessment of facilitators and infrastructure provided by the university. The online questionnaire contained a mix of dichotomous questions (yes or no), gradings (ranging from 0 to 10), multiple-choice questions and open questions (**Appendix 1**).

This data collection was approved by our institution's research ethics committee (protocol number 37360820.8.0000.0064) on September 9, 2020. Quantitative data were analyzed using descriptive analysis, and qualitative data were analyzed using content analysis, as proposed by Bardin.[10] After categorizing the answers for each open-ended questions, we used descriptive analysis to present the results.

RESULTS

Among the 180 students who were involved in this activity, only 11% (n = 20) answered the questionnaire. Out of those students, 18 participated in the scenario (face-to-face activity), one did not want to participate in the scenario and one wanted to participate but did not have the chance. All the students who answered the questionnaire considered simulation important in medical training and agreed that the clinical cases selected for the activities were good.

Concerning the implementation of telesimulation, 50% reported that it was below their expectations and 45% reported that it was in line with their experience (5% reported that it was above their expectations). Half of the students felt safe during the simulation and had a good experience and 10% did not like it. 30% of the students felt exposed, although they felt that it was a good experience. Most of the students considered that Microsoft Teams was an adequate tool. The students also reported that teachers were accessible (70%). The workload was considered adequate by 50% of the students; adequate but would have been better with more hours, by 30%; and inadequate and ought to have been extended for more hours, by 20%.

Although most of the students (85%) reported that telesimulation did not provide the possibility of leading to reflection in the same way as would occur with face-to-face simulation, most of them (70%) said that they would be willing to take some other course using telesimulation. Lastly, the qualitative analysis showed that the main barriers reported were the following: infrastructure (35%); applied methodology (25%); volunteers' performance (20%); workload (10%); proposed scenarios (5%); and teachers (5%).

DISCUSSION

Despite the students' understanding about the pandemic and the teaching effort needed to adapt to simulated training, they showed great frustration regarding telesimulation. This was probably because simulation training is one of the activities most eagerly expected by students. It takes place while the students are still in the preclinical phase. This is the time at which clinical reasoning becomes integrated with procedural and behavioral conduct.

We identified some barriers that impeded the activity. Oscillations in internet connections, inconsistencies in using Microsoft Teams and difficulties in sound recording in the simulated environment hampered the students' understanding. These barriers have also been reported elsewhere, especially in low and middle-income countries.[11]

One limitation of the present study was that the students who participated in the research were more likely to rate this activity positively, since most of them participated in the face-to-face simulation. Another limitation was that this study focused only on the students' satisfaction without measuring their learning.

CONCLUSION

Use of telesimulation has supported clinical training to some degree during the COVID-19 pandemic. Although telesimulation may provide a valid alternative for replacing simulation training during COVID-19, more face-to-face activities should be offered to students, when possible.

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Appendix 1. Medical student experience instrument for use of a new telesimulation-based educational tool during the SARS-CoV-2 pandemic.

(Start by reading and signing the free and informed consent statement. This is MANDATORY for continuing the process).

Date: _____

Group:
A. blue [ ] green [ ]
B. blue [ ] green [ ]

Gender:
Female [ ]
Male [ ]
Non-binary [ ]
I don't want to answer [ ]

Age (in years): _____

Stage: _____

Do you know of any other university institution that is carrying out telesimulation during this pandemic?
No [ ]
Yes [ ]
– Please write the name of the institution and its location, city, state and country: _____

We ask you to answer all of the following items about your experience with the telesimulation training that was implemented. You must answer all items. Each item has only one valid answer. (Note: for some items, the responses are nested because they depend on the previous answer).

1. Do you consider simulation important for your undergraduate medical training?
Yes [ ]
No [ ]
Indifferent [ ]

2. Because of the pandemic, telesimulation was included as a curriculum subject. How has this experience been?
Above your expectations [ ]
Below your expectations [ ]
Indifferent [ ]

3. Have you volunteered for any in-service training?
No [ ]
I wanted to but I couldn't because of the pandemic [ ]
I didn't ask for this method [ ]
Yes [ ]

In this telesimulation experience:
I felt exposed [ ]
I did not like it [ ]
I considered it valid [ ]
I didn't feel exposed. It was a good experience [ ]

4. Analysis on the process experienced:

Regarding the cases discussed, I considered that they:
Were very complex [ ]
Could have been more complex [ ]
Were very simple [ ]

Regarding the technological tool selected for telesimulation (Microsoft Teams), I considered that it was:
Suitable. No problem [ ]
Poor. I would prefer another app [ ]
– State which app: _____

Continue...

Appendix 1. Continuation.

Regarding the professor who facilitated the telesimulation/telefeedback, I considered that this person was:
Always accessible [ ]
Not always accessible [ ]

Regarding the workload, I considered that:
It was good/adequate [ ]
It should have been greater [ ]
It should have been smaller [ ]
It was poor/inadequate [ ]

I considered that the infrastructure of the hospital simulation during the pandemic was:
Adequate [ ]
Inadequate [ ]
Justify your response in a few words: _____

5. Do you consider that telesimulation has contributed to your undergraduate medical training?
Yes [ ]
No [ ]

6. Do you consider that telesimulation can lead to reflection in the same way as occurs with face-to-face simulation?
Yes [ ]
No [ ]

7. Would you take another training course in telesimulation?
Yes [ ]
No [ ]

8. Check the factor that describes your biggest difficulty with this methodology:
The technology (which hampered my development) [ ]
The method (which did not favor my understanding) [ ]
The teacher (who did not facilitate the process) [ ]

9. In your opinion, what had the most positive impact on these telesimulation activities?
Workload [ ]
Proposed scenario [ ]
Infrastructure [ ]
Professor [ ]
Technology applied [ ]
Your performance [ ]

10. In your opinion, what had the most negative impact on these telesimulation activities?
Workload [ ]
Proposed scenario [ ]
Infrastructure [ ]
Professor [ ]
Technology applied [ ]
Your performance [ ]

11. Choose a score from 0 (worst) to 10 (best) for your overall assessment of the process that you experienced: _____

12. Choose a score from 0 (worst) to 10 (best) for your self-assessment of this process: _____

13. Describe, in one paragraph, your experience over the course of this process:
_____
_____
_____

Thank you for your participation in this process.

INSTRUCTIONS FOR AUTHORS

Scope and indexing

São Paulo Medical Journal (formerly Revista Paulista de Medicina) was founded in 1932 and is published bimonthly by Associação Paulista de Medicina, a regional medical association in Brazil.

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Authorship

The Journal supports the position taken by the ICMJE (http://www.icmje.org) regarding authorship. All authors should read ICMJE's recommendations to obtain clarifications regarding the criteria for authorship and to verify whether all of them have made enough contributions to be considered authors.[10]

All authors of articles published in *São Paulo Medical Journal* need to have contributed actively to the discussion of the study results and should review and approve the final version that is to be released. If one author has not contributed enough or has not approved the final version of the manuscript, he/she must be transferred to the Acknowledgement section.

The corresponding author is the primary guarantor of all ethical issues relating to the manuscript, before, during and after its publication. However, *São Paulo Medical Journal* and ICMJE consider that all authors are held fully responsible for the study, regarding the accuracy or integrity of data and data interpretation in the text. Contributions such as data collection only do not constitute authorship.

The addition or deletion of authors' names in the manuscript byline is possible only if the corresponding author provides the

reason for the rearrangement and a written signed agreement from all authors. Modifications to the order of the authors are possible, but also need to be justified. Authors whose names are removed or inserted must agree with this in writing. Publication of the article cannot proceed without a declaration of authorship contributions signed by all authors.

São Paulo Medical Journal supports the ORCID initiative. All authors should create an ORCID identification (ID) record (in www.orcid.org) before submitting their article and should link the submission to their existing ORCID ID in the electronic submission system. ORCID identifications help to distinguish researchers with similar names, give credit to contributors and link authors to their professional affiliations. In addition, this may increase the ability of search engines to retrieve articles.

São Paulo Medical Journal supports Open Science practices. Authors must therefore complete an open science compliance form, which is available from: https://wp.scielo.org/wp-content/uploads/Open-Science-Compliance-Form_en.docx.

Redundant or duplicate publication

São Paulo Medical Journal will avoid publishing redundant or duplicate articles. The Journal agrees with the ICMJE definition of redundant publication,[11] i.e. an attempt to report or publish the same results from a study twice. This includes but is not limited to publication of patient cohort data that has already been published, without clear reference to the previous publication. In situations in which authors are making a secondary analysis on data that has already published elsewhere, they must state this clearly. Moreover, the outcomes assessed in each analysis should be clearly differentiated.

The Journal's peer review policy and procedures

After receipt of the article through the electronic submission system, it will be read by the editorial team, who will check whether the text complies with the Journal's Instructions for Authors regarding format. The Journal has adopted the *CrossRef Similarity Check* system for identifying plagiarism and any text that has been plagiarized, in whole or in part, will be promptly rejected. Self-plagiarism will also be monitored.

When the general format of the manuscript is deemed acceptable and fully compliant with these Instructions for Authors, and only then, the editorial team will submit the article to the Editor-in-Chief, who will firstly evaluate its scope. If the editor finds that the topic is of interest for publication, he will assign at least two reviewers/referees with expertise in the theme, to evaluate the quality of the study. After a period varying from one to several weeks, the authors will then receive the reviewers' evaluations and will be required to provide all further information requested and the corrections that may be necessary for publication. These reviewers, as well as the Editorial Team and the Editor-in-Chief, may also deem the article to be unsuitable for publication by *São Paulo Medical Journal* at this point.

At the time of manuscript submission, the authors will be asked to indicate the names of three to five referees. All of them should be from outside the institution where the authors work and at least two should preferably be from outside Brazil. The Editor-in-Chief is free to choose them to review the paper or to rely on the *São Paulo Medical Journal's* Editorial Board alone.

Articles will be rejected without peer review if:

- they do not present Ethics Committee approval (or a justification for the absence of this);
- they fail to adhere to the format for text and figures described here.

After peer review

Peer reviewers, associated editors and the Editor-in-Chief may ask for clarifications or changes to be made to the manuscript. The authors should then send their article back to the Journal, with the modifications made as requested. Changes to the text should be highlighted (in a different color or using a text editor tool to track changes). Failure to show the changes clearly might result in the paper being returned to the authors.

The modified article must be accompanied by a letter answering the referees' comments, point by point. The modified article and the response letter are presented to the editorial team and reviewers, who will verify whether the problems have been resolved adequately. The text and the reviewers' final evaluations, along with the response letter, will then be sent to the Editor-in-Chief for a decision.

Manuscripts that are found to be suitable for publication through their scientific merit will be considered "provisionally accepted". However, all articles will subsequently be scrutinized to check for any problems regarding the reporting, i.e. sentence construction, spelling, grammar, numerical/statistical problems, bibliographical references and other matters that may arise, especially in the Methods section. The adherence to reporting guidelines will be checked at this point, and the staff will point out any information regarding methodology or results that the authors should provide. This is done in order to ensure transparency and integrity of publication, and to allow reproducibility.

The editorial team will then provide page proofs for the authors to review and approve. No article is published without this final author approval. All authors should review the proof, although the Journal asks the corresponding author to give final approval.

Submission

Articles should be submitted only after they have been formatted as described below. Texts must be submitted exclusively through the Internet, using the Journal's electronic submission system, which is available at http://mc04.manuscriptcentral.com/spmj-scielo. Submissions sent by e-mail or through the post will not be accepted.

The manuscript should be divided into two files. The first of these, the main document ("blinded"), should contain the article title, article type, keywords and abstract, article text, references and tables, but must omit all information about the authors. The second of these, the "title page", should contain all the information about the authors.

To format these documents, use Times New Roman font, font size 12, line spacing 1.5, justified text and numbered pages.

The corresponding author is responsible for the submission. However, all authors should approve the final version of the manuscript that is to be submitted and should be aware of and approve any changes that might be made after peer review.

Covering letter

All manuscripts must be submitted with a covering letter signed at least by the corresponding author. The letter must contain the following five essential items relating to the manuscript:

1. a declaration that the manuscript is original and that the text is not under consideration by any other journal;
2. a statement that the manuscript has been approved by all authors, who agree to cede the copyrights to the Journal, disclose all sources of funding and declare all potential conflicts of interest;
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5. a list of a minimum of five potential referees outside of the authors' institutions, who could be invited, at the Editor-in-Chief's discretion, to evaluate the manuscript.

General guidelines for original articles

The following are considered to be full-text original articles: clinical trials; cohort, case-control, prevalence, incidence, accuracy and cost-effectiveness studies; case series (i.e. case reports on more than three patients analyzed together); and systematic reviews with or without meta-analysis. These types of article should be written with a maximum of 3,500 words (from the introduction to the end of the conclusion).

Typical main headings in the text include Introduction, Methods, Results, Discussion and Conclusion. The authors can and should use short subheadings too, especially those concerning the reporting guideline items.

Trial and systematic review registration policy

São Paulo Medical Journal supports the clinical trial registration policies of the World Health Organization (WHO) and the International Committee of Medical Journal Editors (ICMJE) and recognizes the importance of these initiatives for registration and international dissemination of information on randomized clinical trials, with open access. Thus, since 2008, manuscripts on clinical trials are accepted for publication if they have received an identification number from one of the public clinical trial registration database (such as ClinicalTrials.gov and/or REBEC and/or the World Health Organization; the options are stated at http://www.icmje.org). The identification number should be declared at the end of the abstract. Articles describing systematic reviews must provide the protocol registration number from a reliable database, such as PROSPERO, Open Science Framework, Cochrane, Joanna Briggs and others. Articles presenting clinical trials or systematic reviews without registration protocols will be promptly rejected without peer review.

Results from cases with DNA sequences must be deposited in appropriate public databases. The protocol number or URL can be requested at any time during the editorial review. Publication of other research data in public repositories is also recommended, since it contributes towards replicability of research, increases article visibility and possibly improves access to health information.

Sample size

All studies published in SPMJ must present a description of how the sample size was arrived at. If it was a convenience or purposive sample, the authors must declare so and explain the characteristics of this sample and recruitment method. For clinical trials, for instance, it is mandatory to inform each of the three main values used to calculate sample size:

- power (usually 80% or more);
- level of significance (usually 0.05 or lower);
- clinically meaningful difference (effect size targeted), according to the main outcome measurement.

Regardless of study results (if "positive" or "negative"), the journal will probably reject articles of trials using underpowered samples, when sample size has not been properly calculated or the calculation has not been fully described as indicated above.

Abbreviations, acronyms and products

Abbreviations and acronyms must not be used, even those in everyday use, unless they are defined when first used in the text. However, authors should avoid them for clarity whenever possible. Drugs or medications must be referred to using their generic names (without capital letters), with avoidance of casual mention of commercial or brand names.

Interventions

All drugs, including anesthetics, should be followed by the dosage and posology used.

Any product cited in the Methods section, such as diagnostic or therapeutic equipment, tests, reagents, instruments, utensils, prostheses, orthoses and intraoperative devices, must be described together with the manufacturer's name and place (city and country) of manufacture in parentheses. The version of the software used should be mentioned.

Any other interventions, such as exercises, psychological assessments or educational sessions, should be described in enough details to allow reproducibility. The Journal recommends that the TIDieR reporting guidelines should be used to describe interventions, both in clinical trials and in observational studies.[13]

Supplementary material

Because supplementary material comprises documents that do not form part of the text of the manuscript, São Paulo Medical Journal will not publish it. The authors should cite an access link that allows readers to view the supplementary material.

Short communications

Short communications are reports on the results from ongoing studies or studies that have recently been concluded for which urgent publication is important. They should be structured in the same way as original articles. The authors of this kind of communication should explain, in the covering letter, why they believe that publication is urgent. Short communications and case reports must be limited to 1,000 words (from the introduction to the end of the conclusion).

Case reports, case series, narrative reviews and letters to the editor

Starting in June 2018, only individual case reports dealing with situations of public health emergencies will be accepted by *São Paulo Medical Journal*. Case reports that had already been accepted for publication up to May 2018 will still be published in a timely manner.

After initial evaluation of scope by the editor-in-chief, case reports, case series and narrative reviews will be considered for peer-review evaluation only when accompanied by a systematic search of the literature, in which relevant studies found (based on their level of evidence) are presented and discussed.[12] The search strategy for each database and the number of articles obtained from each database should be shown in a table. This is mandatory for all case reports, case series and narrative reviews submitted for publication. Failure to provide the search description will lead to rejection before peer review.

The access route to the electronic databases used should be stated (for example, PubMed, OVID, Elsevier or Bireme). For the search strategies, MeSH terms must be used for Medline, LILACS, and Cochrane Library. DeCS terms must be used for LILACS. EMTREE terms must be used for Embase. Also, for LILACS, the search strategy must be conducted using English (MeSH), Spanish (DeCS) and Portuguese (DeCS) terms concomitantly. The search strategies must be presented exactly as they were used during the search, including parentheses, quotation marks and Boolean operators (AND, OR, and NOT). The search dates should be indicated in the text or in the table.

Patients have the right to privacy. Submission of case reports and case series must contain a declaration that all patients gave their consent to have their cases reported (even for patients cared for in public institutions), in text and images (photographs or imaging examination reproductions). The Journal will take care to cover any anatomical part or examination section that might allow patient identification. For deceased patients whose relatives cannot be contacted, the authors should consult the Editor-in-Chief. All case reports and case series must be evaluated and approved by an ethics committee.

Case reports should be reported in accordance with the CARE Statement,[7] including a timeline of interventions. They should be structured in the same way as original articles.

Case reports must not be submitted as letters. Letters to the editor address articles that have been published in the *São Paulo Medical Journal* or may deal with health issues of interest. In the category of letters to the editor, the text has a free format, but must not exceed 500 words and five references.

FORMAT: FOR ALL TYPES OF ARTICLES

Title page

The title page must contain the following items:

1. Type of paper (original article, review or updating article, short communication or letter to the editor);
2. Title of the paper in English, which should be brief but informative, and should mention the study design.[14] Clinical trial, cohort, cross-sectional or case-control study, and systematic review are the most common study designs. Note: the study design declared in the title should be the same in the methods and in the abstract;
3. Full name of each author. The editorial policy of the *São Paulo Medical Journal* is that abbreviations of authors' names must not be used; therefore, we ask that names be stated in full, without using abbreviations;
4. Place or institution where the work was developed, city and country;
5. Each author should indicate the way his/her name should be used in indexing. For example: for "João Costa Andrade", the indexed name could be "Costa-Andrade J." or "Andrade JC", as preferred;
6. The author's professional background (Physician, Pharmacist, Nurse, Dietitian or another professional description, or Undergraduate Student); and his/her position currently held (for example, Master's or Doctoral Student, Assistant Professor, Associate Professor or Professor), in the department and institution where he/she works, and the city and country (affiliations);
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8. Each author must inform his contribution, preferably following the CRediT system (see above in Authorship);
9. Date and venue of the event at which the paper was presented, if applicable, such as congresses, seminars or dissertation or thesis presentations.

10. Sources of financial support for the study, bursaries or funding for purchasing or donation of equipment or drugs. The protocol number for the funding must be presented with the name of the issuing institution. For Brazilian authors, all grants that can be considered to be related to production of the study must be declared, such as fellowships for undergraduate, master's and doctoral students; along with possible support for postgraduate programs (such as CAPES) and for the authors individually, such as awards for established investigators (productivity; CNPq), accompanied by the respective grant numbers.
11. Description of any conflicts of interest held by the authors (see above).
12. Complete postal address, e-mail address and telephone number of the author to be contacted about the publication process in the Journal (the "corresponding author"). This author should also indicate a postal address, e-mail address and telephone number that can be published together with the article. *São Paulo Medical Journal* recommends that an office address (rather than a residential address) should be informed for publication.

Second page: abstract and keywords

The second page must include the title and a structured abstract in English with a maximum of 250 words. References must not be cited in the abstract.

The following headings must be used in the structured abstract:

- Background – Describe the context and rationale for the study;
- Objectives - Describe the study aims. These aims need to be concordant with the study objectives in the main text of the article, and with the conclusions;
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- Clinical Trial or Systematic Review Registration – Mandatory for clinical trials and systematic reviews; optional for observational studies. List the URL, as well as the Unique Identifier, on the publicly accessible website on which the trial is registered.
- MeSH Terms - Three to five keywords in English must be chosen from the Medical Subject Headings (MeSH) list of Index Medicus, which is available at http://www.ncbi.nlm.nih.gov/sites/entrez?db=mesh.These terms will help librarians to quickly index the article.
- Author keywords - The authors should also add three to six "author keywords" that they think express the main article themes. These keywords should be different from the MeSH terms and preferably different from words already used in the title and abstract, so as to improve the discoverability of the article by readers doing a search in PubMed. They provide an additional chance for the article to be retrieved, read and cited. Combinations of words and variations (different wording or plurals, for example) are encouraged.

References

For any manuscript, all statements in the text that do not result from the study presented for publication in the *São Paulo Medical Journal* but from other studies must be accompanied by a quotation of the source of the data. All statements regarding health statistics and epidemiological data should generally be followed by references to the sources that generated this information, even if the data are only available electronically.

São Paulo Medical Journal uses the reference style known as the "Vancouver style," as recommended by the International Committee of Medical Journal Editors (ICMJE). Follow the instructions and examples at www.icmje.org, item "References", for the format.

In the text, the references must be numbered in the order of citation. The citation numbers must be inserted after periods/full stops or commas in sentences, and in superscript (without parentheses or square brackets). References cited in the legends of tables and figures must maintain sequence with the references mentioned in the text.

In the list of references, all the authors must be listed if there are up to and including five authors; if there are six or more, the first three should be cited, followed by the expression "et al." For books, the city of publication and the name of the publishing house are mandatory. For texts published on the internet, the complete uniform resource locator (URL) or address is necessary (not only the main home page of a website or link), so that by copying the complete address into a computer internet browser, the Journal's readers will be taken to the exact document cited, and not to a general website.

At the end of each reference, please insert the "PMID" number (for papers indexed in PubMed) and the link to the "DOI" number if available.

Authors are responsible for providing a complete and accurate list of references. All references cited in the text must appear in the reference list, and every item in the reference list must be cited in the text. Also, citations must be in the correct sequence.

Manuscripts that do not follow these guidelines for references will be returned to the authors for adjustments.

The reference list should be inserted after the conclusions and before the tables and figures.

Figures and tables

Images must be submitted at a minimum size that is reproducible in the printed edition. Figures should be sent at a resolution of 300 DPI and minimum size of 2,500 pixels (width) and be recorded in ".jpg" or ".tif" format. Images submitted in inadequate formats will not be accepted.

Images must not be embedded inside Microsoft PowerPoint or Microsoft Word documents, because this reduces the image size. Authors must send the images separately, outside of .doc or .ppt documents. Failure to send the original images at appropriate sizes leads to paper rejection before peer review.

Flowcharts are an exception: these must be drawn in an editable document (such as Microsoft Word or PowerPoint), and should not be sent as an image that can't be changed.

Figures such as bars of line graphs should be accompanied by the tables of data from which they have been generated (for example, sending them in the Microsoft Excel spreadsheets, and not as image files). This allows the Journal to correct legends and titles if necessary, and to format the graphs according to the Journal's style. Graphs generated from software such as SPSS or RevMan must be generated at the appropriate size, so that they can be printed (see above). Authors must provide internal legends/captions in correct English.

All the figures and tables should be cited in the text. All figures and tables must contain legends or titles that precisely describe their content and the context or sample from which the information was obtained (i.e. what the results presented are and what the kind of sample or setting was). The reader should be able to understand the content of the figures and tables simply by reading the titles (without the need to consult the text), i.e. titles should be complete. Acronyms or abbreviations in figure and table titles are not acceptable. If it is necessary to use acronyms or abbreviations inside a table or figure (for better formatting), they must be spelled out in a legend below the table or figure.

For figures relating to microscopic findings (i.e. histopathological results), a scale must be embedded in the image to indicate the magnification used (just like in a map scale). The staining agents (in histology or immunohistochemistry evaluations) should be specified in the figure legend.

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O **XIX Congresso Paulista de Medicina do Sono 2022**, um evento que está se tornando clássico em nosso calendário, já tem data confirmada!

Nos dias 27 e 28 de maio vai acontecer mais uma edição do **Congresso Paulista de Medicina do Sono**. Essa edição foi preparada para trazer uma nova experiência aos congressistas, com um evento diferente e inovador, abusando do que a tecnologia pode oferecer e, claro, da importância da interação. Assim, nosso tradicional Congresso Paulista de Medicina do Sono em 2022 será apresentado como uma "Arena Sono", onde palestrantes trarão insights atuais, de forma dinâmica e objetiva, diferente das tradicionais aulas em congressos, para uma grande discussão temática com os vários especialistas. O tema principal será **"Do sintoma ao diagnóstico e Do diagnóstico ao tratamento"**.

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*ANSs e condições no site.

Simule seu plano:

APM ASSOCIAÇÃO PAULISTA DE MEDICINA

quali corp