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Frailty syndrome and healthcare for older adults

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In the early 2000s, Linda Fried et al.,[1] then affiliated with Johns Hopkins University, described the pathophysiological bases and a phenotype of frailty syndrome in older adults. This research has served as a landmark in the field of geriatrics and has significantly influenced the healthcare provided for older adults across various medical specialties over the years. Despite its ongoing evolution, the concept of frailty syndrome remains crucial and should not be underestimated or overlooked by physicians caring for patients.

Interest in understanding and precisely defining the difference between chronological and biological age predates the work of Fried et al. In the 1980s, for example, frail older adults were already characterized as those dependent on others for daily activities or survival.[2] Adults of the same age exhibit varying levels of functionality and robustness profiles, indicating diverse rates and trajectories of aging. However, Fried et al. not only defined the processes underlying these differences but also established diagnostic criteria for frailty syndrome at earlier stages.[1] This enables the early recognition of older patients at higher risk of developing complications due to clinical and surgical interventions, unplanned hospitalization, falls, functional decline, institutionalization, or death. Such criteria facilitate the implementation of interventions to identify the causes of frailty and propose intervention plans to prevent disease progression and the development of dependency, as exemplified by the Frailty Clinic led by the Geriatrics and Gerontology group of the University of Toulouse.[3]

Although frailty syndrome has been defined in various ways, especially by Professor Kenneth Rockwood and his group,[4] the foundational definition remains consistent: frailty syndrome denotes an age-related state of physiological vulnerability characterized by diminished homeostatic reserve in multiple systems, rendering individuals vulnerable to stressful events. Initially, Fried proposed that the syndrome is caused by immunological and endocrine disorders and sarcopenia. However, extensive scientific literature on frailty has since revealed associations with genetic and epigenetic factors, socioeconomic conditions, life history, chronic diseases, physical activity levels, and other factors interfering with healthy aging.[5]

Several tools have been developed to diagnose frailty syndrome over the years, such as the Fried criteria, which include unintended weight loss, reduced muscle strength, reduced walking speed, fatigue, and low physical activity levels; the Rockwood frailty index; and other practical and concise tools available for use in routine clinical practice.[6] The optimal approach to diagnosing frailty syndrome and assessing its impact on clinical practice and the prognosis of older adults in primary care and other healthcare levels has been a longstanding debate. More recent studies have highlighted that the effectiveness of diagnostic instruments depends on the context in which they are applied. For example, the Fried phenotype method is an excellent predictor of short-, medium-, and long-term outcomes in primary care settings, where ample time and resources allow for comprehensive testing. In emergency settings, where time is extremely limited and patients may have significant functional impairments (such as reduced mobility hindering gait speed measurement), conducting thorough tests becomes challenging, potentially compromising the information obtained. In such context, instruments such as the Clinical Frailty Scale, also proposed by Rockwood et al., prove to be more informative and practical.[6,7]

Irrespective of the diagnostic tool used, current literature highlights frailty syndrome as a significant prognostic indicator in older adults with heart disease, peripheral vascular disease,

cancer, kidney disease, diabetes, hypertension, neurological disorders, and other chronic conditions; in older patients with acute disease admitted to emergency rooms, intensive care units, and other emergency services; and in those undergoing invasive surgical procedures. Therefore, frailty syndrome should be considered in healthcare and complication prevention protocols in all medical practice scenarios.[8]

We have transitioned from relying solely on chronological age to using physiological reserves and functionality as pivotal guides for medical decision-making. The association of this syndrome with acute morbidity indicators proved to be a significant predictor of prognosis during the coronavirus disease 2019 pandemic.[9] The management of frailty syndrome considerably improves outcomes after femur fracture.[10] The accurate diagnosis of frailty syndrome facilitates the implementation of effective interventions that reduce the risk of developing various types of surgical complications.[11] Lastly, addressing the causes of frailty syndrome enables the development of primary care plans aimed at preventing or delaying the onset of dependence.[3]

In conclusion, the degree of the issue in Brazil reflects a rapidly aging population. Brazilian studies indicate that the prevalence of frailty syndrome among community-dwelling older adults ranges from 8% to 10%, with nearly half of older adults classified as pre-frail according to the Fried phenotype criteria.[12] These rates are significantly higher in long-term care institutions, emergency rooms, hospitals, and outpatient clinics.[13] Another crucial issue is that the diagnosis of frailty syndrome is dynamic rather than definitive, with multidisciplinary interventions helping pre-frail adults regain non-frail status and frail adults transitioning between states.[14] This underscores the importance of early detection and multidisciplinary therapeutic interventions to decrease the risk of clinical complications and functional decline.

For these reasons, physicians from all specialties, along with their interdisciplinary teams, should strive to diagnose frailty syndrome in older patients using the most recommended instruments in the literature. This approach enhances planning and the management of interventions, ultimately improving the prognosis and short-, medium-, and long-term outcomes of older patients in routine clinical practice.

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Effects of probiotics on gastrointestinal symptoms, anthropometric measurements, and breastfeeding duration in infants with colic: a randomized control trial

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ABSTRACT

BACKGROUND: Infantile colic has a multifactorial etiology. Recent studies have suggested that probiotics may be effective in its management.

OBJECTIVE: This study was carried out to evaluate the effect of the Actiregularis strain ($5x10^6$ cfu\ml) included in maternal nutrition on gastrointestinal problems, growth development, and breastfeeding outcomes in infantile colic.

DESIGN AND SETTING: The study was a randomized controlled trial conducted in the neonatal outpatient clinic of a training and research hospital in Turkey.

METHODS: A probiotic drink containing the Actiregularis ($5x10^6$ cfu\ml) strain was added to the diet of mothers in the probiotics group once daily for 15 consecutive days. Data were collected for each infant's 0th (birth), 1st, 4th, and 6th months.

RESULTS: Infants whose mothers were administered Actiregularis for 15 days had decreased crying intensity ($P = 0.000$). When the difference in breastfeeding rates between the groups was significant at the 4th and 6th months ($P = 0.044$; $P = 0.035$). There was no difference in anthropometric values except the babies' weights at the 6th month. ($P < 0.001$).

CONCLUSION: Infants treated with Actiregularis, which was added to their mothers' diet for 15 days, showed a decrease in the frequency of crying, and the difference in breastfeeding rates between the groups was significant at the 4th and 6th months. There was no difference in anthropometric values except the babies' weights at the 6th month.

CLINICAL TRIALS REGISTRATION: NCT04374955 (https://clinicaltrials.gov/ct2/show/).

INTRODUCTION

Infantile colic was first described by Wessel et al.[1] in 1954 as "crying attacks that last three hours a day, three days a week, and continue for three weeks." Infantile colic is a benign newborn problem characterized by self-limiting, unpreventable crying attacks that affect 25% of babies in the first three months of life. It is a very troublesome process for parents and babies.[2] It is associated with short- and long-term negative outcomes such as postpartum maternal depression, early cessation of breastfeeding, parental guilt and frustration, shaken baby syndrome, multiple doctor visits, medication use, growth and development problems, allergies, and behavior and sleep problems.[3,4]

The pathogenesis of infantile colic and the conditions that cause its disorder have not yet been elucidated. Some studies have suggested that maternal malnutrition, consumption of foods containing allergens, and lactase deficiency in infants are responsible.[5] In recent years, it has been thought that the gastrointestinal tract flora may play a role in infantile colic.[6,7] It has been suggested that deteriorated intestinal flora (dysbiont), which is thought to be responsible for infantile colic, can be improved by probiotics and ameliorated colic pain in infants.[8,9] Probiotics play an important role in changing the flora, possibly by causing bacterial diversity in the gastrointestinal tract via the gut-brain axis.[8] Some studies have suggested the valuable effects of maternal intake of probiotics during the perinatal or postpartum period to change the intestinal flora of infants in the prevention of infantile colic.[8,10,11] Many studies have been carried out to evaluate the use of probiotic supplements in the treatment of infantile colic and have shown their effectiveness on crying duration, gastrointestinal problems, growth and development parameters, and breastfeeding duration of the baby.[10,12] However, in infantile colic studies, probiotic products are generally included in infant nutrition, while studies on including probiotic products in maternal nutrition are almost non-existent.[13,14]

OBJECTIVE

This randomized controlled trial was carried out to evaluate the effect of the Actiregularis strain ($5x10^6$ cfu\ml) included in maternal nutrition on gastrointestinal problems, growth development, and breastfeeding outcomes in infantile colic.

METHODS

Study population and design

Study is the only blind, randomized, controlled clinical trial conducted to evaluate the effect of maternal probiotic administration on gastrointestinal issues, growth-development, and breastfeeding outcomes in infants with colic. The study was conducted in the children outpatient clinic of a training and research hospital in Istanbul, Turkey, between August 2020 and February 2021. Inclusion criteria required to comply with the study protocol were as follows: (a) age less than 60 days, (b) vaginal delivery, (c) breastfeeding more than 8–10 times a day (more than 50%) and (d) being born at term (e) diagnosis of colic (one week before the start of the study, and the crying duration lasting more than three hours a day for at least three days in a week). Exclusion criteria were as follows: (a) presence of major acute or chronic diseases in the mother and infant, (b) gastrointestinal diseases and gastro-esophageal reflux, (c) maternal use of probiotics/antibiotics one week before or during randomization, (d) gastrointestinal malformations, (e) presence of maternal depression. The exclusion criteria also included not using the probiotic product twice simultaneously, using medication for infantile colic, stopping breastfeeding during supplementation, and using formula.

This study was approved by the Training and Research Hospital Clinical Research Ethics Committee (10.06.2020. Ethics Committee No: 2020-85), and institutional permission was obtained from the same institution. Written informed consent was obtained from the mothers accordingly.

When the infants who presented to the clinic with a complaint of crying were referred by a pediatrician with a diagnosis of infantile colic and fulfilled all criteria for registration, the parents were given a 7-day questionnaire that assessed crying duration, sleep, wakefulness, and feeding. Seven days later, an appointment was made for diagnosis; infants and their mothers who met the criteria for colic were enrolled in the study (150 mothers and infants who were diagnosed).

The minimum sample size was calculated using the power analysis statistical software package version 9.3 (SAS Institute, Cary, North Carolina, United States) based on a 50% reduction in daily crying time in infants in the intervention group (those administered a probiotic product).[13] According to this calculation, it was determined that 30 mother-infant pairs should be included in this study, with alpha level of 0.05 and 80% power at 95% confidence interval. However, considering case losses, the sample size was set at 36 mother-infant pairs. At the end of the data collection process, five mother-infant pairs were excluded for various reasons (**Figure 1**), and 31 mother-infant pairs were finally included in the study sample.

All mothers included in the study were randomized into two groups: probiotic and control groups. Randomization was performed in a 1:1 ratio to groups A and B according to a randomization list created by a specific software (www.randomizer.org). An independent researcher prepared the randomization program. According to a lottery method, the patients were assigned to probiotics group (A) or control group (B). Randomization codes were kept confidential until all data were analyzed (**Figure 1**).

All participants, as well as statisticians who evaluated the results of the research, were blinded to the group assignment. Data were collected by a researcher who was not blinded to the study. To avoid bias, the researcher did not participate in any statistical analyses.

Mothers assigned to the probiotics group were instructed to drink a probiotic product containing one can (80 ml) of Actiregularis ($5x10^6$ cfu\ml) strain once a day for 15 days, directly by mouth, preferably in the morning on a full stomach, and without consuming any liquid, including food and water, for an hour. Instructions for storing and handling the product were provided in accordance with the manufacturer's instructions. The mothers were given a diary and taught how to record their administration of the daily dose of the study product as well as the infant's severity of crying and the frequency of stools daily. The mothers were asked to return the used bottles to the researcher after 15 days. Although maternal diet affects the frequency of colic, no special dietary restrictions were recommended during breastfeeding, except to avoid any commercial products containing probiotics.[15] The mothers were also instructed to avoid other methods of treating infantile colic. The mothers in the control group received routine care (behavioral therapy) from the institution. Prebiotics were given free of charge to mothers.

Analysis of symptoms of infantile colic

A baby diary form was given to each parent to record gastrointestinal events, such as the baby's feeding schedule, daily fuss/crying attacks, frequency of uncontrollable crying per day, intensity of daily crying, number of daily bowel movements, and consistency of stools. The frequency of crying was reported as "increased" or "decreased," and was evaluated using the same terms as the previous day, while crying intensity was scored between 0 and 10 points.

At the time of registration (day 0), a pediatric medical examination was performed, and the following information was collected: (1) gestational age, (2) mode of birth, (3) birth weight, (4) anthropometric data at admission, (5) family history of gastrointestinal disease, and (6) family history of atopy.

Follow-up visits were planned at 15 days, 4 months, and 6 months after the start of the application of the study product. At the end of the 15th day of the study, all diaries were collected. Study analysis and data entry were performed independently by the researchers, both of whom were blinded to the treatment allocation.

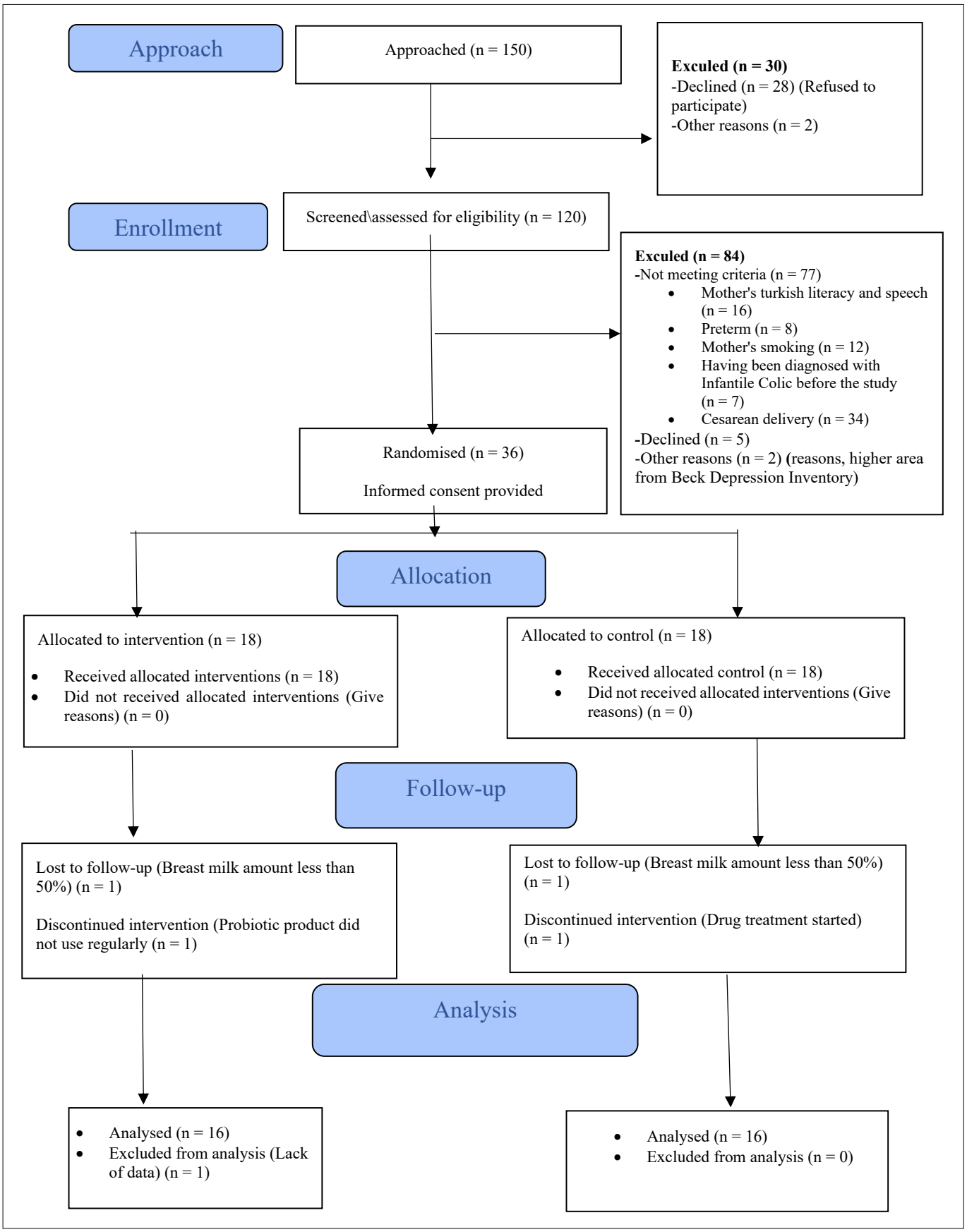

Figure 1. Flowchart of patients considered for the trial of probiotics supplementation for mothers of infants with colic.

Statistical analysis

The primary outcome measures were the ratio of those who responded and those who did not respond to treatment in improving colic symptoms. Success rate was defined as a 50% reduction in the average daily crying frequency and intensity, expressed as frequency and severity.

The secondary outcome measures were the baby's (1) daily defecation consistency, (2) anthropometric evaluations (6-month outcomes), and (3) breastfeeding status (6-month results).

Data from initial visit forms, logs, and the results of the analysis of fecal samples were reported in a database created using Google Drive software (Google ILC, Mountain View, California, United States). The data obtained in this study were analyzed using SPSS (IBM, Armonk, NY, USA) for Windows 25.0.

Quantitative variables with a normal distribution were compared using an independent samples t-test. The Mann–Whitney U test was used for variables without a normal distribution, a comparison was carried out using the analysis of variance for repeated measurements when the variables had a normal distribution. In addition, the Friedman test was applied when the variables did not have a normal distribution, and the Bonferroni test was used to find the time that made the difference in the case where a difference was found. The ratios were compared using the χ^2 test or the Fisher's exact test as appropriate. For all tests, $P < 0.05$ value was considered significant.

RESULTS

For eligibility, 36 mothers and their infants were enrolled and randomized into the control (n = 18) or probiotic (n = 18) group. After 15 days of follow-up, failure was recorded in five infants; therefore, 31 infants (16 in the control group and 15 in the probiotics group) completed the study (**Figure 1**). No significant differences were observed in age, sex, birth weight, and diet **(Table 1).**

Initially, the average crying times of the two groups were similar. On day 15, a higher and statistically significant treatment success rate was observed in the probiotics group compared with the control group (**Table 2**). There was a statistically significant difference between the groups in the frequency of stools on the second, third, sixth, eighth, tenth, and fourteenth days ($P < 0.05$) (**Table 3**).

Table 2. Distribution of crying intensity of infants in the probiotics and control groups (n = 31)

		$\bar{X} \pm$ SD [Min-Max]	Test value	P
1	Intervention	9.93 ± 0.26 [9.00–10.00]	112.000**	0.770
	Control	10.00 ± 0.00[10.00–10.00]		
2	Intervention	9.87 ± 0.35 [9.00–10.00]	111.500**	0.740
	Control	9.94 ± 0.25[9.00–10.00]		
3	Intervention	9.73 ± 0.46[9.00–10.00]	95.500**	0.338
	Control	9.94 ± 0.25[9.00–10.00]		
4	Intervention	9.33 ± 0.49[9.00–10.00]	62.500**	**0.021***
	Control	9.81 ± 0.40[9.00–10.00]		
5	Intervention	9.00 ± 0.85[8.00–10.00]	70.00**	**0.049***
	Control	9.63 ± 0.62[8.00–10.00]		
6	Intervention	8.60 ± 0.83[7.00–10.00]	39.000**	**0.001***
	Control	9.63 ± 0.62[8.00–10.00]		
7	Intervention	7.93 ± 1.03[7.00–10.00]	34.000**	**0.000***
	Control	9.38 ± 0.62[8.00–10.00]		
8	Intervention	7.20 ± 1.01[6.00–9.00]	13.000**	**0.000***
	Control	9.13 ± 0.62[8.00–10.00]		
9	Intervention	6.80 ± 1.15[5.00–8.00]	9.000**	**0.000***
	Control	9.06 ± 0.68[8.00–10.00]		
10	Intervention	6.27 ± 1.22[5.00–8.00]	9.500**	**0.000***
	Control	8.94 ± 0.85[7.00–10.00]		
11	Intervention	5.60 ± 0.99[4.00–7.00]	3.000**	**0.000***
	Control	8.50 ± 0.82[7.00–10.00]		
12	Intervention	5.33 ± 1.18[4.00–7.00]	4.500**	**0.000***
	Control	8.19 ± 0.83[7.00–10.00]		
13	Intervention	5.13 ± 0.99[4.00–7.00]	1.500**	**0.000***
	Control	8.38 ± 0.89[7.00–10.00]		
14	Intervention	4.73 ± 0.70[4.00–6.00]	0.000**	**0.000***
	Control	8.31 ± 0.95[7.00–10.00]		
15	Intervention	3.93 ± 0.70[3.00–5.00]	0.000**	**0.000***
	Control	8.19 ± 1.22[6.00–10.00]		

*P < 0.05; **Mann Whitney U test. Min = minimum; Max = Maximum; X = mean; SD = standard deviation.

Table 1. Distribution of socio-demographic and obstetric characteristics of mothers in the probiotics and control groups (n = 31)

		Probiotics		Control		Test Value	P
		n	%	n	%		
Mother's age	24 years and under	7	46.7	8	50.0	0.034**	0.853
25.58 ± 5.04	25 years and older	8	53.3	8	50.0		
Mother's weight	9 pounds and less	7	46.7	8	50.0	0.034**	0.569
10.35 ± 5.90	10 pounds and more	8	53.3	8	50.0		
Postnatal age of the baby	Day	30.20 ± 2.21		29.56 ± 1.82		0.878**	0.387
Gestational age	Week	39.32 ± 0.87		39.10 ± 1.16		0.591**	0.559
Sex of the baby	Girl	10	66.7	0.059**	0.553	0.059**	0.553
	Boy	5	33.3	6	37.5		
Sum		**15**	**100.0**	**16**	**100.0**		

*P < 0.05; **χ^2 = Chi-square analysis.

Table 3. Comparison of infant stool counts according to the groups (n = 31)

		$\bar{X} \pm$ SD [Min-Max]	Test value	P
1	Intervention	1.80 ± 1.15[1–5]	82.000**	0.140
	Control	1.19 ± 0.40[1–2]		
2	Intervention	2.13 ± 1.81[0–7]	61.500**	**0.019***
	Control	0.81 ± 0.83[0–2]		
3	Intervention	2.00 ± 1.36[0–4]	64.00**	**0.027***
	Control	0.88 ± 0.62[0–2]		
4	Intervention	2.00 ± 1.85[0–8]	75.500**	0.078
	Control	1.06 ± 0.85[0–3]		
5	Intervention	1.93 ± 1.83[0–7]	99.000**	0.423
	Control	1.19 ± 0.75[0–3]		
6	Intervention	1.73 ± 1.49[0–6]	66.500**	**0.033***
	Control	0.75 ± 0.77[0–2]		
7	Intervention	1.93 ± 1.39[1–5]	80.500**	0.119
	Control	1.13 ± 0.89[0–3]		
8	Intervention	2.13 ± 1.46[0–5]	58.000**	**0.014***
	Control	0.88 ± 0.96[0–3]		
9	Intervention	2.20 ± 1.61[0–6]	75.500**	0.078
	Control	1.19 ± 0.75[0–3]		
10	Intervention	1.93 ± 1.22[1–5]	63.000**	**0.024***
	Control	0.94 ± 0.68[0–2]		
11	Intervention	1.87 ± 1.41[0–5]	86.500**	0.188
	Control	1.13 ± 0.81[0–3]		
12	Intervention	2.07 ± 1.53[0–5]	77.000**	0.093
	Control	1.13 ± 0.96[0–3]		
13	Intervention	2.07 ± 1.53[0–5]	75.000**	0.078
	Control	1.06 ± 0.77[0–3]		
14	Intervention	1.80 ± 1.32[0–5]	66.500**	**0.033***
	Control	0.81 ± 0.66[0–2]		
15	Intervention	1.93 ± 1.28[1–5]	83.500**	0.151
	Control	1.19 ± 0.66[0–3]		

*P < 0.05; **Mann Whitney U test. Min = minimum; Max = Maximum; X < in = mean; SD = standard deviation.

The weights of the babies in the probiotics and control groups were compared. **Table 4** shows a statistically significant difference in the sixth-month weight of the babies between the groups (P < 0.05). The babies in the probiotics group weighed more at six months than did the babies in the control group. However, there was no statistically significant difference in the height and head circumference of the infants (P > 0.05). The results showed a statistically significant difference between the groups in the nutritional status of the infants at the third month, sixth month, and time of initiation of additional food (P < 0.05) **(Table 5).**

DISCUSSION

Infantile colic has a multifactorial etiology, and the sociodemographic and obstetric conditions of the parents, especially the mother, are factors related to the etiology.[8,12,13] In this section, the sociodemographic and obstetric data of mothers, crying intensity of the baby, frequency of stools, anthropometric measurements, and breastfeeding status are discussed according to the probiotics and control groups and compared with the literature.

In this study, the mean age of the mothers was 25.58 ± 5.04 (**Table 1**), and approximately half of the mothers (51.61%) whose infants had colic were over the age of 25 years. There was no significant difference between the groups (P = 0.853). In a study of 1955 mothers in whom infantile colic, fetal growth, and other potential risk factors were evaluated, the risk of infantile colic increased as the age of the mother increased.[14] Several studies have shown high incidence rates of colic in infants of mothers over the age of 30 years.[15-18] The findings of the current study are in line with those in the literature.

Infantile colic is a behavioral neonatal problem manifested as crying bouts.[19] Crying time is also an important parameter in the evaluation of the effectiveness of the treatment methods for infantile colic.[20-22] In the present study, when the crying times of the babies in the probiotics and control groups were compared, a significant difference was found between the groups (P = 0.000). Although the difference in crying intensity between the groups became significant from day 4, the largest difference was observed on day 15. At the end of the fifteenth day, there was an approximately 80% improvement in crying intensity in the infants in the probiotics group, while there was a 12.5% improvement in the control group (**Table 2** and **Table 3**). This finding suggests that the probiotic product was more effective at reducing crying time in infants with colic. In a systematic review evaluating seven randomized controlled trials, probiotics intake was associated with treatment success and reduced crying time by approximately 50 minutes per day when compared with placebo intake.[23]

In recent literature, the presence of anthropometric parameter disorders that an infant cannot develop without any health problems has been added to the diagnostic criteria for infantile colic.[24,25] In our study, there was a statistically significant difference in the sixth-month weight of the babies between the groups. The sixth-month weight of the babies in the probiotics group was higher than that of the babies in the control group, and there was no significant difference between the height and head measurements of the babies (**Table 4**). In one study, the growth curves of infants with colic who received probiotic products and formula were evaluated, and it was found that the growth curves of formula-fed infants were low, and the babies were affected.[11] In a study conducted by Costro-Rodriguez et al.,[26] there was no significant difference in weight, height, and head measurements when the nutrition of infants with gastrointestinal problems (the most commonly reported gas complaint) was compared with that of the control group. In the same study, the infant growth outcomes in both formula groups were similar to those of breastfed infants in

Table 4. Comparison of 6-month-old weight between infants in the probiotics and control group (n = 31)

		Min	Max	Median	$\bar{X}$	SD	Test value	P
Birth weight	Probiotics	2755.00	3790.00	3115.00	3182.00	280.95	-0.706**	0.486
	Control	2250.00	4015.00	3382.50	3293.44	547.24		
1st month	Probiotics	3300.00	5000.00	4100.00	4089.67	501.53	-0.278**	0.783
	Control	3400.00	5200.00	4200.00	4139.38	492.62		
4th month	Probiotics	5280.00	7200.00	6450.00	6383.33	456.41	1.746**	0.091
	Control	4800.00	7420.00	5925.00	6008.13	705.01		
6th month	Probiotics	6450.00	8800.00	7850.00	7699.33	651.15	50.500***	**0.005***
	Control	1050.00	8800.00	6800.00	6680.63	1675.19		
Birth Size Measure	Probiotics	46.00	52.00	51.00	50.27	1.87	-0.371**	0.713
	Control	47.00	55.00	50.00	50.56	2.50		
1st month	Probiotics	50.00	56.00	54.00	53.33	2.09	0.098**	0.923
	Control	49.00	58.00	53.00	53.25	2.59		
4th month	Probiotics	59.00	69.00	65.00	63.87	2.95	1.798**	0.083
	Control	57.00	68.00	62.00	61.88	3.20		
6th month	Probiotics	64.00	73.00	71.00	69.93	2.76	1.395**	0.174
	Control	64.00	74.00	69.00	68.56	2.71		
Head Circumference on Birth	Probiotics	33.00	36.00	35.00	34.87	0.64	114.500**	0.830
	Control	33.00	37.00	35.00	34.88	0.89		
1st month	Probiotics	35.00	38.00	36.00	36.43	0.86	120.000**	1.000
	Control	35.00	38.00	36.50	36.44	0.81		
4th month	Probiotics	38.00	40.00	39.00	38.60	0.63	99.000**	0.423
	Control	38.00	40.00	39.00	38.81	0.66		
6th month	Probiotics	39.00	43.00	41.00	40.93	1.10	113.000**	0.800
	Control	39.00	42.00	41.00	41.00	0.89		

*P < 0.05; **Independent t-test; ***Mann Whitney U test; Min = minimum; Max = Maximum; X < in = mean; SD = standard deviation.

Table 5. Comparison of feeding status between infants in the probiotics and control groups by month (n = 31)

Variables		Probiotics		Control		Test Value	P
		n	%	n	%		
4th month nutritional status	Breast milk only	14	93.3	9	56.3	5.727**	**0.044***
	Breast milk + formula	1	6.7	5	31.2		
	Just food	0	0.0	2	12.5		
6th month nutritional status	Breast milk only	1	6.7	0	0.0	7.642**	**0.035***
	Just food	0	0.0	1	6.2		
	Breast milk + additional food	14	93.3	10	62.5		
	Additional food only	0	0.0	5	31.3		
Cessation of breastfeeding	Yes	2	13.3	6	37.5	2.362**	0.220
	No	13	86.7	10	62.5		
Time of initiation of additional food	4th month	0	0.0	3	18.8	8.471**	**0.018***
	5th month	4	26.7	9	56.2		
	6th month	10	66.6	4	25.0		
	7th month	1	6.7	0	0.0		
Sum		**15**	**100.0**	**16**	**100.0**		

*P < 0.05; ** Chi square analysis.

the reference group. In a study conducted by Szajewska and Drly, there was no significant difference in weight between the probiotic-supplemented and non-reinforced (control) groups.[27] Similarly, the mean changes in height, head circumference, and body mass index were not significantly different between the groups. Our findings are consistent with those of previous studies showing that formulas containing specific prebiotic mixtures and/or fermented formulas are well tolerated and promote adequate infant growth.[27,28]

Excessive crying, which is the most prominent feature of infantile colic, can cause mothers to feel inadequate, decrease breast milk intake, and increase formula use and early transition to additional food.[29,30] All mothers included in our study fed their babies more than 50% breast milk daily. In the first month, four (26.4%) mothers in the control group compared with those in the probiotics group were giving breast milk and formula to their babies. By the sixth month, 31.3% of the mothers in the control group had

stopped breastfeeding (**Table 5**). In one study, the main reason mothers found their milk to be insufficient was that their babies were crying, causing them to feed their babies formula.[31] Another study found that frequent crying bouts of infants raised anxiety in mothers about adequate milk intake and were the most important factor that caused mothers to use supplemental products with breast milk.[32] Contrary to the literature, a study showed no relationship between infantile colic and breastfeeding duration and insufficiency.[33] Moreover, gastrointestinal discomfort in infants also leads to changes, especially in the transition from breastfeeding to bottle feeding.[11] In our study, it was found that the babies in the control group (75%) used more pacifiers (40%) than did the babies in the probiotics group. This shows that mothers turn to the use of pacifiers/bottles to reduce the severity of crying. In most cases in one study, mothers decided to stop breastfeeding before seeking medical attention to alleviate gastrointestinal discomfort, and in the same study, reduced diversity and stability of the gut microbiota was associated with the onset of infantile colic.[11]

CONCLUSION

Infants treated with Actiregularis through their mother's diet for 15 days showed a decrease in the frequency and intensity of crying, and this had a positive effect on breastfeeding outcomes and anthropometric measurements.

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Effect of hyperchloremia on mortality of pediatric trauma patients: a retrospective cohort study

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ABSTRACT

BACKGROUND: Hyperchloremia is often encountered due to the frequent administration of intravenous fluids in critically ill patients with conditions such as shock or hypotension in the pediatric intensive care unit, and high serum levels of chloride are associated with poor clinical outcomes.

OBJECTIVES: This study aimed to determine the association between hyperchloremia and in-hospital mortality in pediatric patients with major trauma.

DESIGN AND SETTING: This retrospective cohort study was conducted at a tertiary university hospital in Turkey.

METHODS: Data were collected between March 2020 and April 2022. Patients aged 1 month to 18 years with major trauma who received intravenous fluids with a concentration > 0.9% sodium chloride were enrolled. Hyperchloremia was defined as a serum chloride level > 110 mmol/L. Clinical and laboratory data were compared between the survivors and nonsurvivors.

RESULTS: The mortality rate was 23% (n = 20). The incidence of hyperchloremia was significantly higher in nonsurvivors than in survivors (P = 0.05). In multivariate logistic analysis, hyperchloremia at 48 h was found to be an independent risk factor for mortality in pediatric patients with major trauma.

CONCLUSIONS: In pediatric patients with major trauma, hyperchloremia at 48-h postadmission was associated with 28-day mortality. This parameter might be a beneficial prognostic indicator.

INTRODUCTION

Intravenous fluids are commonly used to restore hypovolemia and ensure maintenance fluid for pediatric patients in emergency departments, intensive care units, and operating rooms.[1] Hypertonic saline is frequently chosen for cerebral antiedema treatment, especially in severely injured patients with trauma.[2] Familiar alternative fluids are 0.9% sodium chloride (NS), Ringer's lactate solution, and 3% hypertonic saline (HTS).[3] Although abundant knowledge has been gathered about the advantages of these fluids over time with their widespread use, a small number of adverse effects related to their biochemical composition, osmolarity, and volume overload have been identified. Acid–base disorders, electrolytic disorders (hyperchloremia, hypernatremia), volume overload, and hemodilution of proteins are major characterized side effects of crystalloid solutions.[4,5] HTS used for cerebral antiedema treatment and the most commonly used resuscitation fluid NS contains supraphysiological concentration of chloride (Cl^-).[6] Hyperchloremia in critically ill patients used to be considered harmless evidence; However, cases during the last decade the current animal model studies have shown that hyperchloremia is related to immune system paralysis, several coagulation problems, and pulmonary disorders.[7] Pediatric patients with major trauma are predisposed to hyperchloremia in the postresuscitation period because they are usually required to be treated with NS during the resuscitation period of shock, and the increase in HTS use may cause a exposure to high amounts of chloride ion and an elevation in the level of serum chloride. An observational study showed that hyperchloremia served as an independent risk factor for hospital mortality in patients with major trauma.[8] Although many studies have investigated the effects of hyperchloremia on mortality in adult trauma patients, few studies have addressed this in the pediatric age group.

OBJECTIVE

This study aimed to determine whether serum chloride levels were related to mortality in pediatric patients exposed to major trauma.

METHODS

Research type and sampling

This retrospective, observational, single-center study analyzed the association between hyperchloremia and mortality in pediatric patients with major trauma admitted to the pediatric intensive care unit of a tertiary university hospital between March 2020 and April 2022. The study was approved by the faculty ethics committee (date: April 1, 2022; no: 2022/4).

Children aged 1 month to 18 years with major trauma who received intravenous fluids with a concentration of > 0.9% sodium chloride were enrolled. Patients with an injury severity score (ISS) of < 15, receiving dialysis, missing serum chloride records, staying for < 48 h, or with baseline hyperchloremia (chloride > 110 mmol/L) were excluded. At our institution, an intravenous 3% NaCl bolus is administered every 6 h to prevent an increase in intracranial pressure (ICP) for every major trauma patient without measuring the ICP. The cutoff level of hyperchloremia was defined as a serum chloride level > 110 mmol/L. This threshold definition was used based on published literature.[9] Delta chloride (Δchloride) was described as the difference between the chloride level 48-h postadmission and the baseline level. All reasons for death within 28 days of admission to the pediatric intensive care unit (PICU) admission were defined as hospital mortality.

Data collection tools

Patients were divided into survivor and nonsurvivor groups based on the outcome. The total fluid balance was calculated by adding all volumes of fluid administered over 48 h. Fluid management involves both the bolus and continuous infusion routes. Pre-PICU fluid management was not included in this study. Age, sex, vital signs on arrival (mean arterial pressure, heart rate, and temperature), peripheral oxygen saturation, the need for mechanical ventilation, length of the intensive care stay, and outcomes were recorded. The ISS was calculated to determine disease severity on admission. Serum levels of chloride, sodium, potassium, and creatinine were recorded upon admission to the PICU and at 48 h. Counts of white blood cells and platelets, level of hemoglobin, alanine aminotransferase (ALT), and aspartate aminotransferase (AST), and the international randomized ratio (INR) were included. Data were acquired from the patient files and an electronic hospital data management system.

Evaluation of data

Data were analyzed with SPSS Statistics 22 software (IBM Co., Armonk, NY, USA). In the univariate analysis, continuous parameters were described as mean ± standard deviation (SD) for normal distribution, median, and interquartile range (IQR) for skewed distribution and nonparametric data. Categorical parameters were evaluated using the chi-squared or Fisher's exact tests. Student's t-test was performed for normally distributed data, and the Mann-Whitney U test was used for nonparametric parameters. Significant parameters identified in the univariate comparison were then included in a multivariate logistic regression model to describe the independent risk factors for 28-day mortality using the maximum likelihood method and backward stepwise selection. Hosmer–Lemeshow goodness-of-fit was used to determine the logistic regression model fit. Significance was set at $P < 0.05$ for all results.

RESULTS

Eighty-six pediatric patients with major trauma (56 boys and 30 girls) that met the inclusion criteria were included: 66 (77%) survived and 20 (23%) died within 28 days of PICU admission. Nine patients with an ISS of < 15, two patients with hyperchloremia at presentation, and two patients who stayed in the PICU for < 48 h were excluded. No significant differences were present between the mechanisms of trauma, traffic accidents (n = 41, 48%), or falls (n = 45, 52%), and no significant differences were found between the trauma types in terms of survival (P = 0.95). The mean age of the survivors was 91.32 ± 54.27 months while the age of nonsurvivors was 96.90 ± 55.49 months (P = 0.77). The main characteristics of the patients with major trauma are shown in **Table 1**. No significant differences were found between survivors and nonsurvivors for respiratory rate, heart rate, mean arterial blood pressure, peripheral oxygen saturation, or body surface area (BSA). The total infused fluid volume was greater in nonsurvivors than in survivors (5.1(2.8) vs. 5.8(3.7); P = 0.04). The ISS differed significantly between the two groups (P = 0.01). The median duration of mechanical ventilation was significantly longer in nonsurvivors (4(2) vs. 5(3); P = 0.80). No significant difference was present in the mean duration of the total length of PICU stay (8.61 ± 2.60 versus 5.90 ± 2.23; P = 0.09). Initial electrolyte levels were similar in the two groups; however, the 48-h sodium level was significantly higher in nonsurvivors (149.9 ± 6.88 versus 142.67 ± 2.62 mmol/L, P ≤ 0.01). Chloride levels > 110 ng/mL were significantly higher in nonsurvivors at 48 h (P = 0.05). Patients in the nonsurvivor group did not have significantly higher serum chloride levels on admission compared with survivors although these levels in nonsurvivors were significantly higher at 48 h (103.76 ± 2.52 versus 104.60 ± 2.83 mmol/L, P = 0.16; 108.55 ± 4.37 versus 113.60 ± 5.71 mmol/L, P = 0.02). Nonsurvivors had significantly higher Dchloride levels (9.70 ± 4.62 versus 6.79 ± 3.59, P = 0.04). Lactate measurements and pH levels did not significantly differ between the groups (**Table 2**). The initial base deficit was similar between groups but the measurement of the 48-h base deficit was significantly higher in nonsurvivors (-4.80 ± 3.95 versus

Table 1. Baseline demographic and clinical variables of survivors and nonsurvivors

Variables	Survivors (n = 66)	Nonsurvivors (n = 20)	P value
Age (month), mean ± SD	91.32 ± 54.27	96.90 ± 55.49	0.77
Sex (female n /%, male n/%)	22 (40%); 44 (60%)	8 (33.3%); 12 (66.7%)	0.61
Respiratory rate (rate/min), mean ± SD	25.62 ± 4.60	24.60 ± 2.45	0.50
Peripheral oxygen saturation, %*	94.50 (5)	89.50 (5)	0.89
Heart rate (rate/min), mean ± SD	132.06 ± 23.15	134.7 ± 27.31	0.13
Mean arterial pressure (mmHg), mean ± SD	67.18 ± 12.43	64.10 ± 6.87	0.21
Type of trauma			
Fall from height	34 (51.5%)	11 (55%)	0.95
Traffic accident	32 (48.5%)	9 (45%)	
Duration of mechanical ventilation (day)*	4 (2)	5 (3)	0.80
Duration of PICU (day), mean ± SD	8.61 ± 2.60	5.90 ± 2.23	0.09
BSA, mean ± SD	0.96 ± 0.39	0.94 ± 0.40	0.89
GCS, mean ± SD	8.56 ± 3.12	7.3 ± 2.31	0.70
ISS*	16(3)	21(5)	0.01
Total infused fluid volume during 48 h (liter)	5.1 (2.8)	5.8 (3.7)	0.04

*Median (interquartile range).

PICU = pediatric intensive care unit; BSA = body surface area; GCS = Glasgow coma scale; ISS = injury severity score; SD = standard deviation.

Table 2. Comparison of laboratory findings between survivors and nonsurvivors

Variables	Survivors (n = 66)	Nonsurvivors (n = 20)	P value
Creatinine (mg/dL)			
Initial, mean ± SD	0.46 ± 0.29	0.61 ± 0.45	0.23
At 48 h, mean ± SD	0.48 ± 0.25	0.64 ± 0.25	0.07
Sodium (mmol/L)			
Initial*	140 (3.25)	141.5 (2.75)	0.26
At 48 h, mean ± SD	142.67 ± 2.62	149.90 ± 6.88	<0.01
Potassium (mmol/L)			
Initial, mean ± SD	4.01 ± 2.89	4.13 ± 0.31	0.31
At 48 h, mean ± SD	4.04 ± 0.26	3.95 ± 0.53	0.42
Chloride (mmol/L)			
Initial, mean ± SD	103.76 ± 2.52	104.60 ± 2.83	0.16
At 48 h, mean ± SD	108.55 ± 4.37	113.60 ± 5.71	0.02
Chloride (mmol/L)			
At 48 h			
< 110	31 (77.5%)	3 (25%)	0.05
≥ 110	9 (22.5%)	9 (75%)	
Δchloride (mmol/L), mean ± SD	6.79 ± 3.59	9.70 ± 4.62	0.04
pH			
Initial*	7.35 (0.08)	7.33 (0.19)	0.14
At 48 h, mean ± SD	7.40 ± 0.02	7.38 ± 0.03	0.10
Base deficit (mmol/L)			
Initial, mean ± SD	-4.80 ± 3.95	-6.56 ± 4.53	0.08
At 48 h, mean ± SD	-1.89 ± 1.51	-4.18 ± 1.79	0.04
Lactate (mmol/L)			
Initial*	4.30 (1.6)	5.1 (4.3)	0.12
At 48 h*	2.5 (1.3)	3.0 (2.1)	0.10
Hgb gr/dl *	11.45 (2.68)	10.60 (4.08)	0.43
PLT × 10^3/μL, mean ± SD	237 ± 112	167 ± 92	0.08
WBC × 10^3/μL, mean ± SD	16.41 ± 5.37	16.74 ± 9.40	0.88
ALT (U/L)*	128 (27)	132 (258)	0.42
AST (U/L)*	137 (66)	148 (51)	0.36
Albumin g/dL, mean ± SD	3.71 ± 0.58	3.40 ± 0.38	0.11
INR (U/L), mean ± SD	1.48 ± 0.37	1.71 ± 0.27	0.07

*Median (interquartile range).

Hb = Hemoglobin; WBC = white blood cell; PLT = platelet; INR = international normalized ratio; AST = aspartate aminotransferase; ALT = alanine aminotransferase; SD = standard deviation.

-6.56 ± 4.53, P = 0.08; -1.89 ± 1.51 versus 4.18 ± 1.79, P = 0.04). Counts of white blood cell and platelets, levels of hemoglobin, ALT, and AST, and the INR were similar between both groups. Finally, multivariate logistic regression analysis was used to evaluate the relationship between hyperchloremia and 28-day hospital mortality by calculating the 95% confidence interval (95%CI) and odds ratio (OR). For the multivariate analysis, possible factors identified by the bivariate analysis were included in the model to detect independent predictors of the outcome. In multivariate analysis, hyperchloremia at 48 h was shown to be an independent risk factor for in-hospital mortality (OR 1.8; 95%CI 1.05–2.1, P = 0.04) (**Table 3**). ISS and totally infused fluid volume at 48 h were also found to be independent risk factors for mortality (OR 1.85; 95%CI 1.02–2.69, P = 0.01; OR 1.43; 95%CI 0.97–2.03, P = 0.05, respectively).

DISCUSSION

The current study was a retrospective clinical study that evaluated the correlation between hyperchloremia and hospital mortality of seriously injured pediatric patients. The strengths of this research are the choice of a representative model of patients with major trauma admitted to the PICU and the performance of multivariate analysis for clinical confounders such as total infused fluid volume, base deficit, and injury severity score that are directly associated with hyperchloremia and mortality,

The main outcomes of this study were as follows: First, all patients had normal serum chloride levels on admission; however, hyperchloremia occurred more commonly in nonsurvivors after the initiation of medical therapy. Second, hyperchloremia, ISS, and the total volume of fluid infused in the first 48 h were found to be independently associated with mortality in children admitted to the PICU in multivariate logistic regression analysis.

The ISS score is frequently used to estimate the probability of mortality and survival ratio for patients with trauma.[10] In a study comparing mortality scores in pediatric patients with major trauma, the ISS score was superior to the pediatric trauma score, pediatric risk of mortality III score, and base deficit, INR, and Glasgow coma scale score.[11] The ISS scoring system has been shown to be better than the RTS in predicting mortality.[12] In our study, although mortality scores were not compared to determine the accuracy of survival prediction, the ISS score was identified as an independent risk factor for mortality.

Table 3. Multivariate logistic regression analysis of survivors versus nonsurvivors

Variables	Odds Ratio	95% Confidence Interval	P value
ISS	1.85	1.02–2.69	0.01
Total infused fluid volume during 48 h (liter)	1.43	0.97–2.03	0.05
Chloride at 48 h	1.8	1.05–2.1	0.04
Δchloride	0.95	0.84–1.08	0.07

ISS = Injury severity score; Δchloride = delta chloride.

The most common cause of hyperchloremia after admission to the intensive care unit is an infusion of chloride-rich solutions, such as HTS and/or NS in patients with trauma.[13] Normal saline is frequently used for the dilution of medications, and HTS is used for cerebral antiedema treatment, which may be the cause of the unnoticed origins of chloride in the PICU. The chloride concentration of normal saline is 154 Eq/L while the chloride concentration of 3% hypertonic saline is 513 Eq/L and is higher than the normal plasma chloride concentration.[14] The very close chloride values among the groups in the present research may complicate the clinical applicability. However, the cutoff level of hyperchloremia was defined as a serum chloride level of > 110 mmol/L in this study. When the chloride levels of the two groups were compared at 48 h, 75% of the nonsurvivor group had a chloride value > 110 mmol/L compared with only 22.5% of the survivor group, which was a significant difference.

Many studies have shown that an increased mortality ratio might be associated with elevated serum chloride levels in patients with major trauma.[15,16] Hyperchloremia can be harmful to tissues, causing an iatrogenic threat to the metabolic energy of cells and contributing to the risk of mortality and morbidity.[17] However, avoiding excessive serum chloride overload is necessary. The pathophysiological mechanisms underlying the relationship between hyperchloremia and mortality from trauma have not yet been elucidated. Proinflammatory reactions are mediated by nitric oxide and have a higher ratio of interleukin (IL)-6 to IL-10 in lactic acidosis than in hyperchloremic metabolic acidosis.[18] Serum chloride levels play a critical role in neutrophil function. Neutrophils need a permanent chloride influx to ensure sufficient is available substrate for hypochloric acid production,[19] and an insufficient chloride concentration has been related to diminished neutrophil function.[20] Although the cause of coagulopathy induced by isotonic saline is not exactly known, the administration of large volumes of isotonic saline may trigger coagulopathy.[21] While fluid overload has been associated with morbidity and mortality in surgical patients, resuscitation volumes < 1,500 mL are not associated with increased mortality.[6,22] Our study shows that nonsurvivors had a significantly larger volume of infused fluid than survivors. An increased risk of mortality was associated with an increase in the amount of fluid used for resuscitation, which may be a risk associated with using chloride-rich fluids for resuscitation. The volume-adjusted chloride load could not be calculated because of missing blood product data on the transfusion amount. However, higher chloride levels in the nonsurvivor group may be associated with patients in this group receiving more resuscitation volumes.

Administration of a large volume of chloride-rich solutions may have harmful outcomes for kidneys.[23] Several experimental studies have demonstrated that large intravenous chloride loads decline renal blood flow, glomerular filtration rate, and renal cortical tissue perfusion.[24] Similarly, a previous study showed that hyperchloremia at 48 h was prominently associated with acute kidney injury and hospital mortality in an ICU.[25] Moreover, the glomerular filtration rate and extended hyperchloremia at rather than the highest serum chloride value or the duration of hypertonic fluid infusion were related to the development of acute kidney injury in patients with traumatic brain injury.[26] In the current study, the serum creatinine level of nonsurvivors was insignificantly higher than that of survivors.

Besides hyperchloremia at 48 h, Δchloride was associated with 28-day mortality in major trauma patients.[8] Huang et al. presented that new-onset hyperchloremia and increases of every 5 mmol/L in Δchloride were related to the increased probability of mortality.[27] Similar to this research, Δchloride was markedly different between survivors and nonsurvivors in our study. This confirms the critical practical effect of using chloride-rich fluids, which can increase serum chlorine levels.

The present study had several limitations. First, this study was performed at a single medical institute, and the relatively small patient count may have limited our ability to clearly illuminate the association between hyperchloremia and mortality. Second, we evaluated serum chloride values only at admission and 48 h after PICU admission, and not at other time points during the stay in the PICU. Third, the total volume and concentration of the fluid infused before admission to the intensive care unit could not be measured. Finally, a larger number of patients is required to achieve considerable power and reliability.

CONCLUSION

This study contributes to the increasing number of studies demonstrating that hyperchloremia may have inconvenient effects, especially in patients with trauma. Attention has recently focused on understanding the effect of serum chloride abnormalities on unfavorable outcomes. This study shows that chloride disorders and unfavorable outcomes appear to be associated with the ICU. Therefore solutions containing electrolytes separate from physiological solutions should be used more appropriately, and the development of hyperchloremia should be considered as a prognostic marker, taking into account the severity of the patient.

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Contamination of equipment and surfaces in the operating room anesthesia workspace: a cross-sectional study

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ABSTRACT

BACKGROUND: Contamination of the breathing circuit and medication preparation surface of an anesthesia machine can increase the risk of cross-infection.

OBJECTIVE: To evaluate the contamination of the anesthetic medication preparation surface, respiratory circuits, and devices used in general anesthesia with assisted mechanical ventilation.

DESIGN AND SETTING: Cross-sectional, quantitative study conducted at the surgical center of a philanthropic hospital, of medium complexity located in the municipality of Três Lagoas, in the eastern region of the State of Mato Grosso do Sul.

METHODS: Eighty-two microbiological samples were collected from the breathing circuits. After repeating the samples in different culture media, 328 analyses were performed.

RESULTS: A higher occurrence of *E. coli, Enterobacter spp., Pseudomonas spp., Staphylococcus aureus, and Streptococcus pneumoniae* ($P < 0.001$) were observed. Variations were observed depending on the culture medium and sample collection site.

CONCLUSION: The study findings underscore the inadequate disinfection of the inspiratory and expiratory branches, highlighting the importance of stringent cleaning and disinfection of high-touch surfaces.

INTRODUCTION

The transmission of microorganisms in healthcare settings, including perioperative areas, has become a potential concern in recent studies. The occupancy of spaces by previously colonized or infected patients increases the risk of acquiring multidrug-resistant microorganisms, emphasizing the critical importance of robust cleaning and disinfection procedures.[1-2] The persistence of contamination on environmental surfaces has been linked to insufficient cleaning practices in operating rooms and anesthesia workspaces. This is of particular concern as eliminating multidrug-resistant microorganisms from these surfaces is a key strategy to mitigate the prevalence of healthcare-associated infections (HAIs) that significantly contribute to morbidity, mortality, and extended hospital stays.[2-5]

The existing[3-6] cleaning practices in operating rooms and anesthesia work areas are inadequate; therefore, environmental surfaces remain contaminated. Removing multidrug-resistant microorganisms from operating room surfaces is essential to minimizing the risk of HAIs. Furthermore, HAIs contribute to major public health problems by increasing morbidity and mortality rates and prolonging the hospitalization time of patients.[1-5]

In particular, surgical patients with open wounds are at a higher risk;[6] therefore, the potential for cross-transmission in the intraoperative environment poses a threat to patient safety.[7] Contamination in the anesthesia work area, including the anesthesia cart, faucets, laryngeal masks, laryngoscope blades, touchscreens, keyboards, and the hands of professionals, can result in the transmission of infections that promote health risks, highlighting pneumonia associated with mechanical ventilation (PAVM).[1-4,8]

Ventilator-associated pneumonia (VAP) is characterized as an infectious disease with an imprecise diagnosis and multiple causes, which allows divergent recommendations related to preventive measures, diagnosis, and treatment.[9-11] Because 24 h of intubation in invasive mechanical ventilation (IMV) favor the colonization of microorganisms in the lower airways, orotracheal

intubation performed during surgeries that require general anesthesia may also be a risk factor for PAVM.[10-11]

Despite practicing high-level cleaning and disinfection protocols, breathing circuits and anesthesia cart surfaces are contaminated by microorganisms such as gram-negative bacteria.[10-15] Patients undergoing general anesthesia with IMV have variable health conditions, which increases the risk of cross-infection in the intraoperative period due to contamination of the respiratory circuit and the surface for medication preparation.[16]

However, despite advances in addressing HAIs, substantial gaps in understanding the dynamics of contamination persist within the confines of operating rooms as well as the intricate landscape of anesthesia-related equipment.[1-3] Of these gaps, the precise level of contamination in operating rooms, more specifically, surfaces designated for the preparation of anesthetic drugs, is of particular concern. Similarly, anesthesia-related devices, which are crucial components of patient care, hold utmost importance in this context, necessitating a deeper exploration of their potential roles in transmitting HAIs in this environment [2-5,8]

Comprehensive studies investigating operating room environments, especially those involving the intricate interaction of instrumental surfaces in the preparation of anesthetic drugs, are lacking. Therefore, constructing a comprehensive picture of the persistent contamination levels in these critical areas remains challenging. Although commendable efforts have been made to develop guidelines for reducing HAIs, their implementation and adoption in the surgical environment are inconsistent. This could lead to a variation in infection prevention practices, which increases the possibility of potential oversights that could compromise patient safety.[2,4-5,17-19]

Furthermore, the shortage of routine compliance audits exacerbates this issue. The potential for misguided practices increases owing to the lack of regular and rigorous evaluation of adherence to infection prevention measures.

OBJECTIVE

The objective of this study was to evaluate the contamination of anesthetic medication preparation surfaces, respiratory circuits, and devices used in general anesthesia with assisted mechanical ventilation.

METHODS

This was a cross-sectional, quantitative study conducted at the surgical center of a philanthropic hospital of medium complexity, located in the municipality of Três Lagoas, in the eastern region of the State of Mato Grosso do Sul. This research was derived from a master's thesis submitted to the graduate program in Nursing at the Federal University of Mato Grosso do Sul.[14]

The institution has 188 active beds, 60% of which are allocated to the Unified Health System (in Portuguese, Sistema Único de Saúde, SUS). This facility has been used for teaching, research, and extension purposes for the students at the Universidade Federal do Mato Grosso do Sul (UFMS) for more than 20 years. The surgical center has four operating rooms for elective, non-stop urgent, and emergency surgeries.

The study center did not have a protocol for changing the breathing circuit of the anesthesia machine, and some equipment had heat and humidity exchange filters (HMEF), whereas others did not. Devices that do not use an HMEF have breathing circuits that were changed after each surgery involving IMV. For devices with a filter, only the filter was changed after each procedure.

Additionally, the institution's protocols and practices for processing the respiratory circuit of the anesthesia machine are not performed using a single method. In general, high-level disinfection was performed using autoclaves at 121°C and/or 134°C or automatic thermo-disinfector washers.

The exchange of components between the common gas outlet and patient (corrugated tubes, inspiratory branch, expiratory branch of the circuit, Y-piece, and connectors) is only performed when a bacterial filter is not used in general inhalatory anesthesia. Notably, this filter was placed between the anesthesia equipment and the patient's airways to prevent postoperative pneumonia.

The requirement for ethics committee approval was waived because this study did not involve human participants and included only surfaces that make up the anesthetist's work area, namely the anesthesia machine and the surface for drug preparation. Prior to the coronavirus disease 2019 (COVID-19) pandemic, approximately 500 surgical procedures every month were performed in this hospital, of which approximately 150 used IMV. However, data were collected during the severe acute respiratory syndrome coronavirus 2 pandemic, which directly affected the final samples obtained.

Respiratory circuits and surfaces in the anesthetic medication preparation area used in surgeries with indications for general anesthesia with IMV were considered eligible for collection of microbiological material. These surfaces were selected for analysis because they were frequently touched by the hands.

Breathing circuits and drug preparation surfaces used in surgeries for previously diagnosed lung disease and/or orotracheal intubation performed outside the surgical center were excluded.

Data were collected between August and September 2020 by the researchers themselves. Using non-probabilistic sampling for convenience, 82 samples were included for microbiological evaluation from four different locations, and a total of 328 analyses were obtained after repetition in different culture media.

In the first stage, samples were collected from the distal portion of the inspiratory branch before anesthesia; the distal portion of the expiratory branch after anesthesia; the breathing circuit canister at the end of anesthesia; and the surface of the anesthetic

medication area before preparation, following a procedure recommended in the literature.[15]

The samples were obtained by performing circular friction with a sterile cotton swab, soaked in sterile saline solution, across the inner surface of the tracheas as far as the swab shaft reached. Circular movements were also used within the canister to apply friction from the sterile cotton swab to the internal walls of this device.[15] Different swabs were used to collect samples from the inspiratory branch, the expiratory branch, the canister, and the surface of the anesthetic medication area.

After rubbing each component of the respiratory circuit of the anesthesia machine, the pre-molded lid of the transport tube, which made up the swab, was removed, and the cotton swab was submerged into Stuart's transport medium. The transport tube was then identified based on the date, name of the surface collected, and the number that represented the collection. After the surgical procedure, three granules of soda lime were collected for each analyzed respiratory circuit, and stored in a sterile 60 mL plastic bag with a stripe and sealed. The plastic bags received an identification label with the date and collection representation number.[15]

All samples were packed in a box for transporting biological samples. The box was washable, resistant to disinfection and bearing the identification of "infectant" or "biological risk." Subsequently, the samples were transported to the Laboratory of Microbiology and Molecular Genetics, located at the Universidade Federal do Mato Grosso do Sul, Campus of Três Lagoas, for processing.

In the first stage, Stuart medium was used as the transport medium to transport the samples to the microbiology laboratory. The Stuart medium is a semi-solid medium that contains thioglycolate, glycerol phosphate, and sodium chloride. Although it does not have a nutrient medium, the viability of most pathogens can be preserved. Because of the non-uniformity of the studied components, the minimum and/or maximum surface for collection was not defined, and friction was carried out up to the point where the swab rod reached.

The second stage took place in the laboratory, where the collected material was seeded into culture media for the growth and isolation of microorganisms. Blood agar, chocolate agar, MacConkey agar, and CLED agar were used as culture media.

In the laboratory, a homogenized culture medium was used to transport the samples using an automatic pipette with a disposable tip, distributed in Petri dishes containing the culture means for the differentiation of microorganisms. Subsequently, using a plastic and sterile bacteriological loop, sowing was performed using successive striations. The plates were then identified with the date, name of the collected surface, and the number that represented the collection, and then incubated in a bacteriological oven at 37 °C. The plates were observed at the following time intervals: 12 h, 24 h, 36 h, 48 h, and 60 h. An electronic colony counter was used to read the plates.

For data analysis, the information was submitted to the appropriate coding and entered into the database through the elaboration of a code dictionary in the Microsoft Excel 2016 worksheet. The growth time of the microorganisms was analyzed according to the microbiological sample collection site and at the second moment using the chi-square test with a significance level of $P < 0.05$. Univariate analysis, the Shapiro–Wilk test, and, when possible, correspondence analysis, a multivariate tool that analyzes all the variables together, were also performed to optimize the exploratory profile of the data.

RESULTS

The average time of surgical procedures that used IMV and served as a parameter for data collection in this study was 96.34 min (± 36.52). The minimum and maximum surgical times were 38 and 311 min, respectively.

Table 1 summarizes the types of surgeries performed during the study and their respective frequencies. Gastric bypass surgery for morbid obesity was the most performed, accounting for 10.98% of all procedures, followed by cholecystectomy, local incisional hernia, herniorrhaphy, and breast augmentation, each representing 8.54% of the surgeries. Mastectomy, which accounted for 7.32%, was another notable procedure in terms of frequency.

Table 2 shows a significant association between the growth times of the microorganisms in relation to the sample collection site ($P < 0.001$). The growth of microorganisms occurred within 36 h on most analyzed surfaces, except for the soda lime, which presented a higher growth frequency in 48 h.

Table 1. Proportions of the type of surgeries performed in the study (n = 82)

Type of surgery	n	%
Gastroplasty for morbid obesity	9	10.98
Cholecystectomy	7	8.54
Local incisional hernia	7	8.54
Herniorrhaphy	7	8.54
Breast augmentation	7	8.54
Breast resection	6	7.32
Tonsillectomy	4	4.88
Glossectomy	4	4.88
Segmentectomy	4	4.88
Thyroidectomy	4	4.88
Video arthroscopic acromioplasty	3	3.66
Benign tumor excision	3	3.66
Exploratory laparotomy	3	3.66
Third ventriculostomy	3	3.66
Laser uretero-reno-lithotripsy	3	3.66
Video cholecystectomy	3	3.66
Spine arthrodesis with instrumentation	2	2.44
Video endoscopy septoplasty	2	2.44
Elbow osteosynthesis	1	1.22

Table 3 presents the microorganisms identified at different biological sample collection locations, with a total of 328 samples. The presence of these microorganisms was analyzed in relation to the selected culture medium and sample collection site. Notably, the variation in the occurrence of microorganisms depends on the culture medium and sample collection site. Interestingly, a high level of contamination was observed in certain areas, such as the distal inspiratory part before anesthesia and the anesthetic medication surface before preparation. In all correspondence analyses, the P value was < 0.001 indicating that the observed differences were statistically significant.

Table 4 presents the percentages of fungi in the canister, with a total of 47 samples analyzed. This study was conducted to complement bacterial growth research, with a focus on identifying the presence of fungi in biological samples collected from canisters. Of the 82 samples collected, only 9 (10.9%) did not show fungal growth, and *Candida spp*. were the predominant fungus, identified in 24 of the 47 samples, representing an occurrence of 51.06%. This indicates that more than half of the canister samples contained this fungus.

Table 4. Percentage of occurrence of fungi in the canister (n = 47)

Microorganism	n	%
Candida spp.	24	51.06
Aspergillus spp.	7	14.89
Penicillium spp.	5	10.64
Fusarium spp.	2	4.26
Other fungi	9	19.15

Table 2. Occurrence of microorganisms in relation to growth time and analysis site (n = 82)

Growing times	Distal portion of the inspiratory branch before anesthesia	Distal portion of the expiratory branch after anesthesia	Breathing circuit canister at the end of anesthesia	Surface area of anesthetic medication (before preparation)	Soda lime
12 h	1 (2.70%)	4 (10.53%)	2 (6.06%)	10 (19.23%)	0 (0.00%)
24 h	14 (37.84%)	14 (36.84%)	10 (30.30%)	12 (23.08%)	0 (0.00%)
36 h	19 (51.35%)	16 (42.11%)	14 (42.42%)	19 (36.54%)	5 (17.24%)
48 h	3 (8.11%)	3 (7.89%)	5 (15.15%)	5 (9.62%)	15 (51.72%)
60 h	0 (0.00%)	1 (2.63%)	2 (6.06%)	6 (11.54%)	9 (31.03%)
P value*			< 0.001		

* P value representing the chi-square test.

Table 3. Microorganisms identified in each of the biological sample collection sites (n = 328)

Culture medium	Microorganism	Distal portion of the inspiratory branch before anesthesia	Distal portion of the expiratory branch after anesthesia	Breathing circuit canister at the end of anesthesia	Surface area of anesthetic medication (before preparation)
CLED agar	*E. coli*	35 (42.17%)	41 (41.41%)	28 (42.42%)	41 (22.78%)
	Enterococcus spp.	0 (0.00%)	0 (0.00%)	0 (0.00%)	39 (21.67%)
	Klebsiella spp.	24 (28.92%)	28 (28.28%)	10 (15.15%)	16 (8.89%)
	Enterobacter spp.	0 (0.00%)	0 (0.00%)	3 (4.55%)	39 (21.67%)
	Staphylococcus aureus (coagulase negative)	24 (28.92%)	30 (30.30%)	25 (37.88%)	45 (25.00%)
	P value*		< 0.001		
MacConkey agar	*E. coli*	8 (50.00%)	10 (52.63%)	0 (0.00%)	19 (25.00%)
	Klebsiella spp.	0 (0.00%)	0 (0.00%)	0 (0.00%)	12 (15.79%)
	Enterobacter spp.	8 (50.00%)	9 (47.37%)	0 (0.00%)	18 (23.68%)
	Proteus spp.	0 (0.00%)	0 (0.00%)	0 (0.00%)	11 (14.47%)
	Pseudomonas spp.	0 (0.00%)	0 (0.00%)	0 (0.00%)	16 (21.05%)
	P value*		< 0.001		
Blood agar	*Staphylococcus aureus*	18 (46.15%)	17 (41.46%)	7 (50.00%)	19 (19.39%)
	Streptococcus pneumoniae	21 (53.85%)	24 (58.54%)	7 (50.00%)	24 (24.49%)
	Streptococcus pyogenes	0 (0.00%)	0 (0.00%)	0 (0.00%)	28 (28.57%)
	Enterococcus faecalis	0 (0.00%)	0 (0.00%)	0 (0.00%)	27 (27.55%)
	P value*		< 0.001		
Chocolate agar	*Streptococcus pneumoniae*	18 (100%)	20 (100%)	15 (100%)	39 (75.00%)
	Neisseria gonorrhoeae	0 (0.00%)	0 (0.00%)	0 (0.00%)	13 (25.00%)
	P value*		< 0.001		

[a] correspondence analysis.

Despite the bacterial growth observed in other parts of the analysis, the soda lime granules did not show microorganism growth. This suggests that although the canister is subject to fungal contamination, the soda lime remains sterilized or free from contamination under the study conditions.

DISCUSSION

Decreasing the microbial load in the operating room can reduce the risk of surgical wound contamination and general surgical site infections. Sources of environmental contaminants include the skin, hair, and hands of healthcare professionals or the physical environment, such as operating tables, auxiliary tables, and anesthesia machines.[20]

In this study, we observed the growth of the main microorganisms of epidemiological importance at different incubation times and sample collection sites. These results were consistent with the findings of previous studies on the contamination of the inspiratory and expiratory branches[21] that may be associated with processing that proves ineffective in disinfection.

The processing of corrugated tubes from the inspiratory and expiratory branches of the respiratory circuit of anesthesia machines may become invalid when the norms and protocols recommended by the national and international bodies for processing are not properly followed.[16,21-23] This reprocessing is performed and conditioned by the human factors responsible for the proper removal of dirt and correct dilution of the products.[16,22-23]

In general, anesthesia machine design makes routine cleaning and disinfection difficult, and complete decontamination is practically impossible in daily practice. Pathogenic microorganisms survive in anesthesia machines after standardized routine cleaning, with the bacterial load reduced but not eliminated even after advanced cleaning practices are initiated.[24-25]

The second possibility of bacterial contamination is the internal handling and storage of reprocessed products. Conducting direct observations in the loco of the reprocessing is necessary to evaluate a series of potential moments that lead to possible failures. Keeping health products unprotected and dry in the air after cleaning and disinfection using a thermodisinfector can influence surface contamination.[25-26]

Our results for the respiratory circuit canister collected at the end of anesthesia, differ from those in the existing literature.[26-27] In contrast to a previous study that highlighted only the presence of fungi, we observed the growth of both bacteria and fungi at different points in time. A possible explanation[26-27] is that while the medium in the canister is alkaline, it might not be regularly rinsed. However, certain microorganisms remain viable even under these conditions. Notably, *Candida spp.* was the predominant fungus detected in our study, which has the ability to cause focal invasive infections.[23]

Surface contamination, especially in the area of anesthetic drug preparation before the actual preparation, is alarming. This underscores the importance of cleaning and disinfecting frequently touched surfaces within the anesthesia care area and workspace between procedures using approved hospital disinfectants. The importance of prioritizing surfaces that undergo frequent hand contact, such as drug preparation tables, has been advocated by the global consensus, as noted in various international studies.[28]

Remarkably, our findings demonstrated the consistent presence of *Neisseria gonorrhoeae* on surfaces designated for the preparation of anesthetics and other intravenous drugs, which is rather unusual as the presence of *Neisseria gonorrhoeae* in such an environment is unprecedented in the reviewed studies. Although the exact origin of this microorganism in our samples remains uncertain, its consistent presence is a cause for concern and may indicate lapses in the cleaning process, compromising patient safety. Further investigation is necessary to determine whether this is an anomaly in data collection or indicative of a broader contamination issue.

The study center did not have a protocol for changing the breathing circuit of the anesthesia machine; therefore, certain equipment had HMEF, whereas others did not. These devices are installed between the endotracheal tube and the "Y" connector to reserve part of the heat and steam from expiration, which are available during the inspiration process. The use of this method has several advantages, such as the reduction of gas loss, reduction of water condensation in the respiratory circuit, and cost-efficient.[29-30] In addition, HMEF is considered efficient in filtering microorganisms; that is, it functions as a physical barrier to protect against microbial contamination, both for patients and mechanical ventilators.

Changing the breathing circuit between patients is recommended to minimize the risk of cross contamination.[27] However, for the prevention and control of VAP to be efficient, it is necessary to standardize and carry out careful processing of the items that make up this circuit, which was not followed in the study center. The American Association of Nurse Anesthesiology (AANA) recommends that the disinfection process be performed on all components of the anesthesia equipment.[27,31-32]

Ineffective cleaning and disinfection of surfaces is capable of superficially removing the polymeric matrix from the biofilm, which can help release microorganisms of epidemiological importance, such as *Staphylococcus aureus, Staphylococcus pneumoniae,* and *E. coli*.[33] Formulating a Standard Operating Procedure (SOP) is necessary for the effective execution of this routine. However, evidence to determine the best inputs for use on hospital surfaces is scarce.[34]

In this study, *E. coli and Staphylococcus aureus* were observed in the inspiratory and expiratory branches. However, there was a higher incidence of *E. coli, Enterococcus spp., and Enterobacter spp.* on the surface of the anesthetic medication area. Therefore, it was possible to identify a variety of bacteria and fungi on these

surfaces. Despite the numerous potentially pathogenic microorganisms identified, the presence of *Streptococcus pneumoniae,* the most prevalent bacteria implicated in pneumonia warrants special attention.[35-36]

Among the other bacteria identified was *Staphylococcus aureus*, which is the most prevalent clinically relevant infectious agent and a major cause of HAIs. This microorganism can survive for long periods, from 7 days to 7 months, on hospital surfaces.[33] In addition, most of the fungi identified were of the genus *Candida spp*., which can become an opportunistic agent and cause severe pneumonia in immunocompromised patients, thereby increasing the risk of mortality in such patients.

Study limitations

Our study had certain limitations. First, this was a single-center study, and therefore the results cannot be generalized. Second, the number of samples collected was influenced by a decrease in surgical procedures owing to the COVID-19 pandemic.

Contributions to the practice

The microbiological findings of this study indicate that patients on IMV undergoing a surgical procedure may be at a greater risk of developing HAI, reinforcing the importance of standardizing the cleaning, disinfection, and sterilization processes of respiratory circuits.

CONCLUSION

This study evaluated the contamination of anesthetic medication preparation surfaces, respiratory circuits, and devices used in general anesthesia with assisted mechanical ventilation. These results highlight the importance of ensuring proper cleaning and disinfection of all high-touch surfaces. Contamination by microorganisms can be minimized by creating protocols that define criteria related to the work process, allowing for systematic and frequent analysis of infection prevention and control practices in operating rooms.

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Frequency of skin diseases in renal transplant recipients and patients with chronic kidney disease in a tertiary center: a cross-sectional study

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ABSTRACT

BACKGROUND: The prevalence of chronic kidney disease (CKD) has increased in the recent decades, along with the number of patients in the terminal stages of this disease, requiring transplantation. Some skin disorders are more frequent in patients with CKD and in renal transplant recipients (RTR).

OBJECTIVES: To evaluate the frequency of skin diseases in RTR and patients with CKD receiving conservative treatment.

DESIGN AND SETTING: This observational cross-sectional study recruited consecutive patients with CKD and RTR from a nephrology clinic at a teaching hospital in Brazil between 2015 and 2020.

METHODS: Quantitative, descriptive, and analytical approaches were used. The sample was selected based on convenience sampling. Data were collected from dermatological visits and participants' medical records.

RESULTS: Overall, 308 participants were included: 206 RTR (66.9%, median age: 48 years, interquartile range [IQR] 38.0–56.0, 63.6% men) and 102 patients with CKD (33.1%, median age: 61.0 years, IQR 50.0–71.2, 48% men). The frequency of infectious skin diseases (39.3% vs. 21.6% P = 0.002) were higher in RTR than in patients with CKD. Neoplastic skin lesions were present in nine (4.4%) RTR and in only one (1.0%) patient with CKD. Among the RTR, the ratio of basal cell carcinoma to squamous cell carcinoma was 2:1.

CONCLUSIONS: This study revealed that an increased frequency of infectious skin diseases may be expected in patients who have undergone kidney transplantation. Among skin cancers, BCC is more frequently observed in RTR, especially in those using azathioprine.

INTRODUCTION

Chronic kidney disease (CKD) is a significant global public health problem with a major socioeconomic impact.[1,2] Its worldwide prevalence is estimated at 10–16%;[1,3,4] additionally, its prevalence has increased in the recent decades, along with the number of patients with terminal CKD requiring transplantation,[3,6] mostly due to the increase in the prevalence of hypertension and diabetes mellitus.[4,5]

Patients with CKD are prone to skin abnormalities.[7,8] These manifestations are often associated with impaired renal function and are more prevalent in end-stage disease,[7,8] when the kidneys are unable to maintain appropriate levels of metabolic products, such as urea, creatinine, sodium, calcium, and phosphate, causing damage to several organs, including the skin.[9]

Kidney transplantation is the best treatment for patients with end-stage CKD;[3,10] however, the immunosuppression required to maintain the graft can lead to various side effects and a greater susceptibility to infectious and neoplastic diseases.[11] Besides immunosuppression itself, the mechanisms of action of immunosuppressive drugs and viral infections (oncogenic viruses) are associated with cutaneous disorders in renal transplant recipients (RTR).[12,13]

There is evidence that dermatological diseases affect the quality of life of patients with CKD[14] and individuals who have undergone kidney transplantation.[15] Several transplant centers do not have a dermatologist working with the transplant team, and dermatological abnormalities are often underdiagnosed and undertreated.[15] Therefore, further research

on the prevalence and presentation of skin diseases in solid organ transplant recipients and patients with CKD is essential. The prevalence and presentation of skin diseases is likely to vary in different regions of the world according to patient genetics, skin phototype, hygiene habits, sun exposure, immunosuppressive medications used, climate, and the prevalence of infectious agents.

OBJECTIVE

This study aimed to evaluate the prevalence of dermatological abnormalities in patients with CKD receiving conservative treatment and in RTR treated at a tertiary academic center in southeastern Brazil.

METHODS

Data source and study participants

For this observational and cross-sectional study, consecutive patients treated between 2015 and 2020 were recruited from a reference center for nephrology and kidney transplantation at a Brazilian academic hospital. The sample was obtained through convenience sampling by inviting consecutive patients who were treated at the nephrology and kidney transplantation outpatient clinics of the hospital.

The eligibility criteria were: RTR regardless of the time elapsed since transplantation, patients diagnosed with CKD (defined as individuals with glomerular filtration rate [GFR] < 60 mL/min/1.73 m^2 for at least 3 months) receiving conservative treatment, or patients with GFR ≥ 60 mL/min/1.73 m^2 associated with markers of kidney damage or structural abnormalities detected by imaging; only patients ≥18 years of age were considered.[16] Patients living with HIV and patients with CKD taking prednisone at ≥5 mg/day or taking other immunosuppressants were excluded from the study. The equation developed by the Chronic Kidney Disease Epidemiology Collaboration group (CKD-EPI) was used to calculate the GFR.[17] CKD was classified into five stages according to the Kidney Disease Outcomes Quality Initiative of the National Kidney Foundation (KDOQI/NKF) classification.[18]

All patients underwent standard screening according to a previously established protocol to provide a solid, standardized assessment that included a dermatological perspective and variables of interest for kidney transplant/disease. Evaluation, diagnosis, treatment of skin diseases, biopsies, and direct mycological examinations were performed by attending dermatology physicians at the teaching hospital.

Outcome measurements and group and subgroup analysis

The following independent variables were collected: age, sex, Fitzpatrick skin phototype, eye color, alcohol and tobacco use, presence of CKD, history of kidney transplantation, kidney function (estimated from the creatinine level or proteinuria), underlying disease that led to kidney transplantation, underlying disease that caused CKD, comorbidities, personal or family history of skin cancer, regular use of sunscreen, and previous sun exposure at work. The level of sun exposure considered was the highest exposure during the workday. Sunscreen application at least once per day was considered regular. Immunosuppressants were collected from in the RTR groups' medical records.

The outcomes of interest were dermatological complaints and diagnoses of cutaneous disease. The skin diseases were divided into four groups: benign non-infectious, infectious (viral, bacterial, or fungal), preneoplastic, and neoplastic.

Statistical analysis

Categorical variables were expressed as numbers and proportions, and continuous variables were expressed as medians and interquartile ranges. As we expected, there was an age difference between groups (RTR and CKD) and the prevalence of skin manifestations was affected by age; thus, the patients were stratified by age into three categories: 18–39 years, 40–59 years, and 60+ years. The Pearson's chi-square test or the Fisher's exact test was performed to determine the association between qualitative variables, and age groups/subgroups were compared using the Mann–Whitney U test. The statistical significance was set at $P < 0.05$. All statistical analyses were performed using the Statistical Package for the Social Sciences software (SPSS) v. 18.0 software for Windows (IBM, Armonk, NY, USA).

Ethics

The study protocol was approved by the Universidade Federal de Minas Gerais Ethics Review Board (CAAE process number 38071114.8.0000.5149) on December 9th, 2014. Written informed consent was obtained from all participants.

RESULTS

Patient characteristics

Overall, 308 participants met the inclusion criteria for the study: 206 (66.9%) RTR and 102 (33.1%) patients with CKD receiving conservative treatment. The median age of the participants in the RTR group was 48.0 years (interquartile range [IQR]: 38.0–56.0), and 63.6% were men. When this group was stratified by age, most participants were in the subgroup of age 40–59 years (54.9%). The median age of the CKD group was 61.0 years (IQR: 50.0–71.0) and 48.0% were men (**Table 1**). Most patients in this group (54.9%) were 60+ years old. The demographic and clinical characteristics of both the groups are presented in **Table 1**.

Table 1. Demographics and clinical characteristics of renal transplant recipients and chronic kidney disease patients (n = 308)

	RTR n = 206	CKD n = 02	P
Age (years)	48.0 (38.0–56.0)	61.0 (50.0–71.0)	< 0.001[c]
18–39	59 (28.6%)	11 (10.8%)	
40–59	113 (54.9%)	35 (34.3%)	
60+	34 (16.5%)	56 (54.9%)	
Men	131 (63.6%)	49 (48.0%)	
Kidney disease etiology			< 0.001[b]
Hypertensive nephropathy	13 (6.3%)	7 (6.9%)	
Diabetes	14 (6.8%)	26 (25.5%)	
Glomerulopathy	55 (26.7%)	24 (23.5%)	
Polycystic kidney disease	10 (4.9%)	3 (2.9%)	
Genetic disease	9 (4.4%)	0	
Unknown	79 (38.3%)	32 (31.4%)	
Other	26 (12.6%)	10 (9.8%)	
Comorbidities			
Diabetes			< 0.001[b]
Type 1 diabetes	12 (5.8%)	3 (2.9%)	
Type 2 diabetes	11 (5.3%)	22 (21.6%)	
Post-transplant diabetes	35 (17.0%)	0	
Hypertension	122 (59.2%)	74 (72.5%)	0.024[a]
CAD	7 (3.4%)	12 (11.8%)	0.10[a]
Heart failure	3 (1.5%)	5 (4.9%)	0.121[a]
Obesity	5 (2.4%)	9 (8.8%)	0.018[a]
Hyperuricemia	25 (12.1%)	5 (4.9%)	0.064[a]
CKD stages*			0.001[b]
1	31 (15.0%)	9 (8.8%)	
2	87 (42.2%)	11 (10.8%)	
3A	32 (15.5%)	26 (25.5%)	
3B	30 (14.6%)	33 (32.4%)	
4	20 (9.7%)	21 (20.6%)	
5	6 (2.9%)	2 (2.0%)	
GFR**	64.2 (43.9–82.3)	44.1 (31.2–56.3)	0.000[a]
Skin phototype			0.286[b]
I	4 (1.9%)	0	
II	25 (12.1%)	6 (5.9%)	
III	86 (41.7%)	44 (43.1%)	
IV	53 (25.7%)	33 (32.4%)	
V	29 (14.1%)	16 (15.7%)	
VI	9 (4.4%)	3 (2.9%)	
Tobacco use	16 (7.8%)	20 (19.6%)	0.004[a]
Alcohol use	13 (6.3%)	12 (11.8%)	0.121[a]
Sun exposure at work			0.897[a]
< 1 hour/day	139 (67.5%)	70 (68.6%)	
≥ 1 hour/day	67 (32.5%)	32 (31.4%)	
Daily sunscreen use	59 (28.6%)	11 (10.8%)	< 0.001[a]
Personal history of skin cancer	13 (6.3%)	4 (3.9%)	0.442[a]
Dermatological complaints	129 (62.6%)	60 (58.8%)	0.536[a]

Data are presented as median (interquartile range) or number (percentage). [a]Fisher's exact test; [b]Pearson chi-square test; [c]Mann–Whitney test; RTR = renal transplant recipients; CKD = chronic kidney disease; GFR = glomerular filtration rate; CAD = coronary artery disease. * According to The Kidney Disease Outcomes Quality Initiative of the National Kidney Foundation (KDOQI/NKF).[18] ** Calculated according to the Chronic Kidney Disease Epidemiology Collaboration group (CKD_EPI equation).[17]

Among RTR, the median time between transplant and the first dermatology visit was 99.9 months (IQR: 55.4–164.7; range: 2.0–482.0). Most of the transplant patients (45.6%) were three to 10 years post-transplant. In terms of the drug treatment, 22.3% of patients in this group were using or had already used azathioprine, 81.5% of patients were using or had used mycophenolate salts (mycophenolate sodium or mycophenolate mofetil), 88.3% of patients were using or had used calcineurin inhibitors

(cyclosporine or tacrolimus), and 30.6% of patients were using or had used mammalian target of rapamycin (mTOR) inhibitors, namely sirolimus and everolimus.

Skin disease diagnosis

Skin disorders were divided into four groups: benign, infectious, preneoplastic, and neoplastic. Non-infectious benign dermatoses were the most frequent disorders in both groups (53.9% in RTR and 60.8% in the CKD group) (**Table 2**). Pigmentation disorders were the most prevalent in the RTR group (11.2%), followed by adverse drug reactions (6.3%) and acne (5.8%). Among patients with CKD, the most frequent diagnoses in this category were pigmentation disorder (16.7%) and xerosis (4.9%) (**Table 2**). Adverse drug reactions were more prevalent in RTR than the CKD group (6.3% vs. 1.0%; P = 0.040) (**Table 2**).

Infectious skin diseases accounted for 39.3% of the diagnoses in the RTR group and 21.6% in the CKD group (P = 0.002). The most frequent ones were, among RTR, dermatophytosis (19.4%) and HPV-related diseases (10.2%), whereas, in patients with CKD, dermatophytosis (14.7%) and candidiasis (4.9%) were prevalent. HPV-related diseases (10.2% vs. 2.0%, P = 0.01) and pityriasis versicolor (8.3% vs. 1.0%, P = 0.009) were more frequent in RTR than in patients with CKD.

Table 2. Dermatological diseases found in renal transplant recipients and chronic kidney disease patients

	RTR n = 206	CKD n = 102	P
*** Benign**	111 (53.9%)	62 (60.8%)	0.273[a]
Xerosis	9 (4.4%)	5 (4.9%)	0.780[a]
Pigmentation disorder	23 (11.2%)	17 (16.7%)	0.208[a]
Sebaceous hyperplasia	9 (4.4%)	0	0.032[a]
Acne	12 (5.8%)	1 (1.0%)	0.067[a]
Adverse drug reaction	13 (6.3%)	1 (1.0%)	0.040[a]
Other benign disorders	64 (31.1%)	43 (42.2%)	0.058[a]
*** Infectious**	81 (39.3%)	22 (21.6%)	0.002[a]
Bacterial			
Bacterial folliculitis	3 (1.5%)	0	0.553[a]
Impetigo	1 (0.5%)	0	1[a]
Viral			
Genital herpes	1 (0.5%)	0	1[a]
Herpes simplex	2 (1.0%)	0	1[a]
HPV	21 (10.2%)	2 (2.0%)	0.010[a]
Molluscum contagiosum	3 (1.5%)	0	0.553[a]
Fungal			
Pityriasis versicolor	17 (8.3%)	1 (1.0%)	0.009[a]
Candidiasis	8 (3.9%)	5 (4.9%)	0.765[a]
Dermatophytosis	40 (19.4%)	15 (14.7%)	0.346[a]
Systemic mycosis	1 (0.5%)	0	1[a]
Pre-neoplastic	21 (10.2%)	8 (7.8%)	0.679[a]
*** Neoplastic**	9 (4.4%)	1 (1%)	0.174[a]
Squamous cell carcinoma	3 (1.5%)	0	0.553[a]
Basal cell carcinoma	6 (2.9%)	1 (1.0%)	0.432[a]

RTR, renal transplant recipients; CKD, chronic kidney disease; HPV, human papillomavirus; [a] Fisher's exact test; preneoplastic (actinic keratosis); *Because some patients had more than one type of dermatosis, the numbers for each condition may not add up to the sum for each disease subtype.

The identified benign dermatological diseases are presented in terms of age subgroups in **Table 3**. Sebaceous hyperplasia was found only in the RTR group (in the subgroups of age: 18–39 years and 40–59 years), while adverse drug reactions were found in all RTR age subgroups, with a small number of cases and only one case in the CKD group (in the 60+ years subgroup).

A few positive cases of two bacterial diseases (impetigo and bacterial folliculitis) were found in the study population, all in the RTR group. In the 60+ years, HPV-related diseases were more prevalent in the RTR than in the CKD group (23.5% vs. 3.6%; P = 0.005) (**Table 3**). Other viral diseases, such as genital herpes, herpes simplex, and molluscum contagiosum are shown in **Table 3**.

Dermatophytosis was the most prevalent fungal disease, predominating in the RTR group over the CKD population, especially in the 60+ age subgroup (32.4% vs. 23.2%, respectively), but without statistical significance (**Table 3**). One RTR patient presented with systemic mycosis (paracoccidioid mycosis) with mucocutaneous, lymph node, and pulmonary involvement.

Actinic keratosis was more predominant in the 60+ age subgroup compared to the other age groups, with a greater prevalence in the RTR group than the CKD group (29.4% vs. 12.5%), but without statistical significance (**Table 3**).

Neoplastic skin lesions were present in nine (4.4%) transplanted patients and only one (1.0%) subject in the CKD group (**Table 2**). Eighteen non-melanoma skin cancer (NMSC) lesions were found in nine patients, and one was observed in a patient with CKD (**Table 2**). One of these nine transplant recipients presented with ten basal cell carcinomas (BCC) at the first dermatology visit (**Figure 1**), while the other cases involved one lesion per patient. All nine RTR with NMSC received their transplants at least four years prior to the skin cancer, and six (66.6%) of these patients already had a history of skin cancer.

Squamous cell carcinoma (SCC) was found in 5.9% of individuals in the 60+ age subgroup in the RTR group and not found in the CKD group in this age range; the frequency of BCC was 4.4% in the RTR group compared to 2.9% in the CKD group among patients in the age range of 40–59 years (**Table 3**).

In terms of immunosuppressive drugs, of the 21 RTR patients with HPV, nine (42.9%) patients were using or had used azathioprine (P = 0.026) (**Table 4**). Of the 17 RTR who presented with pityriasis versicolor, 17 (100%) patients were using or had previously used mycophenolate salts (P = 0.047). Of the nine NMSC patients, six (66.7%) patients were using or had used azathioprine (P = 0.005) (**Table 4**).

Table 3. Diagnosis of skin disorders in renal transplant recipients and chronic kidney disease patients, stratified by age

	18–39 years old (n = 70)			40–59 years old (n = 148)			60+ years old (n = 90)		
	RTR n = 59	CKD n = 11	P-value	RTR n = 113	CKD n = 35	P-value	RTR n = 34	CKD n = 56	P-value
Benign skin diseases									
Xerosis	1 (1.7%)	0	1[a]	6 (5.3%)	2 (5.7%)	1[a]	2 (5.9%)	3 (5.4%)	1[a]
Pigmentation disorder	7 (11.9%)	3 (27.3%)	0.186[a]	14 (12.4%)	6 (17.1%)	0.571[a]	2 (5.9%)	8 (14.3%)	0.308[a]
Sebaceous hyperplasia	1 (1.7%)	0	1[a]	8 (7.1%)	0	0.199[a]	0	0	
Acne	8 (13.6%)	1 (9.1%)	1[a]	4 (3.5%)	0		0	0	
Adverse drug reaction	3 (5.0%)	0	1[a]	7 (6.2%)	0	0.199[a]	3 (8.8%)	1 (1.8%)	0.149[a]
Other benign disorders	15 (25.4%)	4 (36.4%)	0.474[a]	35 (31.0%)	14 (40.0%)	0.411[a]	14 (41.2%)	25 (44.6%)	0.828[a]
Bacterial skin diseases									
Bacterial folliculitis	1 (1.7%)	0	1[a]	2 (1.8%)	0	1[a]	0	0	
Impetigo	0	0		1 (0.9%)	0	1[a]	0	0	
Viral skin diseases									
Genital herpes	0	0		0	0		1 (2.9%)	0	0.378[a]
Herpes simplex	1 (1.7%)	0	1[a]	1 (0.9%)	0	1[a]	0	0	
HPV	4 (6.8%)	0	1[a]	9 (8.0%)	0	0.116[a]	8 (23.5%)	2 (3.6%)	0.005[a]
Molluscum contagiosum	2 (3.4%)	0	1[a]	1 (0.9%)	0	1[a]	0	0	
Fungal skin diseases									
Pityriasis versicolor	8 (13.6%)	0	0.340[a]	7 (6.2%)	1 (2.9%)	0.681[a]	2 (5.9%)	0	0.140[a]
Candidiasis	1 (1.7%)	0	1[a]	6 (5.3%)	3 (8.6%)	0.442[a]	1 (2.9%)	2 (3.6%)	1[a]
Dermatophytosis	8 (13.6%)	0	0.340[a]	21 (18.6%)	2 (5.7%)	0.106[a]	11 (32.4%)	13 (23.2%)	0.461[a]
Systemic mycosis	0	0		0	0		1	0	0.378[a]
Pre-neoplastic and neoplastic skin diseases									
Actinic keratosis	1 (1.7%)	0	1[a]	10 (8.8%)	1 (2.9%)	0.460[a]	10 (29.4%)	7 (12.5%)	0.057[a]
SCC	0	0		1 (0.9%)	0	1[a]	2 (5.9%)	0	0.140[a]
BCC	0	0		5 (4.4%)	1 (2.9%)	1[a]	1 (2.9%)	0	0.378[a]

[a]Fisher's exact test; RTR, renal transplant recipient; CKD, chronic kidney disease; HPV, human papillomavirus; BCC, basal cell carcinoma; SCC, squamous cell carcinoma.

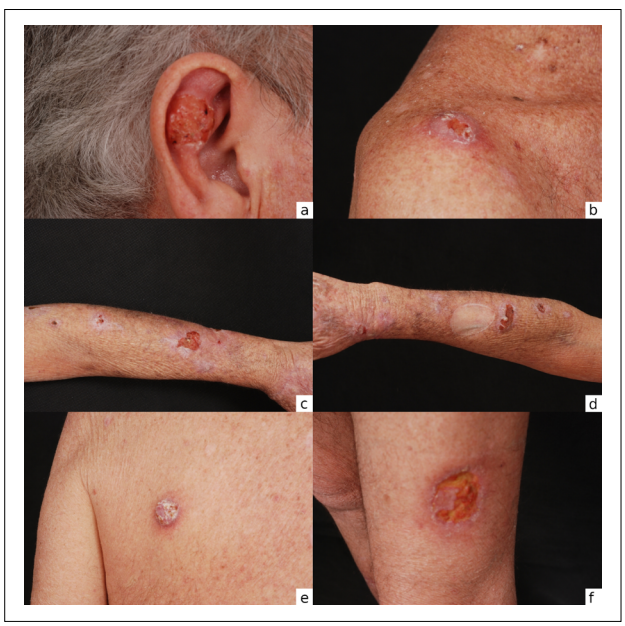

Figure 1. Renal transplant recipient presenting 10 basal cell carcinoma lesions at dermatology visit. Photo credit: HC/UFMG-EBSERH Dermatology Service

Among patients with CKD, no statistically significant association was found between the CKD stage and diagnosed skin diseases.

DISCUSSION

This study investigated patients with CKD and RTR: two populations with a similar distribution of skin phototypes, but distinct demographic profiles and kidney disorders. Patients with CKD were older, had a higher frequency of comorbidities and smoking, and had lower glomerular filtration rates than RTR. In contrast, RTR had a higher frequency of regular sunscreen use than patients with CKD. It is likely that nephrologists may be less persuasive about sun protection measures in patients with CKD than in individuals who have received organ transplants. However, daily sunscreen use is insufficient for adequate protection, and reapplication is necessary every three hours.[30] Additional physical protection measures, such as ultraviolet (UV)-protective clothing, hats, sunglasses, and shade, are simple and effective ways to protect individuals from UV radiation and preventing NMSC.[31]

Table 4. Current and previous use of immunosuppressive drugs and dermatological diseases in renal transplant recipients (n = 206)

Immunosuppressive drugs*	NMSC			Human papillomavirus			Pityriasis versicolor		
	Present (n = 9)	Absent (n = 197)	P-value	Present (n = 21)	Absent (n = 185)	P-value	Present (n = 17)	Absent (n = 189)	P-value
Azathioprine	6 (66.7%)	40 (20.3%)	0.005[a]	9 (42.9%)	37 (20.0%)	0.026[a]	2 (11.8%)	44 (23.3%)	0.372[a]
Mycophenolate salts	5 (55.6%)	163 (82.7%)	0.062[a]	15 (71.4%)	153 (82.7%)	0.234[a]	17 (100%)	151 (79.9%)	0.047[a]
Calcineurin inhibitors	8 (88.9%)	174 (88.3%)	1[a]	17 (81.0%)	165 (89.2%)	0.279[a]	17 (100%)	165 (87.3%)	0.230[a]
mTOR inhibitors	3 (33.3%)	60 (30.5%)	1[a]	5 (23.8%)	58 (31.4%)	0.620[a]	4 (23.5%)	59 (31.2%)	0.594[a]

[a]Fisher's exact test; NMSC = non-melanoma skin cancer; mTOR inhibitors = mammalian target of rapamycin. * Current or previous use was considered.

The frequency of skin infections observed in our population was lower than that in other studies.[15,34,35] Skin infections predominated within the first 3–4 years after transplantation,[28] and more than 3 years had elapsed after transplantation in 84.9% of our RTR sample. There is evidence that increased susceptibility to bacterial, fungal, and viral cutaneous infections in patients with CKD varies between 28 and 70%.[7] Patients with CKD have impaired cellular immunity due to a decreased T lymphocyte cell count, which could explain the high prevalence of infection in those patients.[36] However, the literature on cutaneous infections in individuals with CKD is sparse.

HPV-related skin disease was the most common viral infection, and its frequency was higher in RTR than the CKD group (10.2% vs. 2.0%), in line with previous studies, as a result of chronic immunosuppression.[29,37] Previous research found that 15% of patients present with cutaneous viral warts during the first year after renal transplant, and that this rate reached 92% after a period of 15 years.[38] In approximately 60% of our sample, ten years had not passed since transplantation. In elderly patients, the difference in HPV among RTR and patients with CKD was remarkable (23.5% vs. 3.6%, P = 0.005), which can be partially explained by the persistence of HPV in old age.[37] Furthermore, the age subgroup of 60+ years had proportionally more individuals (50%) at least 120 months post-transplantation, implying a longer period of immunosuppression and increased time of HPV persistence. Despite being a viral skin disease of concern owing to its oncogenic potential to increase the risk of SCC in immunosuppressed patients,[12,29] as it facilitates the accumulation of DNA mutations induced by UV radiation,[39] none of the three patients with SCC in our study had HPV-related disease.

Renal transplant recipients also have a higher frequency of infectious skin diseases, pityriasis versicolor, sebaceous hyperplasia, and adverse drug reactions than patients with CKD. In terms of bacterial skin diseases, only impetigo and folliculitis were diagnosed in the RTR, probably because of the low number of participants in our sample. Moreover, bacterial infections are more prevalent during the first years after transplantation (only 15% of our sample was less than three years post-transplantation), and acute and benign diseases are often treated by an assistant physician without a referral to a dermatologist. No bacterial skin disease was diagnosed in the CKD group.

In terms of immunosuppressant use, NMSC and HPV infections were more frequent in RTR who received azathioprine. Pityriasis versicolor was observed to be associated with the use of mycophenolate salts in RTR. No association was found between the CKD stage and skin disorders. Immunosuppressive agents play important roles in the development of dermatological diseases. With regard to skin cancer in RTR, these drugs have direct carcinogenic action and reduce immunological surveillance.[29] Azathioprine and cyclosporine may directly or indirectly interact with UV radiation to enhance its carcinogenic effects.[29] A higher prevalence of azathioprine use was observed in RTR with NMSC compared to RTR without skin cancer (P = 0.005). Other immunosuppressants were not associated with skin cancer in the present study.

As for immunosuppressive agents and infectious dermatoses, of the 21 RTR with HPV, 42.9% used azathioprine; among the RTR without a diagnosis of HPV, only 20% used the same drug (P = 0.026). This is corroborated by a Brazilian study in which patients who used this drug had a higher incidence of viral warts.[23] The use of mycophenolate salts was more frequent in patients with pityriasis versicolor than in those who were not treated with this medication (P = 0.047). In the RTR group, 81.5% of patients were using or had already used mycophenolate salts. It was, therefore, by chance that 100% of patients who presented with pityriasis versicolor had used this drug.

BCC was more prevalent than SCC in the RTRs. In the general population, BCC predominates over SCC at a ratio of 4:1.[19] However, this ratio is reversed in solid organ transplants, and becomes more pronounced as more time elapses post-transplantation.[19,20] We found a higher proportion of BCC compared to SCC (2:1) in the RTR group; these findings are consistent with observations by Lima et al.[21] Another Brazilian study and a research on organ transplant recipients in the Mediterranean has also reported similar ratios.[22] The genetic background, higher phototypes, and phenotypic characteristics could be responsible for this phenomenon; however, another Brazilian study by Hayashida et al.[23] found a BCC:SCC ratio of 1:2.4 with a minimum follow-up of three years.[23]

Notably, over the past five years, some follow-up studies have found lower BCC:SCC ratios.[20,24,25] These results can be partially explained by the reduced trend of SCC incidence over the past 20 years in solid organ transplant recipients.[26,27] This decline is likely caused by less aggressive and more individualized immunosuppression therapy.[26]

One of the most important extrinsic factors related to the increased incidence of NMSC is exposure to UV radiation.[19,29] Of the RTR patients with NMSC, 66.6% of patients reported no exposure or up to one hour of sun exposure per day at work. There is evidence that in temperate climates, 35–50% of organ transplant recipients will develop one or more skin cancers by the tenth year after transplantation; this number may increase to more than 80% in countries with higher rates of UV radiation.[30] Regular sun protection is of utmost importance for immunosuppressed patients.

Patients with CKD receiving conservative treatment have demonstrated a higher incidence of kidney and urinary tract cancers than the general population;[32,33] however, the incidence of NMSC is unclear.[32] Wang et al.[32] found that predialysis patients (stage 5 CKD) have a greater risk of developing NMSC than the general population, with a standardized incidence ratio (SIR) of 1.14. In our study, only one patient with CKD had BCC.

The most common fungal infections occurring in RTR are superficial mycoses.[28] Dermatophytosis was the most common mycosis found; however, no difference was observed between RTR and patients with CKD or age subgroups. The prevalence of superficial mycoses in RTR varies in the literature (16–60%), probably in accordance with the study type, length of follow-up, and geographic region.[28,34] Charu[7] found a prevalence of 16.9% and Thomas[36] found a prevalence of 1.01% in patients with CKD.

Among benign diseases, sebaceous hyperplasia was more frequent in RTR than in individuals with CKD. Sebaceous hyperplasia was found only in the RTR group, particularly in the age subgroup of 40–59 years. It is observed as a complication in 30% of patients using cyclosporine,[28] as this drug may be partly eliminated through the sebaceous glands, leading to frequent pilosebaceous lesions.[11]

A few cases of xerosis were observed in either group, with no significant differences. Our findings were lower than the prevalence rates observed by other authors, 50–80% in CKD subjects.[7,8] The low prevalence observed in the CKD group may be partly explained by the fact that most of our patients with CKD (77.4%) were stage 3B or less; dermatoses, including xerosis, are more prevalent in the later stages of CKD.[7,8]

This study had some limitations. Acute dermatoses may have been underestimated owing to the study design, as skin lesions may not have been present on the day of the dermatologist's consultation. The data included in this study refer only to the first consultation, which makes it difficult to accurately characterize the spectrum of diseases presented over time. Finally, this study was based on a single-center analysis, which limits the generalizability of the results. However, this method has several strengths. Despite being a single-center study, this is a reference center for transplants in the state of Minas Gerais, and all patients underwent detailed assessment by a team of dermatologists with extensive expertise. Additionally, the study assessed patients from a highly miscigenic population in a tropical region.

CONCLUSIONS

This study of patients monitored at a reference center for nephrology and renal transplantation found more skin infections in kidney transplant recipients than in patients with CKD. A multidisciplinary team, including dermatologists, must know how to diagnose, treat, and implement skin disease prevention measures in these populations. Therefore, the skin of these patients should be routinely evaluated to manage dermatological diseases, especially neoplasms.

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Aspects that facilitate access to care for viral hepatitis: An evaluative research

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ABSTRACT

BACKGROUND: Viral hepatitis is a major public health concern worldwide.

OBJECTIVES: This study aimed to analyze the factors that facilitate access to care for viral hepatitis.

DESIGN AND SETTING: Using a sequential mixed method, this evaluation research was conducted in the state of Mato Grosso, Brazil.

METHODS: Mapping of references and selection of regions were made based on the quantity and heterogeneity of services. The stakeholders, including the managers of the State Department of Health and professionals from reference services, were identified. Nine semi-structured interviews were conducted using content analysis and discussions guided by the dimensions of the analysis model of universal access to health services.

RESULTS: In the political dimension, decentralizing services and adhering to the Intermunicipal Health Consortium are highly encouraged. In the economic-social dimension, a commitment exists to allocate public funds for the expansion of referral services and subsidies to support users in their travel for appointments, medications, and examinations. In the organizational dimension, the availability of inputs for testing, definition of user flow, ease of scheduling appointments, coordination by primary care in testing, collaboration following the guidelines and protocols, and engagement in extramural activities are guaranteed. In the technical dimension, professionals actively commit to the service and offer different opening hours, guarantee the presence of an infectious physician, expand training opportunities, and establish intersectoral partnerships. In the symbolic dimension, professionals actively listen to the experiences of users throughout their care trajectory and demonstrate empathy.

CONCLUSIONS: The results are crucial for improving comprehensiveness, but necessitate managerial efforts to enhance regional governance.

INTRODUCTION

Viral hepatitis is characterized by liver inflammation caused by an infection with five types of viruses.[1] It is considered a significant global public health problem, necessitating the implementation of health management strategies.

Worldwide, approximately 257 million people living with chronic hepatitis B virus infection and 71 million people with hepatitis C virus infection are unaware of their condition. Approximately 57% of liver cirrhosis cases and 78% of primary liver cancer cases are attributed to hepatitis B and C virus infections.[2] In Brazil, from 2000 to 2021, 718,651 patients were diagnosed with human viral hepatitis. Of them, 168,175 (23.4%) had hepatitis A, 264,640 (36.8%) had hepatitis B, 279,872 (38.9%) had hepatitis C, and 4,259 (0.6%) had hepatitis D. From 2000 to 2020, 82,169 deaths due to fundamental causes and linked to hepatitis types A, B, C, and D were reported. The distribution of these deaths was as follows: 1.6% for viral hepatitis A, 21.3% for hepatitis B, 76.2% for hepatitis C, and 0.9% for hepatitis D.[3]

Following the World Health Assembly's recommendation, a global initiative was launched to advance the elimination of hepatitis. This movement aims to achieve a 90% reduction in the new infection rate and a 65% reduction in the mortality rate by strengthening access to diagnosis and treatment to stop virus transmission.[4] In the Americas and Caribbean region, the Pan American Health Organization (PAHO) has advocated for equitable access to preventive and diagnostic care, emphasizing an integrated response from the Health System (HS) to strengthen case surveillance.[5] In recent decades, notable progress has been observed in the adoption of preventive

measures through immunization. However, the primary emphasis has been on the treatment aspect.[6] Strengthening measures to eliminate viral hepatitis is part of the 2030 Agenda.[7]

In Brazil, following the establishment of the National Program for the Prevention and Control of Viral Hepatitis (NPVH), in the Unified Health System (SUS), various coping strategies that permeate disease prevention and diagnosis, and guidelines for the organization of care networks have been implemented.

Considering the extensive scientific research on the clinical aspects and treatment of hepatitis, along with the guidelines provided by the 2030 Agenda for Sustainable Development[7]; the need to enhance access to the diagnosis and treatment of viral hepatitis; and the implications for the organization of healthcare, this study aimed to determine the aspects that facilitate access to care for viral hepatitis. The results are anticipated to make valuable contributions to understanding access to care, influencing health management demands for the organization of care networks and assistance across various points of care from the perspective of prevention, diagnosis, and treatment.

OBJECTIVE

This study aimed to analyze the factors that facilitate access to care for viral hepatitis.

METHODS

This evaluative research[8] utilized the mixed sequential method.[9,10] This study[11] was conducted in the state of Mato Grosso, which comprised 6 macro-regions and 16 Health Regions (HRs). This location was selected owing to its adherence to decentralized management and a regionalization process.[12]

In the quantitative stage, data from the National Register of Health Establishments (NRHE) in the second half of 2020 were reviewed to identify reference services for the treatment of viral hepatitis. The HR demonstrating a higher quantity and heterogeneity of referral services were selected for in-depth analysis, signifying its capacity to provide a comprehensive range of health actions and services. The South Mato Grosso HR not only fulfilled the criteria of having a high population density but also boasted seven dedicated services for the treatment of viral hepatitis, with six actively participating in the study. The service allocated to the penitentiary was an exception, as its organizational conditions for attention require a specific approach.[13]

In line with the criteria employed for HR selection, stakeholders were identified based on the following inclusion criteria: individuals responsible for overseeing health management in the viral hepatitis sector of the State Health Secretariat of Mato Grosso (SHS-MT) and professionals responsible for technical services, specifically the Testing and Counseling Center (TCC) and/or Specialized Assistance Service (SAS), with a minimum of 6 months of active service. Professionals who were absent from their duties for any reason were excluded.

Individual semi-structured interviews were conducted to collect data. The interview script was submitted for face validation and pre-test application. Participants were initially contacted via email and phone. Upon formal acceptance by email along with the submission of the signed informed consent form, the interview was scheduled on a digital platform (WhatsApp, Google Meet, or Zoom) at a mutually agreed upon time.

In addition to the script, a vignette was used to facilitate participant engagement[14]. The interviews lasted for an average of 50 min and were conducted by the lead researcher between August 2020 and January 2021. The material was transcribed, and participants were identified by the letter P, followed by an Arabic numeral. Thematic analysis and data systematization were performed,[15] with the content grouped into cores of meaning. Analysis categories were defined, with a focus on aspects that facilitate attention to viral hepatitis.

Subsequently, to highlight these aspects, the category was compared within the dimensions of the Analysis Model: universal access to health services.[16] To validate the accuracy of the data and ensure the appropriateness of the analyses in capturing the intended messages from the key informants, they were re-invited to provide feedback using *Google Forms*. An agreement rate of >70% on the dimensions was set as the acceptance criterion.

The Analysis Model Universal access to health services[16] was adopted to structure the presentation of results and foster discussion at the interface. This study was approved by the Research Ethics Committee (CAAE: 01481918.0.0000.5393) and the co-participating institution (CAAE: 01481918.0.3001.5164).

RESULTS

Twelve key informants were considered eligible; nine participated in the study, specifically technical professionals from the state management of the SHS involved in coordinating the NPHV and the reference services of the HR. The participants predominantly consisted of individuals with permanent employment status (77,8%), individuals with a service tenure exceeding 3 years (88,8%), women (77,7%), individuals of white ethnicity (66,6%), individuals aged >50 years (44,4%), individuals with a nursing background (44,4%), and individuals who received postgraduate training (88,8%).

Table 1 presents the main findings of the analysis of access dimensions. The level of agreement between the participants and the analyses was higher than 90%.

In the political dimension, the implementation of guidelines and financial support from the Ministry of Health (MH) favored the decentralization of services and increased testing (P3, P4, and P8). Simultaneously, the regional organization process, facilitated

Table 1. Aspects that facilitate access to care for viral hepatitis, according to the dimensions of analysis, Mato Grosso, 2021

ANALYSIS DIMENSION: POLICY		
Aspects analyzed	**Key results**	**Key informant speech strata**
Process monitoring (NPHV) Agreement between the instances (state and municipal)	**- Federal scope:** - Brazilian Ministry of Health acted as a policy inducer that favored the decentralization and implementation of services and increased testing offer.	*"The Ministry encouraged the implementation of TCC. It was important to have this service here".* P4 *"It seems that the Ministry has been providing opportunities for the decentralization of services".* P8 *"The Kits are just ask and they come from the Ministry of Health, this has no cost to the municipality".* P3
	- Inter-municipal agreement: - The health consortium has been the management instrument for agreement favoring access.	*"The consortium was the right strategy. In logistics, the physician attends here at the SAE twice a month for the references of the municipalities. They don't have a hard time buying the consultation; it's not an expensive consultation."* P6 *"With the consortium we were able to hire the specialist."* P5
ANALYSIS DIMENSION: ECONOMIC-SOCIAL		
Investments in the public network by sphere of power and level of complexity. Social, economic, cultural, and physical barriers	**Investment in the health sector has aligned improvements in infrastructure and resources to reduce barriers:** - Recognition of the epidemiological scenario by management, given the increase in reported cases of human immunodeficiency virus infection and sexually transmitted infections, motivated the creation of reference services such as TCC and SAS. - The infrastructure and human resources framework in the SAE that is used as a reference for attention to hepatitis in the health region was considered satisfactory in relation to the other services in the state.	*"The number of people being treated for HIV has increased. So it was proposed to create the TCC to advance testing".* P4 *"Last year (2020) we started a conversation to implement an SAE in the municipality. With the project we moved to a better physical space ".* P7 *"Look, if you are going to make a comparison with other municipalities, we are fine. For a while I got a room for myself".* P3 *"The SAE infrastructure, the multi-professional team is a differential in care. The ease of collecting the material is what gives us strength, we have the laboratory and we collect almost weekly viral load. We reduce the waiting time of the patients, at the latest it is ten days. We also collect for genotyping. The result today is a quick result, before it was 30 or more today from ten to fifteen days".* P6
	Ensuring attention from the perspective of user needs: - The attitude of the municipal manager and the local economy contributes to the displacement of the users to the consultation, covering medications outside the REMUNE (Municipal List of Essential Medicines) and examinations that are not included in the agreement list.	*"Even if the patient has to move from the municipality, transportation is provided and in some cases OHT (Out-of-Home Treatment) has already been offered. The patient has the facility of the examination, of the medication, including those that are out of REMUNE, because sometimes the infectious disease physician passes another medication and the secretary provides it. We had a hepatitis C patient who was given a OHT for her to have tests, which were not in the agreed list in the consortium, she did it in a private service. "* P8
ANALYSIS DIMENSION: ORGANIZATIONAL		
Entry Door Attendance flow Geographical barriers, Regulation Reference Counter-reference Evaluation	**User flow in the regulation system:** - There are no limitations to the offer of testing in TCC and SAE services. - There is a defined flow to the users as soon as they receive the diagnosis. - The expansion of the offer of testing through Primary Health Care and the organization of flows in the municipality is a favorable point. - It is easy to schedule appointments through the consortium. - Female population deprived of liberty has partnerships for actions with the SAE of Rondonópolis. - The care protocol for pregnant women with a flowchart that guides professionals favors the organization of care and access to prevention of vertical transmission.	*"If the user arrives here, there is a test, if he goes to a basic unit and there is no test there, just forward him here and I will attend him."* P4 *"Once this patient is diagnosed, he has a right way."* P5 *"Quick test that gave reagent already has a right way".* P8 *"We have five basic health units here, the rapid test is offered in the units and in the municipal hospital".* P9 *"All FHS (Family Health Strategy) can perform the test, the nurse is trained. This goes a long way in increasing access to testing".* P4 *"The consortium calls and says that there is a patient who is sick; we tell the patient that we can manage to put him in the consultation here. Sometimes it puts this user on the agenda of Rondonópolis, but our concern is that this patient suffers as little as possible and that he has access to medication as soon as possible".* P6 *"For pregnant women, the flow is easier because there is a protocol and all professionals already follow. The quick test is done on the unit, this makes it easier to guide the attention network, it doesn't get so loose".* P5 *"In the SAE we have a partnership with the female penitentiary, with actions there for screening and diagnosis and treatment. Already the male penitentiary they have a team of physicians and nurses there, they seek the SAE more to seek the test and also the medications".* P5

Continue...

Table 1. Continuation.

ANALYSIS DIMENSION: TECHNICAL		
Welcoming Bond Competence Ability Autonomy Commitment Shared therapeutic project Team and user Quality of care	**Strategies to promote welcoming in the reference service:** - Scheduling various time slots at the CTA/SAE is a strategic approach. - Expanding extramural actions enhances testing. - Having an infectious disease physician in the network was recognized as favorable for facilitating diagnosis and treatment. - Professional training within the care network was considered an opportunity to advance in providing testing.	*"I give priority to attending in the morning, but if the patient comes in the afternoon, I attend. You have to consider who works, the sex workers who sleep in the morning".* P3 *"We do a lot of lectures, this region is a farm, so in SIPAT (Internal Week for the Prevention of Accidents at Work) weeks one of the themes is HIV and viral hepatitis, and in addition, lectures go to schools. The lecture is the key car in the municipality, because when we go it is clear that people do not know about hepatitis".* P4 *"We have an infectious disease physician in the unit, this is a gain; many SAE do not have. In the past he attended once a week, today he attends twice. He attended 15 patients; today he attends 20 each day, so there are 40 vacancies weekly. His schedule for other municipalities is 30 vacancies. The chance of treatment being faster and also right is higher with a specialist".* P6 "There are few capacities, but they help to stay on top of the new protocols". P5
	Cross-sector alliances: - - Partnerships between health services and social media, communication channels, and third-sector institutions have helped promote the prevention of hepatitis in society.	*"We have partners like the Catholic Church, AIDS pastoral, partnerships with higher education institutions, with television and radio media. We do interviews talking about epidemiological situation, talking about the importance of prevention and testing. Partnership with Non-governmental organizations that represent LGBT (Lesbian, Gay, Bisexual and Transgender groups and sex workers are intermediaries to get us there".* P7 *"We partner with Rotary for campaigns, especially for hepatitis C. When you do actions with partnerships you can even reach a target audience".* P8
	Professional commitment: - The proactive attitude of the professionals and their commitment to work result in greater benefit to access. - Efforts by professionals to assist the users in their demands increases adherence to treatment.	*"A couple of years ago I started talking to managers about the importance of better structuring the operation and creating a SAE, equipping and putting more professionals".* P7 *"Our social worker does a very detailed job. It is a facilitator for hepatitis patients, especially in scheduling appointments and exams, in medication processes, with documentation and checking so that it works there in the removal of high-cost medications of the pharmacy".* P5 *"The physician gives us a lot of access, we talk to him to solve a problem of a patient that appears, he opens a schedule and we manage to fit in".* P6 *"Sometimes we have leftover HIV medication, because the patient dies and the medication is returned here, so if you have Tenofovir it stays as leftover in the pharmacy and we deliver it. So we stood between the cross and the sword, between doing right or doing wrong, but at that moment if we have leftovers here we will offer, the physician prescribes and we release, it is not the ideal way, but it is also not ideal to leave him in this situation if I have medication here".* P5
ANALYSIS DIMENSION: SYMBOLIC		
Culture Beliefs Values Subjectivity	**The voice of the user after accessing the service mobilizes to qualify the assistance:** - The users' experience in the utilization of the health service and the manner in which they communicate their management and professional interactions have contributed to the expansion of specialized reference services.	*"The patient has a voice, he talks about his difficulties. Although we are close to Rondonópolis, moving there and here is not easy. So he comes and says, when he has an opportunity, he takes it to the board or to the secretary; it helps us to show the manager the need to qualify the service".* P7 *"The idea of bringing an SAE was due to complaints from patients about the times they had to go beyond the conditions of our roads and the wear and tear with the trip".* P9
	- **Profile of the professional to favor access**: - The professional's ability to empathize in serving the vulnerable population is an essential factor in understanding the approach to hepatitis in different territories.	*"For the service to walk, you need to identify yourself a lot with work, you have to like it, you have to break down prejudices, taboos. Example: we have a very large flow of homosexuals, sex workers and if suddenly you are a very closed professional, you may not be able to do the actions that are viable". P4*

through an agreement with the Intermunicipal Health Consortium (IHC), ensured access (P5 and P6).

In the economic-social dimension, the summary of results includes the contributions of public sector investment toward reducing geographical barriers and expanding services in HRs (P3 and P4). This includes ensuring the user's transfer to the reference service through an OHT instrument (P8) and investing in the human resources framework within the health sector of the reference service (P6 and P7).

The findings suggest that in the organizational dimension, providing testing in reference services is not challenging, especially with the expansion of testing offered by PHC (P4). There is a defined flow and rapid forwarding response to users with a positive diagnosis (P5 and P8). The use of IHC ensures appointment scheduling (P6), taking into account the initiatives of the reference service involving the prison sector (P5). Lastly, the organization of the care network flow is facilitated through the implementation of care protocols (P5).

In the technical dimension, the flexibility of TCC/SAS in offering different service hours was recognized as strategic (P3). Extramural actions were deemed essential for enhancing testing (P4). Partnerships with social media, communication channels, and third-sector institutions were identified as crucial for amplifying extramural actions (P7 and P8). The presence of an infectious disease physician in the referral service was considered a differentiating factor in case management, even facilitating access to treatment (P6). Training and updating for teams were emphasized (P5). Lastly, the proactive posture of referral professionals in organizing the service and providing maximum efficiency to treatment and monitoring was highlighted (P5, P6, and P7).

In the symbolic dimension, the results converge on the significance of municipal health management listening to the user regarding their therapeutic itinerary during (P7 and P9) and demonstrating empathy in serving the users within the service (P4).

DISCUSSION

In the political dimension, the inclusion of hepatitis in the Sustainable Development Goals (SDG) has played a crucial role in emphasizing the significance of this disease within health systems. The challenge lies in mitigating inequalities in access to hepatitis care and ensuring treatment, considering the diverse social disparities present in health systems.[17] To formulate public health policies tailored to the reality of HS, incorporating care models that streamline service provision and enhance access, it is necessary to establish indicators that support decision-making.[18]

The collective management guided by the coping model adopted in Europe serves as an exemplary experience for achieving micro-elimination by 2030.[19] Low- and middle-income countries encounter challenges related to unequal access. However, Egypt, Georgia, Rwanda, and Mongolia have made notable progress by implementing targeted strategies aimed at serving priority groups.[20] These successful experiences are linked to the expansion of infrastructure, the implementation of comprehensive public policies for testing and treatment, the systemic integration of various services, and the application of concerted efforts to reduce the cost of medicines in these countries.[20]

In Brazil, the MH initiatives are outlined through the NPVH. Since its inception, the program has established guidelines that emphasize prevention, surveillance, and assistance across various health services, including the organization, regulation, monitoring, and evaluation of the program's actions. Since 2008, in technical collaboration with the World Health Organization (WHO), goals have been set to address this disease in Brazil.[21] In terms of prevention, vaccination has been incorporated into the SUS immunization schedule, indicated for different population groups and age categories. This culminated in 2016, with expansion to the entire population, regardless of age.[22]

In the SUS, from 2005 to 2010, there was a noticeable expansion of the care network for testing and counseling of hepatitis B and C. This expansion was supported by Clinical Protocol of Therapeutic Guidelines publications and involved the incorporation of direct antiviral agents (DAAs), recognized by PAHO as a significant advancement in the care of patients with hepatitis C.[4,23] Since 2020, to facilitate the expansion of access and adherence to treatment, the MH has enabled the migration of hepatitis drugs from the Specialized Component to the Strategic Component of Pharmaceutical Assistance.[24]

To enhance access, the Brazilian MH published Ordinance 1.537/2020 MH/GM,[24] which underscores the necessity for greater coordination between the NPVH and the Health Surveillance Secretariat. This collaboration involves shared actions with the Secretariat of Primary Health Care, Secretariat of Specialized Health Care, National Health Foundation, National Health Surveillance Agency, and Secretariat for Science, Technology, and Strategic Inputs. Technical note number 369/2020 CGAHV/DCCI/SVS/MS was published in order to support the decentralization of testing and treatment through the involvement of nurses.[25] Municipal health management has used the IHC to ensure access to specialty centers for confirmatory tests and the initiation of treatment. The IHC operates based on the logic of political-economic cooperation with territorial agreements, aiming to minimize bureaucratic processes and interfederal barriers.[11,26]

In the economic-social dimension of access, there is consensus that advancing policies to combat hepatitis can generate an economic benefit.[27] The results demonstrate a strong emphasis on epidemiological aspects to expand services, primarily propelled by the robustness of the HIV program. Despite progress in decentralizing SAS-type services, HR care remains centralized

in the regional reference service. Participants rarely explored this dimension, possibly due to the centrality of the treatment and the distant involvement of the state program management in coordinating the economic and social aspects of HR.

To advance regionalization, it is necessary to consider the criteria that can organize attention through regional planning. The European Union has prioritized organizing attention based on epidemiological data that subsidize referencing and creating services that offer focal actions to specific populations and risk groups to achieve microelimination.[28]

One aspect impacting any HS is the provision of treatment, with economic considerations arising from the high cost of medications. To expand guidelines that strengthen access, it is necessary to analyze costs. Alongside this, an investment strategy can be implemented to estimate treatment expenditures, recommending centralized purchasing supported by clinical practice guidelines.[29] This approach, implemented in Colombia, resulted in a reduction of more than 90% in prices.[30] In Brazil, a country with a universal public system playing a significant role in the distribution of hepatitis treatment, this practice has been adopted since 2006.[31] It has facilitated the technological incorporation of treatment, particularly for hepatitis C treatment.[32] Since 2014, access to treatment with DAA has been guaranteed, despite the high cost.[31]

In low- and middle-income countries, the number of studies addressing the costs and continuous follow-up of users from hepatitis diagnosis to cure is low.[33] This limitation poses a challenge for hepatitis control policies in countries confronting significant social inequalities and dealing with fiscal austerity on the political agenda. The Brazilian MH has developed an economic model aligned with strategies considered potential to achieve the goals estimated by the WHO. This initiative aims to support an analysis of the costs associated with eliminating hepatitis C in the country by 2030.[34]

In addition to the infrastructure of the referral service, the participants identified professionals in the services and training for actions as crucial factors that enhance the possibility of advancing access. Allowing users to access reference services with OHT support ensures access and contributes to the realization of the principle of universality, promoting equity.[35] However, the economic and logistical aspects of care networks have weakened, in many cases, the guarantee of OHT.[36]

In the organizational dimension, it is perceived that in HRs, a flow based on positive testing is defined, despite being centralized and requiring intersectoral negotiations. In light of this finding, health services should analyze the geographical barriers that may hinder users' access.[37]

In regional organizations, analyzing strategic points for the dissemination of actions and services, with the support of specialist physicians in decentralized teams, increases the likelihood of improving the hepatitis program[38]. For this improvement to occur, effective professional communication is essential, which involves a multi-professional team to facilitate access in the territory, especially for priority groups.[39]

The regulation of care and care management are important in the work process. It is essential to have PHC in place to identify people individuals susceptible to diagnosis, particularly in the context of decentralizing hepatitis treatment[24] and establishing matrix support for PHC teams. In SUS, the importance of PHC and the matrix support for its teams has been discussed since the creation of the NPHV[40]. Since 2021, new investments have been allocated to materialize this proposal, with initiatives aimed at enhancing the competence of clinical and pharmaceutical care professionals and fortifying the care pathway by formulating guidelines for the diagnosis, treatment, and referral of patients.[11]

The TCC in municipalities can strengthen the adoption of testing practices in PHC, support professional training, monitor referred cases, and promote a more organized user flow.[12] In this context, it is essential to minimize the barriers to accessing treatment, especially access to medications that affect adherence. Implementing flexible documentation, monitoring support, and incorporating strategies for screening side effects and drug interactions are potential approaches for collaborative teams.[41]

In the technical dimension, positive aspects of access include organizing the work process to enhance reception, implementing measures to expand testing, and utilizing strategies to disseminate information about the disease. To overcome social, economic, cultural, and physical barriers, it is necessary to coordinate actions that integrate health services with rapid response flows in priority locations that serve socially vulnerable populations, especially those marginalized by HS.[24] The support of specialists extends the connection with users in the territory and allows the dissemination of educational resources through multipliers.[44] Personalization of attention to the users' problem strengthens monitoring and increases the acceptability of treatment.[45]

To align with the WHO Hepatitis Testing Guidelines, various strategies are recommended, such as integrated HIV testing, use of social media to promote acceptance of tests, workplace testing, utilizing emergency departments for testing, directing professionals with notifications in electronic medical records of high-risk patients for testing, and expanding specific services in different populations, such as injecting drug users, prisoners, other high-risk groups, migrants, and relatives of people living with hepatitis B or C.[46] For users born between 1945 and 1965,[47] the study used personalized invitations through letters , which successfully increased the test response rate.

Involving the community is an essential strategy in planning actions that aim to advance the elimination of hepatitis.[27] To achieve this, establishing partnerships with non-governmental organizations (NGOs) and providing training for a multidisciplinary approach

in collaboration with civil society enhance the effectiveness of actions.[48,49] Investing in a multidisciplinary approach to hepatitis care is a recent and rapidly evolving field. Care management that considers dynamic and collaborative practice approaches is likely to strengthen access to testing, diagnosis, and treatment adherence.[50]

In addition to performing testing, ensuring the accuracy of the diagnosis, and training professionals to make appropriate requests and interpret tests correctly for treatment, focusing on hepatitis necessitates a multimodal approach. This involves the use of technology resources and coordination of care.[41] For this, managers can use electronic registration banks of health services to subsidize analyses that guide the selection of strategies.[51]

Nurses are highly effective professionals trained to assume the role of Hepatitis Clinical Nursing Consultants. HepCare Europe considers the role of this professional as crucial, serving as a mediator of communication between levels of care, from prevention to treatment adherence.[49] In Australia,[52] New Zealand,[53] Baltimore,[54] and the United States, the nurses-led strategy has demonstrated success, suggesting the optimization of health resources.

In the symbolic approach, prioritizing listening and providing opportunities for users to share their care experiences in the therapeutic itinerary justifies the increased decentralization of services in HR. Thus, understanding cultural aspects helps health services design actions based on proposals that can be more welcoming in different territories.[55] The communication process that takes place in the professional-user relationship plays a key role in adherence to the recommendations and treatment of hepatitis.[56]

CONCLUSIONS

In terms of the factors promoting access, the political dimension highlights the incentive of decentralizing services and the use of IHC in the agreements. In the economic-social dimension, the expansion of reference services, supported by public resources, involves the transportation of users to consultation and treatment centers, incorporating medications beyond the REMUNE, and covering examinations that are not included in the agreement list. In the organizational dimension, ensuring professionals' confidence in the availability of testing supplies, establishing a clear flow for diagnosed users, and facilitating appointment scheduling are key considerations. Although the organization of care by PHC is beneficial, it necessitates the formulation of guidelines and protocols in collaboration with extramural activities through partnerships with the TCC/SAS, as exemplified in the prison context. In the technical dimension, the commitment of professionals to the service, offering diverse time slots at the TCC/SAS to meet the user's demand, having an infectious disease physician in the network, and providing professional training, in addition to inter-sectoral partnerships, play crucial roles. In the symbolic dimension, listening to users' experiences during the care trajectory and demonstrating professional empathy are emphasized.

Analysis of the participants' discourse did not reveal solid strategies from state coordination guidelines that focused on testing indigenous populations, quilombolas, rubber tappers, artisanal fishermen, riverside dwellers, gays, men who have sex with men, transvestites and transsexuals, people who use drugs, the homeless, and those deprived of freedom. In HR, only two services cited actions with intersectoral partnerships to expand testing to sex workers. This situation highlights the lack of criteria aligned with political guidelines, in addition to the low coordination of actions in health services.

On the contrary, if a discourse is based on the manager's concern with the goals established for the elimination of hepatitis C, the intensity of the provision of actions and services in the SUS care network can be reevaluated from the health promotion to risk and damage control perspectives.

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Isotemporal substitution analysis of time between sleep, sedentary behavior, and physical activity on depressive symptoms in older adults: a cross-sectional study

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ABSTRACT

BACKGROUND: Compared to young individuals, older adults participate more in sedentary behavior (SB) and less in physical activity (PA). These behaviors are associated with numerous adverse health factors.

OBJECTIVE: The purpose of the study was to examine the hypothetical effects of substituting time spent sleeping, performing SB, and performing moderate-to-vigorous physical activity (MVPA) on depressive symptomatology in older adults.

DESIGN AND SETTING: An analytical cross-sectional study employing exploratory survey methods was conducted in the city of Alcobaça in the state of Bahia, Brazil

METHODS: The study included 473 older adults who answered a structured questionnaire during an interview. Exposure time to SB and PA level were assessed using the International Physical Activity Questionnaire, and depressive symptoms were analyzed using the short version of the Geriatric Depression Scale. An isotemporal replacement model was used to evaluate the effects of different SB sessions on depressive symptomatology.

RESULTS: An increase in the risk of depressive symptoms was observed when MVPA and sleep time were substituted for the same SB time at all times tested, with maximum values of 40% and 20%, respectively. Opposite substitution of MVPA and sleep time increments reduced the risk of depressive symptomatology by 28% and 17%, respectively.

CONCLUSIONS: The results of the present study indicate that replacing SB with the same amount of sleep or MVPA may reduce depressive symptoms. The longer the reallocation time, the greater are the benefits.

INTRODUCTION

Depression is considered one of the most prevalent mental disorders in many countries.[1] It frequently occurs in older adults and results from the interaction of several factors, including genetic factors, cognitive deficits, and disturbing situations.[2]

Depression presents a major economic burden, as it ranks 15th among conditions with the highest healthcare costs,[3] causes a reduced functional capacity to perform basic activities of daily living, decreases quality of life, and is associated with increased use of health services, hospitalization, and mortality.[4-6]

Specifically, in older adults, previous studies have noted that several factors contribute to the disease.[2,7] Among behavioral factors, a meta-analysis showed that people with depression are less physically active and engage in higher levels of sedentary behavior (SB).[8] Sleep time has also been shown to be associated with depression, with both short (≤ 6 hours) and long durations (> 9 hours) significantly and more strongly associated with depression than sleep durations between 7 and 9 hours.[9]

Although there is consensus in the literature that intervention strategies based on increased regular physical activity (PA) are effective in reducing depressive symptoms,[10] it is necessary to consider other behaviors during the day. During a 24-hour period, different behaviors may be adopted: sleep, SB, and PA (light, moderate, and vigorous).[11] In addition, the interaction of behaviors (sleep, SB, and PA) over the course of 24 hours is directly related to an individual's health.[12]

The recommended sleep time for older adults is 7–8 hours per day, and the recommended amount of moderate-to-vigorous physical activity (MVPA) is 30 minutes/day.[13,14] Regarding SB, although there is not yet a recommended time per day, its reduction is important for individuals to maintain an active lifestyle, and increases in the time spent in light-intensity activities have been suggested.[15,16]

Statistical isotemporal substitution modelling was used to assess the hypothetical effects of the replacement of time spent on activities on health conditions. Thus, hypothetical isotemporal replacement models have gained prominence in the literature and have been applied to assess the reallocation of a given time spent on one activity to the same time spent on another.[17]

The analysis of isotemporal substitution has been applied in several studies in different populations, as highlighted in a recent systematic review.[18] For example, in a longitudinal study performed in older adults, it was found that the 60-minute replacement of sitting time for standing, walking, MVPA, and sleep for individuals sleeping ≤ 7 hours/day reduced the risk for mortality.[19]

Previous studies have estimated the impact of isotemporal substitution on depressive symptoms, and positive effects were found when 60 minutes of television time was reallocated to walking at an average speed[20] and 60 minutes of SB time was reallocated to vigorous PA.[21] Specifically, in older adults, studies have reported the benefits of replacing time spent in SB with time spent in PA on depressive symptoms,[22,23] and the benefits of the replacement of only 30 minutes/day, not including a replacement with sleep, was verified. In addition, these studies were conducted in developed countries.

OBJECTIVE

This study aimed to examine the hypothetical effects of substituting time spent sleeping, engaged in SB, and performing MVPA on depressive symptomatology in older adults. We hypothesized that the substitution of time spent engaged in MVPA with the same amount of time spent in SB could increase the likelihood of developing depressive symptomatology and that inverse substitution when replacing SB with MVPA reduces the chances of developing depressive symptomatology.

METHODS

Study sample

A cross-sectional population-based epidemiological survey entitled the Longitudinal Study of the Elderly Health of Alcobaça (ELSIA Project) was conducted with individuals aged ≥ 60 years who were registered with the Family Health Strategy of the Health System of the Brazilian government in the municipality of Alcobaça, Brazil. The exclusion criteria were the presence of cognitive impairment according to the Mini-Mental State Examination,[24] inability to ambulate even with the assistance of a cane or walker, severe difficulty in visual and auditory acuity according to the interviewer's perception, wheelchair dependence; and severe sequelae of a cerebrovascular accident with a localized loss of strength.

Among the 743 older adults registered in the Family Health Strategy, 54 refused to participate in the survey, 58 were excluded because they did not meet the inclusion criteria, and 158 could not be located. Thus, the analysis included 473 participants, with data collected from July to October 2015.

Data were collected at an older adult residence by a team of students and trained health professionals. Participants responded to a structured questionnaire during a face-to-face interview. The research protocols were evaluated and approved by the Research Ethics Committee of the Universidade Federal do Triangulo Mineiro (Ordinance No. 966.983/2015; February 25, 2015). All participants provided written informed consent before participation.

Outcome measures: depressive symptoms

Depressive symptoms were assessed using the Brazilian short-form version of the Geriatric Depression Scale (GDS-SF; Spearman's rho test-retest reliability = 0.86).[25] The GDS-SF scale consists of 15 "yes or no" questions. The final score ranges from 0 to 15 points; a score of 6–10 points suggests mild to moderate depression, and a score of 11–15 points suggests serious or severe depression. In this study, the presence of depressive symptoms in older adults was defined as a score of ≥ 6 points.

Outcome measures: sleep, sedentary behavior, and physical activity

Time spent sleeping at night was measured using a single question, which was part of the Brazilian Portuguese version of the Pittsburgh Sleep Quality Index: "How many hours of actual sleep do you get at night?"[26]

The time in SB and physical activity was determined using the long version of the International Physical Activity Questionnaire (IPAQ), which has been validated for the Brazilian older adult population (Kappa coefficient = 0.27 for women and 0.24 for men; Spearman's rho test-retest reliability = 0.78 for women and 0.95 for men).[27,28] The participants were asked to report the time spent sitting during a typical weekday and weekend day and the time spent performing moderate- to vigorous-intensity physical activity in a standard week in four domains: work, transportation, domestic activities, and leisure activities.

The time spent engaged in SB during a typical day was calculated as [(time spent sitting during a typical weekday × 5 + time spent sitting during a typical weekend day × 2)/7]. The total time spent in moderate-to-vigorous-intensity physical activity (MVPA) per day was determined using the following formula: time spent in MVPA/day = [total minutes in moderate-intensity physical activity/week + (total minutes in vigorous-intensity physical activity/week × 2)]/7.[29]

Covariates

Demographic variables included sex (male or female), age (years), and marital status (single/divorced, married, or widowed). These variables were self-reported by participants.

Statistical analysis

Data were entered twice using Epidata software, version 3.1b (EpiData Association, Odense, Denmark). Statistical analyses were performed using Statistical Package for the Social Sciences version 21.0 (SPSS Inc., Chicago, IL, USA).

To compare the participants' characteristics according to depressive symptoms, t-tests or chi-square tests were used. To verify the hypothetical effects of reallocation of time spent on sleep, SB, and moderate and vigorous activities on depressive symptoms, the isotemporal substitution approach was used.[17] The isotemporal substitution analyses were performed by estimating the prevalence ratio (PR) with the respective 95% confidence interval (CI) using Poisson regression with robust variance. Isotemporal substitution models were performed for 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, and 60 minutes/day spent on sleep, SB, and MVPA and depressive symptoms. All models were adjusted for sex, age, and marital status, and the level of statistical significance was set at $P < 0.05$.

RESULTS

Of the 473 participants, 62.6% (n = 296) were women, and 46.0% (n = 217) were married. The average age of the participants was 70.2 (± 8.2) years, with a range of 60 to 97 years. On average, the participants spent 436.14 (± 105.94) minutes per day sleeping, 433.68 (± 162.45) minutes engaged in SB, and 52.12 (± 82.08) minutes performing MVPA.

Table 1 presents the participants' characteristics based on the presence of depressive symptoms. Older adults who were female, were widowers, had longer SB, or had lower MVPA had depressive symptoms (**Table 1**).

According to the isotemporal substitution models, more time spent sleeping or performing MVPA and less time spent engaged in SB reduced the likelihood of depressive symptoms ($P < 0.05$). When more time was substituted, the protective effect was greater, and reallocation to MVPA resulted in a greater reduction in depressive symptoms (**Table 2**).

Table 1. Characteristics of the participants by the presence of depressive symptoms

	Absence of depressive symptoms	Presence of depressive symptoms	P
Sex, n (%)			0.041
Male	163 (39.1)	14 (25.0)	
Female	254 (60.9)	42 (75.0)	
Marital status, n (%)			0.004
Single / divorced	110 (26.4)	15 (26.8)	
Married	201 (48.3)	16 (28.6)	
Widowed	105 (25.2)	25 (44.6)	
Age (years)	70.01 ± 8.15	72.00 ± 8.89	0.091
Sleep (min/day)	438.31 ± 103.24	416.50 ± 116.42	0.145
Sedentary behavior (min/day)	424.43 ± 159.49	498.74 ± 162.29	0.001
MVPA (min/day)	54.82 ± 84.62	31.01 ± 40.22	0.001

MVPA = moderate to vigorous physical activity.

DISCUSSION

This study examined the hypothetical effect of reallocation of time spent on active and sedentary activities on the prevalence of depressive symptoms in older adults. These results suggest that substituting sitting time for MVPA has positive effects on depressive symptoms. In addition, substituting sitting time with sleep resulted in benefits. These findings reinforce the benefits of these measures for preventing depressive symptoms, especially those related to an active lifestyle.[10]

A study on SB in older adults has also been highlighted. This age group spent the most time engaged in SB, as evidenced by the results of studies conducted in developed[30] and developing[31] countries. As a consequence, it has been reported that older people who spend more time in SB have worse health conditions[16] and are at higher risk for depressive symptoms.[32]

Within a 24-hour period, SB comprises a significant portion of an individual's time.[12] Therefore, according to the present study, replacing 60 minutes/day of sitting time with MVPA or sleep can reduce the likelihood of depressive symptoms by 29 and 17%, respectively. Significant differences, albeit of lesser magnitude, were also observed when the amount of time replaced was shorter. Replacing activities with shorter durations may be more feasible for older adults.[33]

Recent studies have verified that replacing 30 minutes of time spent engaged in SB with the equivalent time in low-intensity physical activity and MVPA[22] and transport time (walking/bicycling) and MVPA[23] is also beneficial. Unlike the results of the present study, those presented by Yasunaga et al.[22] showed no beneficial effects of the reallocation of SB time to MVPA in older Japanese adults. However, the authors found that replacing sedentary time with low-intensity physical activity resulted in decreased depressive symptoms. However, a study conducted by Wei et al.[23] in older adults in the U.S. (National Health and Nutrition Examination Survey) showed that replacing SB with walking/bicycling or MVPA was associated with lower severity of depressive symptoms among older adults.

The practice of MVPA in older adults is considered a protective factor against adverse health conditions[34] and mortality.[6] Moreover, the relationship between MVPA and depressive symptoms has been verified both in cross-sectional studies[35,36] and in longitudinal studies;[37,38] thus, it can be considered a protective factor.

In addition, exercise programs reduce depressive symptoms. According to a systematic review and meta-analysis of 41 randomized controlled trials conducted with older adults, exercise is an effective treatment option for older adults with depressive symptoms.[39]

Table 2. Isotemporal substitution models for depressive symptoms

Isotemporal models	Sleep	SB	MVPA
	PR (95%CI)	PR (95%CI)	PR (95%CI)
5 minutes/day			
Replace sleep	Dropped	1.01 (1.01-1.03)*	0.99 (0.96-1.01)
Replace SB	0.98 (0.97-0.99)*	Dropped	0.97 (0.95-0.99)*
Replace MVPA	1.01(0.99-1.04)	1.03 (1.01-1.06)*	Dropped
10 minutes/day			
Replace sleep	Dropped	1.03 (1.01-1.06)*	0.97 (0.92-1.03)
Replace SB	0.97 (0.95-0.99)*	Dropped	0.94 (0.89-0.99)*
Replace MVPA	1.02 (0.97-1.08)	1.06 (1.01-1.11)*	Dropped
15 minutes/day			
Replace sleep	Dropped	1.05 (1.01-1.08)*	0.96 (0.89-1.04)
Replace SB	0.95 (0.92-0.99)*	Dropped	0.92 (0.85-0.99)*
Replace MVPA	1.04 (0.96-1.13)	1.08 (1.01-1.18)*	Dropped
20 minutes/day			
Replace sleep	Dropped	1.06 (1.02-1.11)*	0.95 (0.85-1.06)
Replace SB	0.94 (0.90-0.98)*	Dropped	0.89 (0.80-0.99)*
Replace MVPA	1.05 (0.94-1.17)	1.12 (0.01-1.24)*	Dropped
25 minutes/day			
Replace sleep	Dropped	1.08 (1.02-1.14)*	0.94 (0.82-1.08)
Replace SB	0.92 (0.88-0.98)*	Dropped	0.87 (0.76-0.99)*
Replace MVPA	1.06 (0.93-1.22)	1.15 (1.01-1.31)*	Dropped
30 minutes/day			
Replace sleep	Dropped	1.10 (1.03-1.17)*	0.93 (0.79-1.09)
Replace SB	0.91 (0.85-0.97)*	Dropped	0.85 (0.72-0.99)*
Replace MVPA	1.08 (0.92-1.27)	1.18 (1.01-1.38)*	Dropped
35 minutes/day			
Replace sleep	Dropped	1.11 (1.03-1.20)*	0.92 (0.76-1.11)
Replace SB	0.89 (0.83-0.97)*	Dropped	0.82 (0.68-0.98)*
Replace MVPA	1.09 (0.90-1.32)	1.22 (1.01-1.46)*	Dropped
40 minutes/day			
Replace sleep	Dropped	1.13 (1.04-1.24)*	0.90 (0.73-1.12)
Replace SB	0.88 (0.81-0.97)*	Dropped	0.80 (0.65-0.98)*
Replace MVPA	1.10 (0.89-1.37)	1.25 (1.01-1.54)*	Dropped
45 minutes/day			
Replace sleep	Dropped	1.15 (1.04-1.27)*	0.89 (0.70-1.14)
Replace SB	0.87 (0.79-0.96)*	Dropped	0.77 (0.61-0.98)*
Replace MVPA	1.12 (0.87-1.43)	1.28 (1.01-1.63)*	Dropped
50 minutes/day			
Replace sleep	Dropped	1.17 (1.04-1.30)*	0.88 (0.67-1.16)
Replace SB	0.86 (0.77-0.96)*	Dropped	0.75 (0.58-0.98)*
Replace MVPA	1.13 (0.86-1.48)	1.32 (1.02-1.72)*	Dropped
55 minutes/day			
Replace sleep	Dropped	1.18 (1.05-1.34)*	0.87 (0.65-1.17)
Replace SB	0.84 (0.75-0.95)*	Dropped	0.74 (0.55-0.98)*
Replace MVPA	1.15 (0.85-1.55)	1.36 (1.02-1.82)*	Dropped
60 minutes/day			
Replace sleep	Dropped	1.20 (1.06-1.37)*	0.86 (0.62-1.19)
Replace SB	0.83 (0.73-0.95)*	Dropped	0.71 (0.52-0.98)*
Replace MVPA	1.16 (0.84-1.61)	1.40 (1.02-1.92)*	Dropped

CI = confidence interval; PR = prevalence ratio; MVPA = moderate-to-vigorous physical activity; SB = sedentary behavior. The PR was adjusted for sex, age, and marital status. * $P < 0.05$.

In addition to demonstrating that increased physical activity can reduce depressive symptoms, the results of the present study indicate that sleep plays a key role. Both long and short sleep durations are negatively associated with health[40,41] and a higher risk of mortality in older adults.[42] Furthermore, according to a meta-analysis of longitudinal studies, short and long sleep durations are risk factors for depressive symptoms.[43]

Among the studies that evaluated the effect of isotemporal substitution on depressive symptoms,[20,22] the reallocation of sitting time was not tested with sleep. However, it has been shown that replacing 30 and 60 minutes of SB with an equivalent amount of time sleeping is associated with benefits in cardiovascular risk biomarkers[43] and a lower mortality risk, respectively, in those who sleep for fewer than 7 hours.[19]

Thus, increasing the time spent sleeping seems to be a protective factor against depressive symptoms as sleep plays a role in homeostasis and body regulation, and its imbalance is associated with depression.[45,46] According to a two-year follow-up study, older adults who sleep for fewer than 6 hours at night have a higher incidence of depressive symptoms than those who sleep for 7–8 hours.[47]

According to the consensus of a National Sleep Foundation expert panel, sleeping for fewer than 6 hours is associated with poorer health conditions, including physical and mental illnesses, compared with sleeping for 6–9 hours. The National Sleep Foundation further notes that sleeping for 9–10 hours or more is associated with diseases and an increased risk of mortality.[13]

Thus, it is noteworthy that the results supporting an increase in the time spent sleeping should be interpreted with caution, as duration and previous sleeping time should be taken into consideration in older adults. It is possible that the older adults in the present study who sleep for fewer than 6 hours would benefit from increased sleeping time.

Finally, the results of this study reinforce the need for additional evidence on interventions to reduce SB in older adults. A recent systematic review found that interventions for SB reduction appeared to be effective in the short-term in adults;[48] however, evidence of this effect in older adults remains incipient.[16]

With the results presented in the study, health professionals and public policies should focus on the regular practice of PA, encouraging decreased time engaged in SB, reducing the risk of various health hazards, including depressive symptoms.

Some limitations of the present study should be considered, as it is a cross-sectional study, which does not allow the determination of cause-and-effect relationships, and subjective measures were used to measure the level of physical activity and SB. However, it is worth noting that the IPAQ instrument has been validated in Brazil and is widely used.[46] A strong point of this study is the inclusion of sleeping time in the estimation of the hypothetical effect of isotemporal substitution and the different durations tested. This

method is valuable and widely used in research, as it allows behavioral changes to be modeled, and can provide important insight for new studies and interventions without expending unnecessary time and resources on inefficient studies.

CONCLUSIONS

Replacing SB time with the same amount of time spent sleeping or performing MVPA can lead to a reduction in depressive symptoms. The longer the reallocation time, the greater are the benefits.

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Effect of N-acetyl cysteine, rifampicin, and ozone on biofilm formation in pan-resistant *Klebsiella pneumoniae*: an experimental study

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ABSTRACT

BACKGROUND: To the best of our knowledge, this is the first study to evaluate the effectiveness of specific concentrations of antibiofilm agents, such as N-acetyl cysteine (NAC), rifampicin, and ozone, for the treatment of pan-resistant *Klebsiella pneumoniae* (PRKp).

OBJECTIVES: We evaluated the effectiveness of antibiofilm agents, such as NAC, rifampicin, and ozone, on biofilm formation in PRKp at 2, 6, 24, and 72 h.

DESIGN AND SETTING: This single-center experimental study was conducted on June 15, 2017, and July 15, 2018, at Istanbul Faculty of Medicine, Istanbul University, Turkey.

METHODS: Biofilm formation and the efficacy of these agents on the biofilm layer were demonstrated using colony counting and laser-screened confocal microscopy.

RESULTS: NAC at a final concentration of 2 μg/mL was administered to bacteria that formed biofilms (24 h), and no significant decrease was detected in the bacterial counts of all isolates (all $P > 0.05$). Rifampicin with a final concentration of 0.1 μg/mL was administered to bacteria that formed biofilm (24 h), and no significant decrease was detected in bacterial count (all $P > 0.05$). Notably, ozonated water of even 4.78 mg/L concentration for 72 h decreased the bacterial count by $\geq 2 \log_{10}$.

CONCLUSION: Different approaches are needed for treating PRKp isolates. We demonstrate that PRKp isolates can be successfully treated with higher concentrations of ozone.

INTRODUCTION

Nosocomial infections are major causes of morbidity and mortality. These infections and their agents are becoming increasingly difficult to treat. Current antibiotics against multidrug-resistant microorganisms are inadequate, and there is a critical shortage of new antibiotics.[1] Notably, biofilm formation has been implicated as one of the antibiotic-resistance mechanisms.[2] Living within a biofilm provides bacteria with the advantage of protection against nutrient deprivation, dehydration, pH changes, disinfectants, antibiotics, and toxic substances. Bacteria that form biofilms are 100-1000 times more resistant to antibiotics than their free forms.[3] The mechanisms responsible for the resistance of bacteria within biofilms often include enzymatic inactivation, efflux pumps, and mutations in drug targets. A microorganism that is inherently susceptible to antimicrobial agents can become resistant when it forms a biofilm and can revert to susceptibility once it detaches from the biofilm. Factors such as low penetration of antimicrobials into the biofilm, changes in the microenvironment, formation of resistant phenotypic variants specific to the biofilm, slowed bacterial growth within the biofilm, and presence of persister cells contribute to antibiotic resistance.[4,5]

OBJECTIVES

In this study, we aimed to identify an effective method for treating pan-resistant *Klebsiella pneumoniae* (PRKp) isolates, an important nosocomial infection agent in our hospital that has become increasingly difficult to treat. Therefore, there is a need for different approaches to treat potent biofilm-forming pan-resistant PRKp isolates. Antibiotics were hypothesized to be effective after administering effective antibiofilm agents such as N-acetylcysteine (NAC), rifampicin, and ozone.

METHODS

Identification of the bacteria isolates and antibiotic susceptibility tests

K. pneumoniae isolates that formed invasive infections in the samples collected from nine patients aged over 18 years who were hospitalized in the university hospital between June 15, 2017, and July 15, 2018, were identified using current conventional microbiological and biochemical methods. The zone diameter and minimum inhibitory concentration (MIC) values determined by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) for the disk diffusion, gradient, and broth microdilution methods were used to identify the susceptibility of the bacteria to antibiotics. *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, NCTC *E. coli* 13846 (mcr-1 positive), *Staphylococcus aureus* ATCC 29213, and *Enterococcus faecalis* ATCC 29212 were used as quality control isolates.[6]

Carbapenem resistance was determined by using the "In-house polymerase chain reaction (PCR)" method for $bla_{\text{OXA-48}}$, $bla_{\text{NDM-1}}$, bla_{VIM}, bla_{IMI}, and bla_{KPC} genes isolates defined by the meropenem zone diameter of < 28 mm in the disk diffusion test as recommended by EUCAST.[3,8-10]

Identification of the antibiotic resistance of bacteria

Among *K. pneumoniae* isolates, bacteria that are resistant to at least one agent in three or higher antimicrobial categories were defined as "multidrug resistant" (MDR). Bacteria that are sensitive to a maximum of two antimicrobial categories but resistant to at least one agent from other categories are defined as "extremely drug-resistant" (XDR). Finally, bacteria that are resistant to all agents in all antimicrobial categories are defined as "pan-drug resistant" (PDR).[11]

Demonstration of the biofilm formation

Crystal violet stain was used in the microplate to investigate the biofilm-forming capacity of bacteria.[12] *P. aeruginosa* ATCC 27853 and *E. coli* ATCC 25922 isolates were used as positive control, and 1% glucose-containing tryptic soy broth (TSB) was used as the negative control.[13] The values above the optical values measured for negative control were evaluated as biofilm-positive, and the values equal to negative control or below negative control were evaluated as biofilm-negative.[14]

Investigating the efficacy of NAC, rifampicin, and ozone (0.6 μg/mL) on the biofilm layer

Pure cultures of *K. pneumoniae* isolates of 18-24 hours grown in blood agar were cultured in 5 mL TSB tubes to obtain a suspension of McFarland standard turbidity of 0.5. The tubes were incubated for 18-24 hours at 37 °C. The next day, the bacterial suspension was diluted to a ratio of 1:50 in 5 mL tryptic soy broth containing 1% glucose. 200 μL of the sample was aliquoted to each well of a 96-well U-based cell culture microplate. The fluid medium in the wells was emptied and washed with phosphate-buffered saline (PBS) three times after the microplate was incubated at 37 °C for 18-24 hours. The microplates were dried at room temperature. This stage was defined as basal biofilm formation (0 h). After the evaluation of the basal biofilms, 200 μL of TSB containing 1% glucose was added in each well for 2, 6, 24, and 72 h. The medium was incubated for 18-20 hours, and the dividing colonies were counted. The effects of NAC, rifampicin, and ozone on the biofilm layer were evaluated using the 2 $\log_{10}$ method, and comparisons were made to the control group.

The effect of ozone (4.78 μg/mL) on the biofilm layer

To obtain high rates of ozone concentration, as described for NAC and rifampicin, 1 mL was taken from the prepared bacterial suspension and distributed into a 6-cell culture plates, separately for each bacterium. The plates were incubated at 37 °C for 18-24 hours, and the fluid medium in the plates was emptied and washed with PBS three times. This stage was defined as basal biofilm formation (0 h). Ozone solution in distilled water was prepared by holding the oxygen regulator on an ozone device (Longevity Resources, BC, Canada) at 0.12 speed and 40 gammas for 10 minutes. Ozonated water with a final concentration of 4.78 μg/mL was obtained by measuring with the ozone analyzer device, and 1 mL of the solution was distributed into one of the two 6-cell culture plates prepared for each bacterium. The other plate, in which no ozone solution was added, was used as the control. Plates containing the ozone solution were emptied after 10 min. TSB containing 1% glucose was used to make the well volume 1 mL, and biofilm formation was quantified at 2, 6, 24, and 72 h. We evaluated the cell density at the specified times using a colony count when the final concentration of ozonated water was 4.78 μg/mL, similar to the treatments with NAC and rifampicin.

Demonstrating the efficacy of the antimicrobial agents on the biofilm formation using laser scanning confocal microscopy

Pure cultures of *K. pneumoniae* isolates of 18-24 hours grown in blood agar were cultured in 5 mL TSB to obtain a suspension of McFarland standard turbidity of 0.5. The tubes were incubated for 18-24 hours at 37 °C. The next day, the bacterial suspension was diluted to a ratio of 1:50 in 5 mL of TSB containing 1% glucose. Samples (2 mL) were taken from this bacterial suspension and delivered in 6-cell culture plates which included

sterile circular lamellae prepared separately for each bacterium (to include two plates for each bacteria, at 0, 2, 6, and 24 h). The plates were emptied again at the end of the specified times, washed thrice with PBS solution, and subjected to laser scanning confocal microscopy (Leica, TCS SP8 Ted, Leica Microsystems CMS GmbH, Mannheim, Germany, using 10*x* objective and a 1*x* magnification factor) to evaluate the efficacy of the biofilm layer. After a mixture of fluorescein diacetate (FDA)/propidium iodide (PI 25/2.5 µl/ml^{-1} was prepared, the lamellae were stained for microscopic investigation. Biofilms were observed at 2, 6, and 24 h using laser scanning confocal microscopy.

Statistical analysis

IBM SPSS for Windows, Version 21.0 (SPSS, IBM Corp., Armonk, NY, USA) was used for statistical analysis. Student's t-test was used for normal distribution, and the Mann-Whitney U test was used for non-parametric distribution of continuous variables. Wilcoxon Marked Rank Test was performed as a "non-parametric alternative of dependent two-sample t-test," comparing the two means of the same sample to investigate the significance of the difference of the pre-test and post-test scores of the groups. Statistical significance was set at $P < 0.05$.

Ethical approval

All the protocols adhered to the ethical guidelines outlined in the Declaration of Helsinki. This study was approved by the Ethics Committee of Istanbul University and the National Research Committee (approval no: 2017-1414, dated December 8, 2017). Considering the retrospective nature of the study, the requirement for informed consent was waived.

RESULTS

Nine patients were included in the study. The mean age was 57.6 years (range, 20-93 years), and five patients (55.5%) were male. *K. pneumoniae* isolates were obtained from the blood of four patients, the sputum of three patients, the wound area of one patient, and the urine of one patient. Four patients were followed up for bacteremia, three for ventilator-associated pneumonia, one for surgical area infection, and the others for complicated urinary tract infection. The mean length of hospital stay time was 50.4 days (4-80 days). Seven patients (77.7%) died in a mean of nine days (4-21 days) of treatment initiation, although broad-spectrum antibiotics were administered.

Antibiotic susceptibility tests and biofilm formation

All *K. pneumoniae* isolates were defined as PDR isolates because they were resistant to all agents in **Table 1**. The gene responsible for carbapenem resistance in all isolates, identified using the PCR method, was OXA-48, and both OXA-48 and NDM-1 were detected in only one strain (no. 12). The evaluation of all *K. pneumoniae* isolates revealed a biofilm-forming ratio of 100%.

Quantification of biofilm formation using the colony counting method

The results for all the isolates are shown in **Table 2**. 2 µg/mL NAC was administered to bacterial biofilms (24 h), and its effects at

Table 1. The minimum inhibitory concentration values and genes responsible for carbapeneme resistance in *K. pneumoniae* isolates (n = 9), tested using the Gradient Test and Fluid Micrudilution methods

Antibiotic susceptibility test	Antibiotic	Isolate no								
		4	6	7	8	9	12	13	14	15
Gradient test	Meropenem	> 32	> 32	> 32	24	> 32	> 32	16	32	> 32
	Imipenem	32	24	12	8	> 32	> 32	12	8	8
	Ertapenem	> 32	> 32	> 32	> 32	> 32	> 32	24	> 32	> 32
	Amikacine	24	64	64	48	96	> 256	32	48	> 256
	Gentamicin	32	8	12	48	64	> 256	16	16	> 256
	Ciprofloxacine	> 32	> 32	> 32	> 32	> 32	> 32	> 32	> 32	> 32
	Levofloxacine	> 32	> 32	> 32	> 32	> 32	> 32	> 32	> 32	> 32
	Ceftriaxone	> 32	> 32	> 32	> 32	> 32	> 32	> 32	> 32	> 32
	Ceftroline	> 32	> 32	> 32	> 32	> 32	> 32	> 32	> 32	> 32
	Piperacillin-tazobactam	96	64	64	64	64	256	64	128	256
	Cefepime	> 256	> 256	> 256	> 256	> 256	> 256	> 256	> 256	> 256
	Aztreonam	64	96	96	128	128	128	> 256	32	> 256
	Chloramphenicol	64	64	64	128	96	96	64	128	96
	Trimethoprim-sulphamethoxazole	> 32	> 32	> 32	> 32	> 32	> 32	> 32	> 32	> 32
	Rifampicin	> 32	> 32	> 32	> 32	> 32	> 32	> 32	> 32	> 32
Fluid microdilution	Tigecycline	2	2	1	2	1	4	1	1	1
	Colistin	4	8	64	64	32	32	4	8	16
Agar dilution	Fosfomycin	125	125	256	> 256	> 256	> 256	> 256	256	125

Table 2. The logarithmic colony forming units of N-acetyl Cysteine, rifampicin, and ozone-treated basal biofilm layers, calculated using the colony counting method for each isolate at 2 h, 6 h, 24 h, and 72 h compared with their controls

Isolate no	Difference at 2 h	Result	Difference at 6 h	Result	Difference at 24 h	Result	Difference at 72 h	Result	Antibiofilm agent (mg/L)
	log_{10} CFU/mL		log_{10} CFU/mL		log_{10} CFU/mL		log_{10} CFU/mL		
4/4 C	0.17	–	0.03	–	1.49	–	0.04	–	NAC (2)
	0.7	–	0.52	–	0.02	–	0.52	–	RIF (0.1)
	0.14	–	0.8	–	0.52	–	0.42	–	Ozone (0.6)
	0.06	–	0.49	–	0.06	–	2.06	+	Ozone (4. 78)
6/6 C	1.27	–	0.12	–	0.14	–	0	–	NAC (2)
	1.1	–	0.66	–	0.3	–	0.12	–	RIF (0.1)
	0.05	–	0.06	–	0.11	–	0.03	–	Ozone (0.6)
	0.11	–	0.06	–	1.27	–	2.4	+	Ozone (4. 78)
7/7 C	1.85	–	0.6	–	0.24	–	1	–	NAC (2)
	0.05	–	0.21	–	0.12	–	0.43	–	RIF (0.1)
	0.52	–	0.34	–	0.79	–	0.85	–	Ozone (0.6)
	0.34	–	0.1	–	0.92	–	2.07	+	Ozone (4. 78)
8/8 C	1.05	–	0.11	–	0.03	–	0.46	–	NAC (2)
	0.06	–	0.2	–	0.43	–	0.31	–	RIF (0.1)
	0.85	–	0.08	–	0.22	–	0.45	–	Ozone (0.6)
	0.28	–	0.21	–	0.17	–	4.08	+	Ozone (4. 78)
9/9 C	1.3	–	0.39	–	1.23	–	0.69	–	NAC (2)
	0.86	–	0.06	–	0.07	–	0.46	–	RIF (0.1)
	0.58	–	0.14	–	0.1	–	0	–	Ozone (0.6)
	0.39	–	0.11	–	0.59	–	3.4	+	Ozone (4. 78)
12/12 C	0.29	–	1	–	0.41	–	0.71	–	NAC (2)
	0.72	–	0.1	–	0.14	–	0.28	–	RIF (0.1)
	0.27	–	0.12	–	0	–	0.56	–	Ozone (0.6)
	0.05	–	0.45	–	1.07	–	2.15	+	Ozone (4. 78)
13/13C	0	–	0.47	–	0.78	–	0.39	–	NAC (2)
	0.48	–	0.59	–	0.86	–	0.31	–	RIF (0.1)
	1.9	–	1.22	–	0.08	–	0.85	–	Ozone (0.6)
	2.1	–	0.81	–	0.05	–	3.16	+	Ozone (4. 78)
14/14 C	0.1	–	0.4	–	0.37	–	0.18	–	NAC (2)
	0.88	–	0.87	–	0.52	–	1	–	RIF (0.1)
	0.9	–	1.46	–	0.88	–	0.13	–	Ozone (0.6)
	1.65	–	0.13	–	0.04	–	3.12	+	Ozone (4. 78)
15/15 C	0.4	–	0.96	–	0.28	–	0.21	–	NAC (2)
	0.06	–	0.26	–	0.95	–	0.03	–	RIF (0.1)
	0.08	–	0.7	–	0.59	–	0.02	–	Ozone (0.6)
	0.04	–	0.15	–	0.2	–	3.05	+	Ozone (4. 78)

C = Control; (+) = Effective; (–) Ineffective; CFU = colony-forming units; NAC = N-acetyl Cysteine; RIF rifampicin.

2, 6, 24, and 72 h were investigated. No significant decrease was detected in the bacterial count of all isolates compared to their controls (all $P > 0.05$). Similar to NAC, 0.1 μg/mL rifampicin was administered to bacterial biofilms (24 h), and no significant decrease was detected in the bacterial count at 2, 6, 24, and 72 h compared to the controls (all $P > 0.05$).

The efficacy of 0.6 μg/mL and 4.78 μg/mL ozonated water was evaluated on the biofilm layer. When ozonated water of 0.6 μg/mL was administered, a statistically significant decrease in bacterial count was observed at 6 h ($P < 0.05$) and 24 h ($P < 0.05$) compared to the control group. However, it was concluded to be ineffective as no logarithmic decrease of 2 log_{10} or greater was observed. However, no significant decrease was detected in the bacterial count at 2 and 72 h (all $P > 0.05$).

Although a statistically significant decrease was detected (all (all $P < 0.05$) in the bacterial count at 2 and 24 h with 4.78 μg/mL ozonated water, it was considered ineffective, as no decrease over 2 log_{10} was detected. Interestingly, no statistically significant decrease was detected at 6 h at the same concentration, but at 72 h, there was a decrease between 2.06-4.08 (log^{10}) in the bacterial count in all isolates, and it was considered effective.

Demonstrating live and dead/inactive bacteria in the biofilm using laser scanning confocal microscopy

The results for all isolates are shown in **Table 3**. The dead and live bacteria 24 h after biofilm formation by strain no. 7 is shown in **Figure 1** using the laser scanning confocal microscopy method. The effects on strain 8 at 2, 6, and 24 h after ozonated water treatment (4.78 µg/mL) are shown in **Figure 1**. Significant decreases were observed on the living (P = 0.05) and death bacterial count (P = 0.01) when using ozonated water at hour 24 h. However, ozonated water did not achieve $\geq 2 \log_{10}$ decrease and was accepted as ineffective.

Table 3. The logarithmic cell counts of live and dead cells of each isolate at 2 h, 6 h, and 24 h compared with their controls treated with N-acetyl Cysteine, rifampicin, and ozone, evaluated using the laser scanning confocal microscopy

Isolate n	Difference at h 2 $\log_{10}$	Result	Difference at h 6 $\log_{10}$	Result	Difference at h 24 $\log_{10}$	Result	Antibiofilm agent (mg/L)
4/4C	0.02 cell/mL	–	0.33 cell/mL	–	0.38 cell/mL	–	NAC_{dead} (2)
	0.87 CFU/mL	–	0.78 CFU/mL	–	0.19 CFU /mL	–	NAC_{alive} (2)
	0.4 cell/mL	–	0.14 cell/mL	–	0.12 cell/mL	–	RIF_{dead} (0.1)
	0.5 CFU/mL	–	0.96 CFU/mL	–	0.02 CFU /mL	–	RIF_{alive} (0.1)
6/6 C	0.11 cell/mL	–	0.47 cell/mL	–	0.36 cell/mL	–	NAC_{dead} (2)
	0.14 CFU/mL	–	0.67 CFU/mL	–	0.24 CFU /mL	–	NAC_{alive} (2)
	0.67 cell/mL	–	0.36 cell/mL	–	0.12 cell/mL	–	RIF_{dead} (0.1)
	0.43 CFU/mL	–	0.59 CFU/mL	–	0.4 CFU /mL	–	RIF_{alive} (0.1)
7/7 C	0.17 cell/mL	–	0.02 cell/mL	–	0.21 cell/mL	–	$Ozone_{dead}$ (4.78)
	0.03 CFU/mL	–	0.08 CFU/mL	–	0.47 CFU /mL	–	$Ozone_{alive}$ (4.78)
	0.5 cell/mL	–	0.6 cell/mL	–	0.28 cell/mL	–	NAC_{dead} (2)
	0.43 CFU/mL	–	0.08 CFU/mL	–	0.06 CFU /mL	–	NAC_{alive} (2)
	0.48 cell/mL	–	0.63 cell/mL	–	0.23 cell/mL	–	RIF_{dead} (0.1)
	0.22 CFU/mL	–	0.02 CFU/mL	–	0.63 CFU /mL	–	RIF_{alive} (0.1)
8/8C	0.19 cell/mL	–	0.02 cell/mL	–	0.54 cell/mL	–	NAC_{dead} (2)
	0.39 CFU /mL	–	0 CFU/mL	–	0.1 CFU /mL	–	NAC_{alive} (2)
	0.06 cell/mL	–	0.37 cell/mL	–	0.55 cell/mL	–	RIF_{dead} (0.1)
	0.13 CFU /mL	–	0.03 CFU/mL	–	0.38 CFU /mL	–	RIF_{alive} (0.1)
9/9C	0.12 cell/mL	–	0 cell/mL	–	0.23 cell/mL	–	NAC_{dead} (2)
	0.14 CFU /mL	–	0.01 CFU/mL	–	0.45 CFU /mL	–	NAC_{alive} (2)
	0.4 cell/mL	–	0.44 cell/mL	–	0.24 cell/mL	–	RIF_{dead} (0.1)
	0.14 CFU /mL	–	0.83 CFU/mL	–	0.02 CFU /mL	–	RIF_{alive} (0.1)
12/12C	0.53 cell/mL	–	0 cell/mL	–	0.16 cell/mL	–	$Ozone_{dead}$ (4.78)
	0.06 CFU /mL	–	0.09 CFU/mL	–	0.52 CFU /mL	–	$Ozone_{alive}$ (4.78)
	0.68 cell/mL	–	0.23 cell/mL	–	0.33 cell/mL	–	NAC_{dead} (2)
	0.19 CFU /mL	–	0.09 CFU/mL	–	0.3 CFU /mL	–	NAC_{alive} (2)
	0.7 cell/mL	–	0.36 cell/mL	–	0.57 cell/mL	–	RIF_{dead} (0.1)
	0.32 CFU /mL	–	0.11 CFU/mL	–	0.30 CFU /mL	–	RIF_{alive} (0.1)
13/13 C	0.73 cell/mL	–	0.41 cell/mL	–	0.52 cell/mL	–	NAC_{dead} (2)
	0.15 CFU /mL	–	0.13 CFU/mL	–	0.33 CFU /mL	–	NAC_{alive} (2)
	0.4 cell/mL	–	0.37 cell/mL	–	0.29 cell/mL	–	RIF_{dead} (0.1)
	0.5 CFU /mL	–	0.3 CFU/mL	–	0.51 CFU /mL	–	RIF_{alive} (0.1)
14/14 C	0.03 cell/mL	–	0 cell/mL	–	0.16 cell/mL	–	NAC_{dead} (2)
	0.33 CFU /mL	–	1.19 CFU/mL	–	0.64 CFU /mL	–	NAC_{alive} (2)
	0.27 cell/mL	–	0.79 cell/mL	–	0.39 cell/mL	–	RIF_{dead} (0.1)
	0.45 CFU /mL	–	0.75 CFU/mL	–	0.43 CFU /mL	–	RIF_{alive} (0.1)
15/15 C	0.06 cell/mL	–	0.09 cell/mL	–	0.2 cell/mL	–	$Ozone_{dead}$ (4.78)
	0.03 CFU /mL	–	0.15 CFU/mL	–	0.15 CFU /mL	–	$Ozone_{alive}$ (4.78)
	0.82 cell/mL	–	0.41 cell/mL	–	0.64 cell/mL	–	NAC_{dead} (2)
	0.13 CFU /mL	–	0.23 CFU/mL	–	0.45 CFU /mL	–	NAC_{alive} (2)
	0.76 cell/mL	–	0.42 cell/mL	–	0.74 cell/mL	–	RIF_{dead} (0.1)
	0.24 CFU /mL	–	0.71 CFU/mL	–	0.13 CFU/mL	–	RIF_{alive} (0.1)

C = Control; (+) = effective; (–) = ineffective; NAC = N-acetyl Cysteine; RIF = rifampicin.

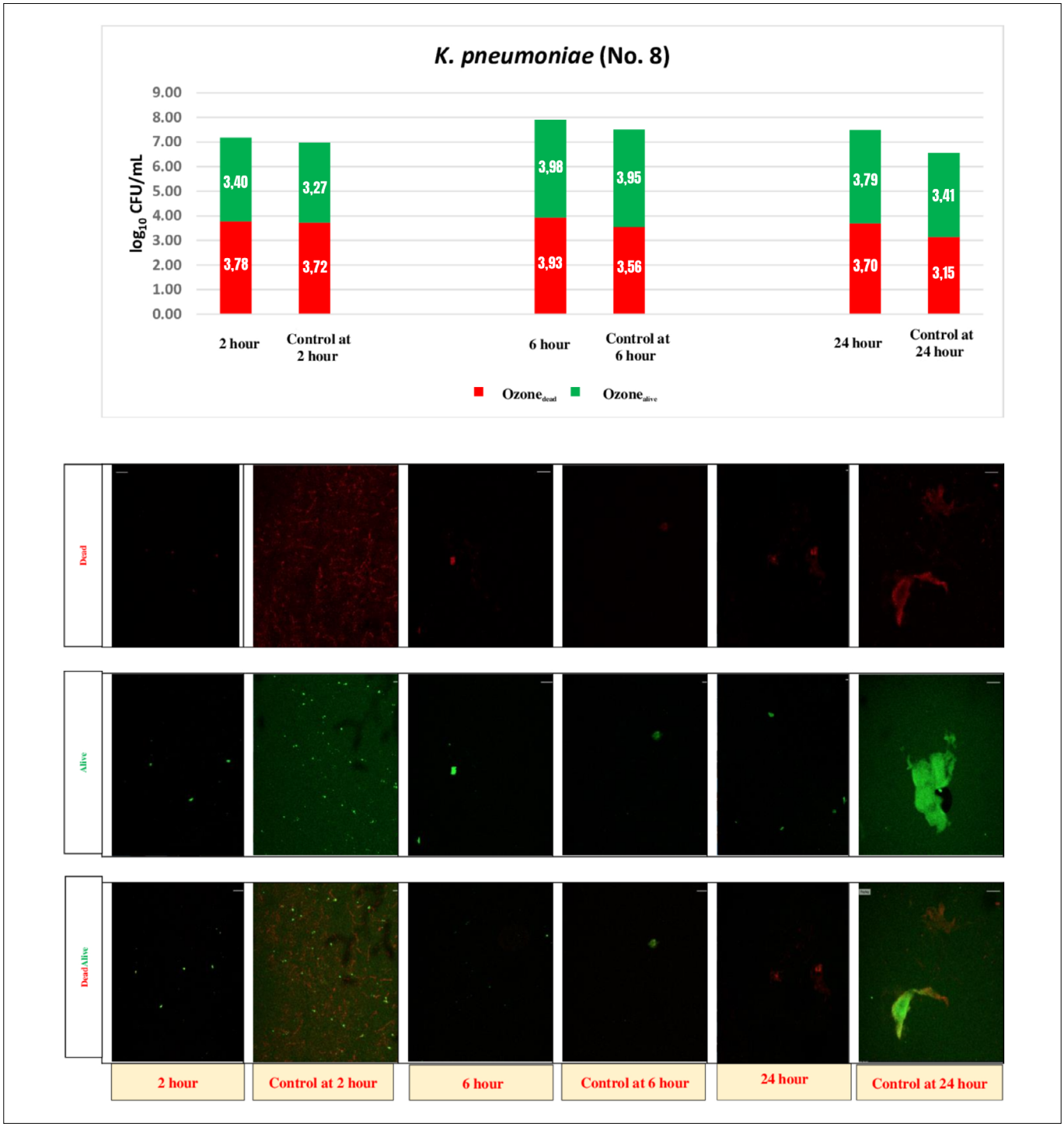

Figure 1. The effects of ozone at 2 h, 6 h, and 24 h on the biofilm of the *K. pneumoniae* strain no. 8. Cell counts and images of live and dead bacteria in the biofilm of the *K. pneumoniae* strain no. 8 treated with 4.78 µg/mL ozonated water and the untreated controls calculated using laser scanning confocal microscopy (Red: dead bacteria. Green: alive bacteria).

DISCUSSION

The biofilm-forming ability of nosocomial opportunistic microorganisms such as *K. pneumoniae* on tissue surfaces is a critical stage in the development of infection. Therefore, it is necessary to obtain detailed information on biofilm formation and biofilm-forming bacteria for the treatment of associated infections.

Copur et al.[15] showed that the most commonly used meropenem and colistin combination did not affect the planktonic and

biofilm forms of *K. pneumoniae* isolates; therefore, meropenem and colistin are not appropriate options for the treatment of *K. pneumoniae*-related infections.

Extracellular polysaccharide (EPS) production increases, particularly at 72 h, as the biofilm becomes older. Diago-Navarro et al.[16] showed a correlation between the antibiotic resistance profile and biofilm-forming ability by demonstrating the mucoid phenotype of *K. pneumoniae* isolates (n = 40). Singla et al.[17] reported that the production of the polysaccharide component of the matrix increased in younger biofilms compared to older biofilms, which might be responsible for antibiotic resistance. The results of the same study indicated that an increase in EPS production made the older biofilm resistant to antibiotics, and early initiation of antibiotics to bacteria in the biofilm is more effective.

Various studies have demonstrated that irreversible adhesion to different surfaces, such as catheters and implants, develops within 20 min to 4 hours, [18-20,] and biofilm develops within a short time, such as 24 hours.[21,22]

NAC is produced from cysteine residues. NAC has been reported to decrease biofilm formation in various bacteria *in vitro*. In addition, NAC disintegrates biofilm and prevents biofilm formation by reducing EPS production.[23,25] However, it was found to be ineffective in our study.

Rifampicin has been shown to inhibit the synthesis of bacterial proteins against Gram-positive bacteria.[26] Furthermore, studies have investigated the efficacy of rifampicin on biofilms formed by Gram-positive bacteria.[27-29] Rifampicin has recently been used in antibiotic combination studies owing to the difficulties encountered in the treatment of PRKp infections. We found no studies on the efficacy of rifampicin on biofilms of gram-negative bacteria belonging to the *Enterobacteriaceae* family. Therefore, the effects of rifampicin on biofilms were investigated in this study. However, we found it to be ineffective.

Ozone is the trivalent (O_3) form of oxygen (O_2) formed in the atmosphere. Some possible explanations for the mechanisms of action are the production of peroxides by ozonolysis, the production or activation of reactive oxygen species, and increased expression of enzymes in cells that have antioxidant activity. The bacteria, fungi, and viruses in the infected tissues are killed by the higher oxygen levels in the tissues, healthy cells reproduce more rapidly, and a stronger immune response is obtained.

Ozone was proven to be useful as an antimicrobial agent in various areas such as medicine, agriculture, maritime, and food sectors.[30,31] The effects of different concentrations of ozone in dynamic and static cultures, in gas (0.1-20 ppm) and fluid (0.5 ppm), on different bacterial isolates were investigated.[32,34] In our study, the two different ozone concentrations used in static conditions were 0.6 mg/L for 15 min and 4.78 mg/L for 10 min. The treatment of 4.78 mg/L ozonated water was effective against each bacteria compared to their controls (2.06-4.08 $\log_{10}$ decrease) at 72 h. The effect of ozone clearly increased with an increase in ozone concentration. However, in this study, the concentration of 4.78 mg/L was the highest obtainable with the device used for ozonated water. Gürsoy et al.[35] established in their study on *E. coli* and *S. aureus* that the bacterial count reached zero at 40 min and 3 h, respectively, with an increase in ozone concentration. We suggest that the effect of ozone might be stronger, and more successful results could be obtained if we could obtain higher ozone concentrations (80-100 mg/L, etc.). However, this should be tested in future studies. Previous studies have used medical ozone at a concentration between 1 and 100 µg/mL (0.05–5% O_3) with a mixture of pure ozone and pure oxygen.[36]

Several studies have reported that the efficacy of ozone varies between new and older biofilms. Bialoszewski et al.[37] showed in their study that older biofilms at 48-72 hours are more sensitive to the bactericidal effect of ozonated water. Similar to other studies, we found that ozonated water was effective on 72 h old biofilm layer.[37,38]

Although a 2.06-4.08 $\log_{10}$ decrease was detected in the bacterial count with ozonated water, complete eradication could not be accomplished in our study. This could be because the ozone concentration was lower, all isolates showed a mucoid phenotype, and the bacterial load in the biofilm was significantly higher. The efficacy of ozone varies according to the bacterial count. Studies have shown that ozone is more effective on biofilms with a lower bacterial load. Gürsoy et al.[35] showed in their study that bacteria might be completely inhibited, particularly at 1.5×10^5 cfu/mL or lower.

In the present study, we obtained quantitative results of the live bacterial load and count in the biofilm using a culturing method, and the ratio of live to dead cells in the biofilm was determined using laser scanning confocal microscopy. Because both methods have advantages and disadvantages, we recommend a combination of both for such biofilm studies.

This is a preliminary study on *K. pneumoniae*. However, in the near future, we intend to conduct molecular genotyping.

CONCLUSION

The efficacy of NAC, rifampicin, and ozone for the treatment of PDR isolates of *K. pneumoniae* was tested at specific concentrations in the present study. The ozonated water at even 4.78 mg/L concentration was found to produce a ≥ 2 $\log_{10}$ decrease in bacterial count in biofilms. Our study is significant in that it suggests that effective clearance is possible at higher concentrations of ozone. The present study lays the foundation for future research as a preliminary study.

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Variability in the perception of palliative care and end-of-life care among hematology professionals from the same reference center in Bahia, Brazil: A descriptive cross-sectional study

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ABSTRACT

BACKGROUND: There are several illness-specific cultural and system-based barriers to palliative care (PC) integration and end-of-life (EOL) care in the field of oncohematology.

OBJECTIVES: This study aimed to investigate the variability in the perceptions of PC and EOL care.

DESIGN AND SETTING: A cross-sectional study was conducted in the Hematology Division of our University Hospital in Salvador, Bahia, Brazil.

METHODS: Twenty physicians responded to a sociodemographic questionnaire and an adaptation of clinical questionnaires used in previous studies from October to December 2022.

RESULTS: The median age of the participants was 44 years, 80% of the participants identified as female, and 75% were hematologists. Participants faced a hypothetical scenario involving the treatment of a 65-year-old female with a poor prognosis acute myeloid leukemia refractory to first-line treatment. Sixty percent of the participants chose to follow other chemotherapy regimens, whereas 40% opted for PC. Next, participants considered case salvage for the patient who developed septic shock following chemotherapy and were prompted to choose their most probable conduct, and the conduct they thought would be better for the patient. Even though participants were from the same center, we found a divergence from the most probable conduct among 40% of the participants, which was due to personal convictions, legal aspects, and other physicians' reactions.

CONCLUSIONS: We found considerable differences in the perception of PC and EOL care among professionals, despite following the same protocols. The study also demonstrated variations between healthcare professionals' beliefs and practices and persistent historical tendencies to prioritize aggressive interventions.

INTRODUCTION

A comprehensive and multidisciplinary approach is crucial in oncohematology. Oncohematologic patients often experience higher symptom burden, increased in-hospital mortality, and higher rates of complex care needs during the cancer-associated end-of-life (EOL) process.[1-3] Palliative care (PC) is uncommon in oncohematology. Although continuous combined care by an interdisciplinary provider team including physicians well-equipped for PC is effective, it is not usually considered.[1,4,5] Furthermore, limited research has been conducted to explore determinants of the variability in EOL care among healthcare professionals, especially in oncohematology practice.[6,7]

Hematologic malignancies consist of a heterogeneous group of diseases characterized by distinct patterns of illness progression, approaches to treatment and prospects for remission, impacting patients' requirements for PC and EOL support.[8-10] The "rollercoaster" nature of life experienced by individuals with these illnesses bring distinct physical and psychological challenges encompassing emotional pain and a multitude of symptoms.[11-14] Particularly in diseases such as acute myeloid leukemias, patients commonly have a high risk of serious complications and negative outcomes along with high possibility for positive outcomes.[11-14]

EOL is the final phase of patients' life when a cure is no longer possible, and the focus of care shifts towards providing comfort, symptom relief, and emotional support for patients and families.[8-10] Several barriers to PC integration and optimal EOL care exist among oncohematologic patients including illness-specificity, cultural differences, and system-based inhibitions.[15-17]

Adequate PC requires appropriate knowledge from the healthcare team, but frequently there are divergences in practice within the same staff, which may hinder the integration and efficiency of patients' clinical course.[1,3,15]

On a larger scale, PC plays an essential role in coordinated patient care, with the primary goal of promoting relief from physical, psychological, and spiritual suffering while providing family support throughout the disease course.[15,18] It is extremely important to consider combined care (PC associated with disease-modifying treatment) to enhance the quality of life and symptom control.[5,15,18]

Even among centers with experienced healthcare professionals and specialized PC teams, significant disparities exist in the clinical management and decision-making process.[4,19,20] A patient-centered approach aims to provide comfort and quality of life regardless of the possibility of a cure.[15,18] However, in the oncohematology field, there is a historical tendency to prioritize aggressive and curative interventions, often delaying PC strategies.[5,15-17] Therefore, a major challenge is finding a therapeutic balance to avoid overtreatment while bypassing premature treatment discontinuation that could lead to potentially avoidable deaths.[21,22]

Various reasons have been described as to why physicians tend not to promote EOL care discussions, for example, the perception of weakening the physician-patient relationship due to the restriction of curative measures, the loss of professional credibility, and the feeling of diminishing hope for the patient and family members.[5,23,24]Moreover, this process is often also dependent on what professionals believe is best for the patient based on their personal convictions about healthcare, PC, and EOL care, previous experiences, and cultural aspects.[5,23,24]

Thus, a growing awareness of the importance of combined care among oncohematologic patients has led to critical reflection on the conduct of healthcare professionals.[25,26] The involvement of the PC team in severe and refractory cases is especially relevant, considering that therapeutic options could generate significant effects that impact patients quality of life.[25,26] In such circumstances, it is necessary to investigate not only the concordance of conduct within the same healthcare team involved in the care of these patients, but also the presence of determinants of the variability in the establishment of PC and EOL care strategies.[27]

OBJECTIVE

This study aimed to investigate variability in the perception of PC and EOL care among 20 hematologists and hematology residents from the Hematology Division of the Universidade Federal da Bahia (UFBA) University Hospital.

METHODS

This cross-sectional web-based survey study was approved by the Ethics Committee of the Medical School of Universidade Federal da Bahia (UFBA) on August 8, 2022 (CAAE 61154522.0.0000.0049) and was conducted according to the Declaration of Helsinki and Good Clinical Practice guidelines (December 2, 2021, number: 12/155).

The hematology service of UFBA's University Hospital has been consolidated as the only hematology reference center in the state of Bahia, Brazil since the 1990s. This service includes annual curricular activities for three medical residency programs and dozens of medical school students. Since 2010, over 400 oncological treatments have been performed monthly at the University Hospital, and almost 50 bone marrow transplantations have been performed annually.

Fifteen hematologists and five hematology residents (four second-year residents and one first-year resident) from UFBA University Hospital were recruited from October 2022 to December 2022. We recruited 20 hematology team members who provided written informed consent and agreed to participate in the study. Participants were provided with a Google Forms electronic survey through a link sent to them via e-mail or WhatsApp Messenger. A reminder was sent to those who did not respond to the initial invitation to participate.

The questionnaire contained 28 questions, with an approximate completion time of 15 minutes. To submit the questionnaire, each participant was required to log into a Google account and submissions were limited to one per account. The questionnaire consisted of two sections that addressed the sociodemographic and clinical factors.

The first section addressed participants' personal, professional, and EOL educational characteristics. The participants were analyzed by age, sex, years since graduation in medical school, function in the service, work hours/week at the University Hospital, training in other medical specialties, whether or not participants had had PC and/or EOL classes during their medical education, whether or not they had had law or ethical classes regarding PC and/or EOL during their medical education, PC and/or EOL-themed articles read in the last two years, PC and/or EOL-themed events participated in the last two years, whether or not they had interest in discussing PC and/or EOL, self-attributed knowledge on PC and/or EOL, religiosity, belief in God, and the presence of children.

The second section adapted a questionnaire from a similar previously published study.[6] It consisted of two fictitious clinical cases constructed by the authors, followed by questions about prognosis and therapeutic possibilities, and the reasons that led the participants to make those decisions. A multidisciplinary team of six members, including palliative and support Care experts, hematologists, and nurses, reviewed the content of the survey to ensure interpretability and applicability to the hematology setting. Four rounds of discussion were necessary to reach

a consensus. The questionnaire was written in Portuguese, the official language of Brazil.

The case scenario presented in the second section described a 65-year-old female patient with a poor prognosis of acute myeloid leukemia refractory to first-line treatment. We analyzed the hypothetical conduct in the face of this scenario and two main elements of EOL care: Q1) whether the decision-making process was conducted using a multi-professional approach and Q2) what approach to life-sustaining therapies would be taken if the patient developed septic shock following chemotherapy. In the second question, two additional sub-options were prompted for selection: Q2a) the care clinicians are most likely to deliver, and Q2b) the care they think is best for the patient, given the exact same alternatives to choose from. If the participants selected different answers for 2a and 2b, they were further prompted to explain their reasoning by choosing one reason from a limited number of options.

The data were combined into a common database to ensure the coding and analysis procedures. Data normality was assessed using Kolmogorov-Smirnov and Shapiro-Wilk tests, descriptive statistical measures, and graphic analysis. Descriptive analyses included frequencies and percentages for categorical variables and means and standard deviations or medians and interquartile ranges for continuous variables. Because the study was a census, only descriptive statistics were necessary, considering that statistical inference to estimate population values from samples is trivial in this type of study. The Wilson score method without continuity correction was used to calculate the confidence interval for a proportion.[28] Statistical analyses were performed using SPSS 25.0 for Windows (Chicago, IL, USA).

Table 1. General characteristics of 20 respondents from the Hematology Division

Characteristics	Results
Age (years) *	44 (12)
Female sex	16 (80%)
Years since graduation †	16 (7-27)
Graduated hematologist	15 (75%)
Work hours/week in the University Hospital †	24 (24-54)
Another specialty	8 (40%)
PC and/or EOL classes	14 (70%)
Law and/or ethic classes on PC or EOL	14 (70%)
PC and/or EOL articles	
2-3 articles read/2 years	6 (30%)
4 or more articles read/2 years	8 (40%)
PC and/or EOL themed events	
2-3 articles events/2 years	5 (25%)
4 or more events/2 years	10 (50%)
Interest in discussing PC and/or EOL	20 (100%)
Self-attributed knowledge	
Regular	12 (60%)
Good	6 (30%)
Religiosity	9 (45%)
Belief in God	17 (85%)
Has children	10 (50%)

* mean (SD); † median (IQR); PC = palliative care; EOL = end of life; SD = standard deviation; IQR = interquartile range.

RESULTS

All professionals completed the questionnaire during the study period and consolidated it as a census. Eight physicians completed the questionnaire using hospital facilities in a private room at the University Hospital. They submitted the questionnaire anonymously and the remaining 12 completed it using private mobile devices. The primary characteristics of the 20 physicians are listed in **Table 1**.

Regarding hypothetical conduct in the face of the fictitious scenario, 12 participants (60%; 95%CI = 38% to 78%) chose to follow other chemotherapy regimens, while eight (40%; 95%CI = 21% to 61%) chose to implement PC, with or without transfusion support. A descriptive analysis of the characteristics of the two groups divided by chosen attitudes is shown in **Table 2**.

All 20 participants (100%) said they would discuss the care pathway with the patient and the multidisciplinary team (e.g., physicians, nurses, psychologists), but 18 (90%; 95%CI = 69% to 97%) said they would predominantly take into consideration the opinion of the patient when making decisions, while only two (10%; 95%CI = 2% to 30%) said they would predominantly take into account the opinion of the multidisciplinary team.

Participants were further asked to consider their conduct when a next-step chemotherapy regimen was adopted, and the patient developed febrile neutropenia, which progressed to septic shock, low peripheral oxygen saturation levels, and a low level of consciousness. Thirteen physicians (65%; 95%CI = 43% to 81%) chose to apply the full code (referral to the intensive care unit, antibiotic treatment, and invasive supportive measures such as cardiopulmonary resuscitation, orotracheal intubation, and hemodialysis, if necessary). However, 7 participants (35%; 95%CI = 18% to 56%) chose to withhold or withdraw treatment interventions. A descriptive analysis of the characteristics of the two groups divided by chosen attitudes is shown in **Table 3**.

When considering the next step of chemotherapy, 8 of the 20 respondents (40%; 95%CI = 21% to 61%) had disagreeing answers about the most likely treatment to be given and the believed to be the best approach for patients experiencing septic shock (**Figure 1A**). All eight participants (100%) believed that it would be better for the patient to limit invasive supportive measures and strategies

Table 2. Descriptive analysis of the initial approach to a 65-year-old female patient with poor prognosis acute myeloid leukemia refractory to first line treatment according to physicians' characteristics

Characteristics	Chosen attitude			
	Other chemotherapy regimen (n = 12)	(95%CI)[a]	Palliative care (n = 8)	(95%CI)[a]
Age (years) *	42 (11)	(35-49)	46 (13)	(35-57)
Female sex	8 (67%)	(39% - 86%)	8 (100%)	(67% - 100%)
Years since graduation †	15 (7-24)	(10-24)	20 (8-32)	(6-34)
Graduated hematologist	9 (75%)	(46% - 91%)	6 (75%)	(41% - 93%)
Work hours/week in the University Hospital †	25 (24-54)	(24-60)	24 (24-52)	(24-70)
PC and/or EOL classes	8 (67%)	(39% - 86%)	6 (75%)	(41% - 93%)
Law or ethic classes on PC and/or EOL	8 (67%)	(39% - 86%)	6 (75%)	(41% - 93%)
PC and/or EOL articles				
4 or more articles read/2 years	5 (42%)	(19% - 68%)	3 (37%)	(13% - 69%)
PC and/or EOL themed events				
4 or more events/2 years	6 (50%)	(25% - 74%)	4 (50%)	(21% - 78%)
Interest in discussing PC and/or EOL	12 (100%)	(75% - 100%)	8 (100%)	(67% - 100%)
Self-attributed knowledge				
Good	2 (17%)	(4% - 44%)	4 (50%)	(21% - 78%)
Religiosity	5 (42%)	(19% - 68%)	4 (50%)	(21% - 78%)
Belief in God	10 (83%)	(55% - 95%)	7 (87%)	(52% - 97%)
Has children	6 (50%)	(25% - 74%)	4 (50%)	(21% - 78%)

* mean (SD); † median (IQR);

[a] Wilson score method without continuity correction was used to calculate the confidence interval for a proportion.

PC = palliative care; EOL = end of life; SD = standard deviation; IQR = interquartile range.

Table 3. Descriptive analysis of the most likely approach for a 65-year-old female patient with poor prognosis acute myeloid leukemia refractory to first line treatment, experiencing septic shock following a next step chemotherapy regimen according to physicians' characteristics

Characteristics	Chosen attitude			
	Full code (n = 13)	(95%CI)[a]	Withholding/ withdrawal (n = 7)	(95%CI)[a]
Age (years) *	41 (11)	(34-47)	49 (13)	(37-61)
Female sex	10 (77%)	(49% - 91%)	6 (86%)	(48% - 97%)
Years since graduation †	15 (5-23)	(10-24)	23 (13-38)	(13-44)
Graduated hematologist	9 (69%)	(42% - 87%)	6 (86%)	(48% - 97%)
Work hours/week in the University Hospital †	24 (24-60)	(24-60)	24 (24-36)	(24-70)
PC and/or EOL classes	8 (61%)	(35% - 82%)	6 (86%)	(48% - 97%)
Law or ethic classes on PC and/or EOL	8 (61%)	(35% - 82%)	6 (86%)	(48% - 97%)
PC and/or EOL articles				
4 or more articles read/2 years	6 (46%)	(23% - 70%)	2 (29%)	(8% - 64%)
PC and/or EOL themed events				
4 or more events/2 years	8 (61%)	(35% - 82%)	2 (29%)	(8% - 64%)
Interest in discussing PC and/or EOL	13 (100%)	(77% - 100%)	7 (100%)	(64% - 100%)
Self-attributed knowledge				
Good	5 (38%)	(17% - 64%)	1 (14%)	(2% - 51%)
Religiosity	6 (46%)	(23% - 70%)	3 (43%)	(15% - 74%)
Belief in God	12 (92%)	(66% - 98%)	5 (71%)	(35% - 91%)
Has children	5 (38%)	(17% - 64%)	5 (71%)	(35% - 91%)

* mean (SD); † median (IQR);

[a] Wilson score method without continuity correction was used to calculate the confidence interval for a proportion.

PC = palliative care; EOL = end of life; SD = Standard deviation; IQR = Interquartile range.

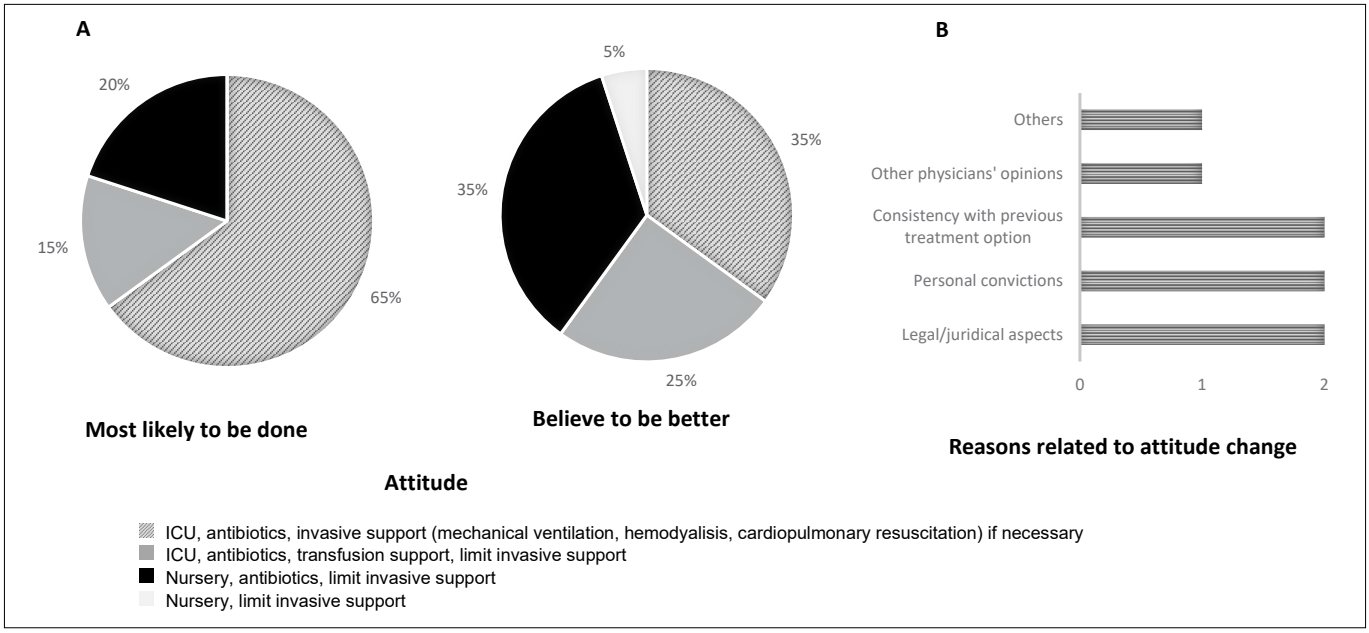

Figure 1. Most likely to be done and believed to be the best treatment for a 65-year-old female patient with poor prognosis acute myeloid leukemia refractory to first line treatment, experiencing septic shock following a next step chemotherapy regimen. **A** Shows the approach most likely to be done and the approach believed to be best for the patient. **B** Shows the reasons related to attitude change in approach of the 8 physicians (40%) that changed answers between the two questions. "Others" denotes one physician who answered that "it is hard to give up".

than they would choose. The number of participants who chose to apply the full code decreased from 13 (65%; 95%CI = 43% to 81%) to 7 (35%; 95%CI = 18% to 56%) comparing these two possibilities (**Figure 1A**). The reasons for the differences in these approaches are shown in **Figure 1B**.

DISCUSSION

PC in the oncohematology team is particularly pertinent when dealing with severe and refractory cases, as treatment interventions may profoundly affect patients' overall quality of life.[25,26] Furthermore, only a limited number of studies have investigated the impact of physicians' characteristics and their respective approaches in clinical practice.[7,29,30] Given that the research done on PC in an oncohematological clinical setting is scarce, our study is crucial and makes a significant contribution to a clinical environment that demands renewed analyses and heightened attention. This study will prompt clinicians to contemplate the underlying paradigms and representations that shape their professional interventions. This introspection is vital to facilitate the initiation of EOL discussions and promote the early integration of PC practices.

In our hypothetical case study, most participants opted to pursue alternative chemotherapy regimens, and a minority opted to pursue PC interventions with or without transfusion support. This highlights the prevailing inclination to explore non-PC options, aligning with the existing literature on this subject.[16,31] We also found a small disparity in the number of years since graduation between those who opted for PC and those who chose alternative chemotherapy regimens. The former group had completed a median of 20 years (interquartile range 8-32) since graduation, whereas the latter group had completed a median of 15 years (interquartile range 7-24). This discrepancy suggests a tendency among more experienced professionals to lean towards PC as an approach.

Furthermore, it is crucial to emphasize the percentage of participants who self-reported their level of knowledge regarding the respective treatment options. Only 17% of those who chose alternative chemotherapy regarded themselves as having a good understanding, whereas 50% of those who opted for PC self-attributed a higher level of knowledge in this area. Additionally, in the other scenario of chemotherapy, a higher percentage of participants (86%) who chose withholding/withdrawal options claimed to have attended both PC and/or EOL classes and law or ethics classes on PC and/or EOL within a span of two years than the group who opted for the full code treatment approach (61%). These findings shed light on the distinct profiles of each group.

In this study, all 20 participants said that they would discuss the care pathway with the patient and multidisciplinary team, which is not commonly seen in daily oncohematology practice in several healthcare services. Moreover, it is important to highlight that our sample comprised a substantial proportion of participants

with prior theoretical or practical exposure to PC and/or EOL care. This observation is contrary to the existing literature and highlights the uniqueness of our study population.[8,26,27] Furthermore, our study included a sample in which 100% of the participants expressed interest in engaging in discussions regarding PC and/or EOL. This high level of interest may be attributed to the fact that our study was conducted in a hospital setting that boasts of robust PC services. This service encompasses a multidisciplinary team that actively involves two palliative doctors in patient discussions within the Hematology Division.

A standout observation in our study was the presence of contrasting responses among nearly 50% of the participants when asked about the most likely course of action and approach they believed to be optimal for the patient. It is crucial to delve deeper into the underlying reasons for such discrepancies, as medical decisions should ideally be guided by evidence-based medicine and established protocols, rather than individual preferences or subjective considerations. This finding raises significant concerns, particularly within a specialized team, where the majority of physicians have received training within the same service and have access to the same patient cohort. Notably, even in the presence of a specialized PC team, the selected approaches varied significantly among nearly 50% of the participants. Considering the broader context of hematologists in cities, states, or countries, it is reasonable to anticipate a greater divergence in clinical practice.

This study has few limitations. First, owing to the cross-sectional design, it was not feasible to establish a causal relationship between the results obtained from the census sample and the observed differences in the percentages and standard deviations. Second, the sample size of 20 respondents is insufficient to provide a reliable estimate for a larger population. This limitation further accounts for the observed differences in percentages, means, and standard deviations. Therefore, it is crucial to evaluate the heterogeneity of hematologists' practices in PC and EOL care within larger populations to establish potential correlations between physician characteristics and their respective approaches. Also, this study employed single-scenario control within a tertiary hospital that already employed a specialized PC team. Consequently, the generalizability of these findings to other healthcare settings is a challenge. Further investigations involving diverse and representative populations are required to confirm the applicability of these results. In addition, to mitigate potential confounding factors and emphasize the impact of physician characteristics, we deliberately utilized a simplified case scenario, excluding the pivotal roles that patients, families, and surrogates may play in shaping the provision of care.

The strengths of the study include a remarkable 100% response rate which is comparatively higher than what could be achieved in a multicenter study. This is a pilot study and is significantly important as an early contributor to the initiation and fostering of discussions in the field of oncohematology. Further, the questionnaire underwent meticulous piloting and refinement guided by expert opinions to ensure its robustness and reliability. Moreover, the anonymous nature of the questionnaire deserves special mention as it likely fostered an environment conducive to candid responses, particularly given that this topic remains relatively underrepresented and is not widely disseminated in Brazil or globally.

Nonetheless, there has been relatively limited research investigating the impact of physician characteristics on clinical practice.[7,29,30] Both personal and professional attributes, such as sex, years since graduation, work hours, and even religious beliefs, have been linked to disparities in EOL decision-making.[32-35] Research conducted in the oncology field has revealed correlations between physicians' educational credentials and their performance in providing EOL care to patients in critical stages, although a specific lack of research exists in the context of oncohematology.[7,8,16] These criteria have emerged as a crucial perspective on the variability of PC and EOL care decisions in hematology.[7,8,16,29]

We found it essential to recognize the potential for discovering and investigating further associations. Such investigations hold immense importance in the existing literature, as they have the potential to identify modifiable factors that can effectively enhance the quality of PC and EOL care within the oncohematology setting. For instance, the identification of ethical and legal considerations in PC or EOL may lead to significant changes in clinical practices, especially when considering the "rollercoaster" nature of evolution of oncohematology, with diseases that present high chances of cure and at the same time strong odds of death or intensive deterioration.[11-13] These strategic findings can subsequently be implemented within healthcare services, along with the potential influence of sociodemographic factors. Therefore, preliminary studies are crucial to reach these significant milestones. This study can be a benchmark for extensive investigations involving larger sample sizes that better represent a larger physician population, to propose new PC models and more specialized clinical and intra-team approaches.[7,29,30]

CONCLUSION

Our study highlights marked differences in the perceptions of PC and EOL care among hematology professionals within the same center. These disparities may be driven by personal beliefs, previous educational experience with PC and EOL care, and ethical considerations. Physicians' personal beliefs and knowledge levels may influence their approaches, although all participants in our study expressed interest in PC and EOL care discussions. The study also demonstrated variations between healthcare professionals' beliefs and practices and persistent historical tendencies to prioritize aggressive interventions in the oncohematology

scenario. Further research is needed to understand how physician characteristics affect the perceptions of PC and EOL care among patients with hematological malignancies, paving the way for improved care practices for their patients.

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Physical condition and perceived fatigue in post-covid patients: An observational descriptive study

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ABSTRACT

BACKGROUND: Patients with severe coronavirus disease 2019 (COVID-19) often require hospital admission and experience sequelae such as chronic fatigue or low muscle mass.

OBJECTIVE: To analyze the functional capacity of a cohort of patients with severe acute respiratory syndrome coronavirus 2 who required hospitalization.

DESIGN AND SETTING: An observational descriptive study was conducted on post-COVID-19 patients referred to the Rehabilitation Department of Gregorio Marañón Hospital (Madrid, SPAIN).

METHODS: Cardiorespiratory fitness, muscle strength, body composition, and perception of fatigue and dyspnea were analyzed. Furthermore, the existing correlations between clinical variables and physical conditions were analyzed.

RESULTS: Forty-two patients who required hospital admission (80 ± 22.45 days) or intensive care unit (ICU) admission (58 ± 10.52 days) were analyzed. They presented with decreased strength, respiratory capacity, and moderate-to-severe perceived fatigue. Additionally, an inverse correlation was found between right-handgrip strength and days in the ICU, as well as the 6-minute walk test for women. Similarly, strength and fitness were negatively associated with perceived fatigue.

CONCLUSIONS: Post-COVID-19 patients showed low muscle function and low levels of physical fitness associated with high perceived fatigue.

INTRODUCTION

Coronavirus disease 2019 (COVID-19) is a viral infection caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The disease causes severe acute respiratory syndrome in 75% of patients and induces acute systemic inflammation,[1] which requires 26% of patients admitted to the intensive care unit (ICU).[2]

Skeletal muscle-related symptoms are common in both acute illness and post-acute sequelae of COVID-19,[3] among which chronic fatigue syndrome stands out, in part due to the significant reductions in muscle mass described after spending 7–20 days hospitalized in the ICU.[4] In a recent study, patients admitted to the ICU for severe COVID-19 showed a 30% decrease in the cross-sectional area of the Rectus Femoris after 10 days.[5] Many works have also described significant reductions in the maximum isometric strength of the Quadriceps and Biceps Femoris.[6]

These data are consistent with those of the first SARS-CoV-2 epidemic almost two decades ago. Patients hospitalized with this infection reported decreases of 32% in maximum isometric grip strength and 13% in the distance walked on the 6-minute walk test (6 MWT) several months after hospital discharge.[7,8] In patients surviving critical illnesses other than SARS-CoV-2, who had suffered an extended hospitalization, significant muscle mass and strength losses were detected, possibly due to a reduction in protein synthesis, an increase in muscle breakdown, and systemic inflammation, the latter caused by the so-called cytokine storm, the same one caused by COVID-19, leading to a drastic rise in protein catabolism.[9] These findings were reported even five years after patients had been released from the hospital.[10] In this frame, ICU-acquired weakness developed above all in patients with more severe disease involvement,[11,12] which are also those with the highest levels of inflammatory markers.[13] Some of these biomarkers, such as C-reactive protein (CRP), interleukins (IL) such as IL-6, IL-1b, or tumor growth factor (alpha-TNF), can directly induce muscle proteolysis and decreased protein synthesis, especially when

the inflammation comes from the lung.[14] Other causes associated with muscle protein catabolism come from the nutritional status and rest associated with the disease.[3]

Performing early rehabilitation to recover muscle mass is of interest. To this end, physical strength exercise should be an essential component.[15] In this sense, in a randomized clinical trial with 133 patients affected by SARS-CoV, in which a 6-week mixed strength and cardiorespiratory exercise program was carried out, with 4–5 weekly sessions of 60–90 minutes, the patients increased their isometric grip strength by 17%, shoulder flexion strength by 38%, and hip extension strength by 250%. Similar programs could benefit patients affected by COVID-19.[8] However, currently, most studies on post-COVID-19 rehabilitation are quasi-experimental studies based on daily cardiorespiratory-type exercises, achieving significant improvements in the 6 MWT, which is associated with decreased perceived fatigue.[16]

Comparing patients admitted to the ICU for COVID-19 with patients admitted to the ICU for other reasons, the former presented higher levels of CRP and a direct correlation between inflammation, lung injury, and muscle breakdown.[17] Patients with multiorgan involvement undergo higher muscle degradation. This systemic involvement is more frequent than that in other types of patients admitted to the ICU because intubated patients experience a more significant inflammatory response in the airways, which can cause inflammation in virtually every organ of the body.[18]

Another noteworthy fact is that elderly patients, who are the majority of patients severely affected by COVID-19, already have a situation of underlying muscle breakdown and systemic inflammation, even suffering from sarcopenia.[19] Excess myokines and adipokines secreted by sarcopenic muscle stimulate oxidative stress and directly induce multiorgan damage, including skeletal muscle.[20] Patients hospitalized for COVID-19 with pre-existing sarcopenia take twice as long to be discharged from the hospital and have a mortality rate eight times higher than those who do not suffer from it.[21]

OBJECTIVE

Given the data previously expressed and the existing gaps regarding functional capacity and fatigue in this type of patient, the objective of the present study was to analyze and describe the functional capacity of a cohort of patients infected with SAR-Cov-2 requiring hospitalization during the 1st and 2nd waves of the pandemic in Spain. Additionally, differences according to sex and possible associations between body composition, physical condition, perception of fatigue, and length of hospitalization were analyzed.

METHODS

A descriptive observational study was conducted. The data were obtained during the first six months of 2021 from the Rehabilitation Service. The study protocol was adjusted to the Helsinki Declaration Ethical Guidelines, whose last modification was written in 2013 and approved by the Clinical Research Ethics Committee of the hospital (Reference 26/2020 date 11/30/2020). The reference guide for manuscript preparation was the Strengthening the Reporting of Observational Studies in Epidemiology.[22] All patients provided signed informed consent to participate in the study.

Participants

Non-probabilistic convenience sampling was performed, where all patients referred by their pulmonologist or internist to the hospital's rehabilitation service were invited to participate. The inclusion criteria were as follows: (i) older than 18 years, (ii) having passed the SARS-CoV-2 infection with negative PRC, and (iii) admitted to the hospital between March 2020 and May 2021. The exclusion criteria were as follows: (i) patients with severe psychiatric illnesses, poor comprehension skills, or severe cognitive impairment; (ii) patients with contraindications for physical activity (acute myocarditis, uncontrolled arterial hypertension, arrhythmias, acute thrombosis, and infection); (iii) patients who revoked the informed consent signature due to not having an interest in participating.

Between March 2020 and May 2021, the Rehabilitation Service received 73 patients, 52 of whom were eligible for inclusion in the study. Of these 52 patients, five withdrew from the study. Therefore, 47 patients were included in the functional assessments and questionnaires, of which five did not complete the assessments; therefore, 42 patients were finally analyzed.

Variables

Clinical History

Clinical variables were collected, such as age, date of hospital admission and discharge in the internal medicine ward, days of admission of those patients who were in the ICU; type of mechanical ventilation provided during hospitalization: (a) orotracheal intubation (OTI) or (b) non-invasive mechanical ventilation with high flow nasal cannula oxygen therapy (HFNOC); reason for admission: (a) due to bilateral SARS-CoV-2 pneumonia, (b) due to unilateral SARS-CoV-2 pneumonia, or (c) without pneumonia.

Assessment of cardiorespiratory capacity

An indirect test to estimate maximum oxygen consumption (VO2max) was performed to assess cardiorespiratory fitness using the 6 MWT according to the guidelines of the American Thoracic Society (ATS).[23] The test consisted of walking as fast as possible (without running) for 6 min on a flat corridor over a

distance of 20 m. Heart rate (HR) and oxygen saturation (SpO2) were recorded with a pulse oximeter (Agptek. Brooklyn, USA) at four times: (1) at rest just before starting the test, (2) immediately at the end of the test, (3) two minutes after recovery began, and (4) five minutes after recovery began.

Assessment of muscle function

Two tests were used to estimate the neuromuscular performance: 1) Manual Handgrip (Jamar. Chicago, USA) in the upper body, the patient was seated on a chair with the elbow at 90°, the dynamometer was pressed with maximum force, and the value obtained was recorded. This procedure was performed twice, and the average values for both the right and left hands were recorded. In addition, the patient's dexterous hand was recorded.[24] 2) The Chair Stand Test (30sCST) in the lower body: the patient was seated in a chair to stand and sit as many times as possible in 30 s, ideally without support, unless the patient needed it. The type of support provided was recorded.[25]

Body Composition Assessment

Body composition data such as body weight (kg), body mass index (BMI) (kg/m2), fat mass (%), and fat-free mass (kg) were measured using a bioelectrical impedance balance (Tanita Ironman InnerScan BC-545N. Amsterdam, the Netherlands). Furthermore, the waist-to-hip ratio (WtHr) was measured using a tape measure (Rosscraft Anthro Tape. St. Paul, MA, USA) to assess the risk of developing cardiovascular diseases. This index is derived from the ratio between the waist circumference and hip circumference in centimeters, with reference values for both men and women.[26]

Self-reported questionnaires

Each patient was given five questionnaires: 1) Rapid Assessment Physical Activity (RAPA), measuring the level of physical activity, consisting of nine items, seven of which sought to determine if the patient met the minimum exercise recommendation[27] (30 minutes or more at least five days a week), assessing the frequency and intensity at which they performed these activities, and two additional items measuring whether the patient performed flexibility and strength exercises.[28] 2) The Brief Fatigue Questionnaire presents a numerical rating scale from 1 to 10, with 10 being the worst fatigue imaginable. It is divided into four items that rate the degree of fatigue perceived in 24 hours and six items that rate how much this fatigue interferes in different life situations.[29] 3) The SARC-F Scale, which assesses muscle weakness by determining the degree of sarcopenia of the patient, uses five items that rate the difficulty that the patient presents in performing actions that involve both the stability and the strength of their upper and lower limbs. If the total score was equal to or greater than 4 points, the presence of sarcopenia was considered.[30] 4) Dyspnea analog scale, in which the patient must assess the perception of breathlessness graphed in drawings and numbers, where 0 = absence of breathlessness except when exercising; 1 = I choke when walking too fast or going up a slight hill; 2 = I choke when walking on the flat at the same pace as other people my age or I have to stop to rest; 3 = The choking forces me to stop before 100 meters or after a few minutes when walking on a flat ground; 4 = I choke when making daily efforts such as getting dressed or leaving the house, and I have to stop.[31]

Assessment protocol

During consultation with a rehabilitation doctor, the patient signed an informed consent form to participate in the study. Subsequently, the patient was scheduled to undergo physical tests and complete the questionnaires. On the appointment day, the patient answered self-reported questionnaires and his body composition was assessed, followed by muscle function tests, ending with the cardiorespiratory fitness test.

Statistical analysis

Data were collected in one notebook per patient and codes were assigned. After data collection, a database was created for further analysis. First, the Shapiro–Wilk test was performed to determine whether the variables were parametric or nonparametric. Descriptive statistics were performed, presenting data of mean and standard deviation (Mean ± SD) of all variables that determine the characteristics of the sample. Furthermore, for the following variables: (i) reason for admission, (ii) mechanical ventilation provided, (iii) estimated maximum oxygen consumption, (iv) Waist-to-Hip Ratio, (v) BMI, (vi) dyspnea scale, (vii) fatigue level, and (viii) sarcopenia frequency, a test was performed to determine the distribution of the sample in percentages (%) by category. A chi-square (X2) test was performed to analyze differences in data distribution between sexes. Finally, to analyze the correlations among the clinical variables, physical condition, muscle function, and perceived fatigue, the Spearman test (rs) was performed, as all variables were non-parametric. A value of coefficient rs smaller than 0.40 is considered weak correlation, 0.40–0.69 moderate correlation, and > 0.70 strong correlation. All analyses were performed using the Statistical Package for the Social Sciences (SPSS 20.1 for Windows, IBM, Armonk, New York, United States).

RESULTS

Forty-two post-COVID-19 patients (mean age 59.9 ± 10.06) derived from the rehabilitation service were analyzed. The mean days admitted to the ICU were 58 ± 10.52 days and 80 ± 22.45 days in admission to the ward. When comparing the

results according to sex, the percentage of men admitted to the ICU was much higher than that of women (85.7% and 47.6%, respectively). Descriptive results are shown in **Table 1**, separated by anthropometric and physical condition variables represented as means and standard deviations.

Comparing male and female participants, there were significant differences in the percentage of fat mass (P = 0.001), fat-free mass (P = 0.001), and right handgrip, both in kg and kg relative to body weight (P = 0.001 and 0.004, respectively), and also in the left handgrip, both in kg and kg relative to body weight (P = 0.001 and P = 0.006, respectively) (**Table 1**).

The sample distribution by category was analyzed for the following variables: clinical variables and (i) reason for admission, where 90.5% of men and 85.7% of women were admitted for bilateral pneumonia; (ii) Types of supplied ventilation. Regarding body composition variables, we observed that 90.4% of men and 80.9% of women were overweight or obese, according to BMI. Concerning the cardiovascular risk index (Waist-to-Hip Ratio), over 70% of the participants were categorized as high risk. Physical fitness levels were low in both men (57.1%) and women (76.2 %). For levels of physical activity (RAPA), most of the participants were categorized as low-active (48% of men and 14% of women) or moderately active (38% of men and 57% of women). According to self-reported questionnaires, only 19% of men and 47.6% of women had sarcopenia. They did not claim significant dyspnea, and most of the cohort analyzed claims that perceived fatigue as moderate (57.1% men and 33.3% women) to severe (19% men and 47.6% women). The data are presented in detail in **Table 2**.

Significant associations were observed between clinical variables and physical conditions. The relative strength of body weight of the right hand showed an inverse correlation with the days of ICU admission for men (rs = 0.156), unlike women, where no correlation was seen between them (rs = 0.00059). However, we found an inverse association between travel distance in the 6 MWT and ICU admission days (rs = 0.165), but not in men (rs = 0.004).

Regarding perceived fatigue, an inverse trend was observed, although not significant, with the right handgrip relative to body weight and distance traveled in the 6MWT in both cases; thus, the higher the perceived fatigue, the lower the force of the handgrip and the lesser the distance traveled in the 6 MWT. Additionally, non-significant but clinically relevant trends were observed for fat mass, ICU admission days, and perceived fatigue. Participants with the highest fat mass percentage reported the greatest increase in perceived fatigue and were admitted to the ICU for more days.

DISCUSSION

COVID-19 presents a tremendous challenge for the global population, particularly at the clinical level. If the disease is overcome, one of the most severe problems is physical deterioration that patients experience after medical discharge, mainly due to the extended time they are admitted with reduced mobility and systemic inflammation caused by the virus itself. This was reflected in the cohort of post-COVID-19 patients analyzed in this study; both men and women had a significant deterioration in body composition, with a very high percentage of fat mass and a mean body mass considered as "obesity" by the World Health

Table 1. Characteristics of the sample

	Mean ± SD Men n = 21	Mean ± SD Women n = 21	P value
Anthropometric Variables			
Age (years)	60.95 ± 9.56	58.9 ± 10.68	0.51
Weight (kg)	91.08 ± 17.91	80.9 ± 20.84	0.09
Height (cm)	170.85 ± 6.61	158.33 ± 5.44	0.001
Body Composition			
BMI (kg/m2)	31.03 ± 4.79	32.08 ± 7.25	0.58
Fat mass (%)	31.67 ± 7.24	41.35 ± 8.3	0.001
Fat-free mass (kg)	58.54 ± 9.92	43.63 ± 6.27	0.001
Physical Condition and Functional Capacity			
6MWT Distance (m)	453.90 ± 111.55	388 ± 90.66	0.04
6MWT VO_2max estimated (ml/kg/min)	27.83 ± 5.07	24.51 ± 7.36	0.09
right handgrip (kg)	25.31 ± 9.94	15.21 ± 6.85	0.001
relative right handgrip (kg/kg weight)	0.30 ± 0.11	0.19 ± 0.11	0.004
left handgrip (kg)	23.34 ± 9.11	13.2 ± 6.79	0.001
Relative left handgrip (kg/kg weight)	0.28 ± 0.10	0.19 ± 0.10	0.006
Sit to Stand Test (nº repetitions)	12.09 ± 5.75	9.66 ± 5.54	0.17

P value: P < 0.001; Data are presented as Mean ± SD; 6 MWT = 6 Minutes' Walk Test; BMI = body mass index. The Mann–Whitney U test was performed to analyze the differences between female and male participants.

Table 2. Distribution by sex of clinical variables, body composition, physical condition, and self-reported questionnaires

	Men n = 21	Women n = 21	X^2
Reason for admission			
Unilateral Pneumonia	4.8%	0.0%	
Bilateral Pneumonia	90.5%	85.7%	0.36
Others	4.8%	14.3%	
Type of ventilation			
MV. OTI	61.9%	33.3%	
MV. HFNCOT	19%	14.3%	0.07
Without MV	52.4%	19%	
Body composition: body mass index			
Normal	9.5%	19%	
Obesity	33.3%	23.8%	0.60
Overweight	57.1%	57.1%	
Cardiovascular risk (Waist-Hip Ratio)			
Low risk	19%	28.6%	0.46
High risk	81%	71.4%	
Physical condition: estimated maximal oxygen consumption			
Low	57.1%	76.2%	0.19
Normal-High	42.9%	23.8%	
Rapid Assessment Physical Activity (RAPA)			
Sedentary	0%	10%	
Little active	48%	14%	0.08
Moderately active	38%	57%	
Active	14%	19%	
Dyspnea Scale			
0	0%	14.3%	
1	57.1%	33.3%	
2	33.3%	42.9%	0.07
3	9.5%	0%	
4	0%	9.5%	
Perceived fatigue			
Mild	23.8%	19%	
Moderate	57.1%	33.3%	0.13
Severe	19%	47.6%	
Sarcopenia			
Yes	19%	47.6%	0.05
No	81%	52.4%	

Data are presented as percentages. MV. HFNCOT: mechanical ventilation. High-Flow Nasal Cannula Oxygen Therapy OTI: mechanical ventilation. orotracheal intubation; The dyspnea scale ranges from 0 to 4, where "0" does not present dyspnea and "4" present dyspnea with daily living activities.

Organization.[32] Furthermore, there was a high Waist-to-Hip Ratio in both men and women, which reflected an increased risk of cardiovascular disease in both.[32] In this line, it is essential to note that women had worse body composition than men, with statistically significant differences in fat mass percentage and fat-free mass. In addition, only 14% of men and 19% of women in this study declared themselves physically active.

In line with the study by Eksombatchai et al., the physical condition assessed with the 6 MWT reflected a low cardiorespiratory capacity, possibly due to lung involvement caused by the virus and the prolonged downtime produced by hospital admission.[33] Furthermore, the present study participants presented with very low handgrip values, which pose a risk of all-cause mortality.[34,35] These findings are in line with studies reported on the SARS-CoV-1 epidemic nearly two decades ago.[7,8]

According to numerous observational studies, one of the main sequelae found in post-COVID-19 patients is the presence of persistent fatigue, which, in many cases, persists even months after hospital discharge.[36,37] In the present study, a high percentage of patients reported experiencing moderate-to-severe fatigue in the tasks of daily living. In addition, the perception of fatigue was negatively associated with muscle strength measured by handgrip; therefore, patients who had less relative strength in the right hand reported more significant fatigue, which was more relevant to this association in women. Similarly, a study analyzing perceived fatigue after post-COVID-19 in patients with type 2 diabetes established the same negative relationship between grip strength and perceived fatigue.[38] In the present study the same association was found concerning the distance achieved in the 6 MWT test. Patients who perceived significant fatigue achieved shorter distances in the test.

In addition to significant pulmonary involvement and its consequences, these patients are admitted for an average of 80 days, which is highly detrimental to muscle health. According to Puthucheary et al., after seven days in bed, 10% of muscle mass is lost.[17] This results in muscle mass brings with it a loss of strength, muscle atrophy, changes in muscle fibers (from type I to type II), worse oxidative capacity, loss of mitochondrial capacity,[39] and a considerable risk of disability.[40] All these findings justify that these patients perceived more significant fatigue. Similarly, we found negative associations between the total number of days admitted to the ICU and lower grip strength, in addition to shorter distance in the 6 MWT. In line with these findings, other studies on post-COVID-19 patients have also found negative correlations between the severity of affectation and muscle weakness.[11,12]

In addition, a positive association was observed between body fat percentage, days admitted to the ICU, and the level of perceived fatigue. Participants with greater adiposity had longer ICU stays and higher perceived fatigue. According to a recent systematic review, obesity is a risk factor for the development of a more severe form of COVID-19 because this infection is highly inflammatory, and these patients already have high levels of systemic inflammation.[41]

Limitations of the study

Despite presenting clinically relevant findings, this study has some limitations. First, as this was an observational study, causal relationships could not be established. There is no certainty whether patients with a lower initial physical condition required a longer hospitalization time and developed a more severe form

of the disease or whether it was the fact that they had a longer hospital admission time or were admitted to the ICU, which led to a more significant functional impairment. Additionally, the wide age range of the participants may have influenced the results. Finally, it would be interesting to compare these results with those of a sample of ICU patients without COVID-19.

CONCLUSION

The post-COVID-19 participants were mostly obese and sedentary older adults who presented with low muscle function and low levels of physical condition associated with moderate-to-severe fatigue. Male participants required ICU admission more frequently than female participants. Obesity was associated with increased perceived fatigue and a longer ICU stay.

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Suicide ideation and psychotropic recreational drug use by adolescents: a systematic review and meta-analysis

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ABSTRACT

BACKGROUND: Adolescence is characterized by complex and dynamic changes, often involving experimentation, including the use of psychotropic substances. Although it is well-established that recreational psychotropic drugs are associated with suicide ideation in adults, evidence of this association in adolescents remains limited.

OBJECTIVE: To investigate the relationship between suicide ideation and psychotropic recreational drug use among adolescents.

DESIGN AND SETTING: Systematic review with meta-analysis developed at Universidade Federal de Uberlândia (UFU) and Universidade Estadual de Campinas (UNICAMP), Brazil.

METHODS: A search across eight electronic databases for observational studies, without language or publication year restrictions, was conducted. The Joanna Briggs Institute tool was used to assess the risk of bias. Random-effects meta-analyses and odds ratios were used to measure the effects.

RESULTS: The search yielded 19,732 studies, of which 78 were included in the qualitative synthesis and 32 in the meta-analysis. The findings indicated that suicidal ideation was 1.96 times more likely (95% confidence interval, CI = 1.47; 2.61) for adolescents who used some drug recurrently and 3.32 times more likely (95%CI = 1.86; 5.93) among those who abused drugs. Additionally, adolescents who used cannabis were 1.57 times more likely (95%CI = 1.34; 1.84) to experience suicide ideation compared with non-users, while cocaine users had 2.57 times higher odds (95%CI = 1.47; 4.50).

CONCLUSIONS: Psychotropic recreational drug use is associated with suicidal ideation among adolescents regardless of current or previous use, abuse, or type of substance used.

SYSTEMATIC REVIEW REGISTRATION: Registered in the PROSPERO database under the identification number CRD42021232360. https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42021232360.

INTRODUCTION

Adolescence marks a period characterized by complex and dynamic changes that directly influence individuals' personalities and social performance development.[1] These transformations contribute to the development of various types of behaviors, including experimentation with drugs,[2] alcohol, medications, and other psychoactive substances,[3] making adolescence a critical phase vulnerable to starting substance use, including psychotropic drugs.[4] Drug use disorders severely affect children and adolescents' physical and mental health.[5]

The scientific literature underscores the widespread prevalence of psychotropic substance use among adolescents globally. A study performed in Poland showed that 10.8% of respondents aged 13 to 17 had consumed psychotropic drugs, such as cannabis, cocaine, or heroin, at least once.[6] Similarly, a survey on drug use among Brazilian adolescents indicated cannabis as the second most commonly used substance, following alcohol.[7]

Different factors contribute to adolescents' susceptibility to drug consumption.[7-10] In the family environment, permissive relationships regarding smoking and alcohol consumption, particularly among males, predict substance use.[8] Furthermore, social determinants such as strain family relationships,[7] low maternal education levels, non-white-collar parental occupations, economic hardships within the community, and high poverty and unemployment levels at 18 years are the risk factors for drug use in adolescence.[10] In the school environment, adolescents who experience or perpetrate bullying demonstrate a higher prevalence of substance use.[9]

A study by the United Nations Office on Drugs and Crime revealed a 30% global increase in drug use between 2009 and 2018,[11] along with a 10% increase in suicide rates between 2006 and 2018.[12] Annually, 800,000 individuals worldwide die by suicide, making it the second principal cause of death among young women and the third among young men.[13]

Suicidal ideation in adolescents is influenced by biological, psychological,[14] and socioeconomic factors.[15] Developing suicidal ideation, meaning the existence of suicidal thoughts by adolescents, is related to the feeling of not belonging to the school environment, low resilience, the existence of stress factors throughout life,[16] the presence of depressive symptoms, bullying and other types of violence, and tobacco and alcohol consumption.[17,18] Symptoms such as sadness, self-hatred, fatigue, self-deprecation, and crying are associated with depression and suicidal ideation, with loneliness being particularly correlated.[19] The prevalence of suicidal ideation among adolescents ranges between 15 and 25%, representing a significant global public health care concern.[20]

A previous systematic review of 108 included studies highlighted positive associations between different substances, such as alcohol, tobacco, cannabis, illicit drugs, and non-medical use of prescription drugs and suicidal ideation in adults.[21] However, robust evidence evaluating the consumption of psychotropic recreational drugs and their relationship with suicidal ideation among adolescents remains limited.

OBJECTIVE

This systematic review aimed to compare suicidal ideation among adolescents who did and did not use psychotropic recreational drugs.

METHODS

Protocol registration

The protocol for this systematic review was based on the Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols (PRISMA-P) guidelines[22] and registered in the International Prospective Register of Systematic Reviews (PROSPERO) database (http://www.crd.york.ac.uk/PROSPERO) under the number CRD42021232360. The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines[23] and the Joanna Briggs Institute (JBI) Manual for Evidence Synthesis[24] were used to conduct this systematic review.

Research question

This systematic review aimed to answer the following guiding question based on the Population, Exposition, Comparator, and Outcome (PECO) acronym: "Is the use of psychotropic recreational drugs associated with a higher chance of suicide ideation among adolescents?"

Inclusion criteria

Observational studies (prospective or retrospective) comparing suicidal ideation (outcome) between adolescents in the school environment (population) who reported psychotropic recreational drug use (exposition) and adolescents who did not use psychotropic recreational drugs (comparator).

Including the classifications of the World Health Organization (10–19 years old) and the United Nations (15–24 years old), the age range of 10–24 years was considered as adolescents in this study.

We defined cannabis, cocaine, ecstasy, LSD, heroin, amphetamine, glue-sniffing, and cracks as psychotropic drugs. The assessment of suicidal ideation can be primary (questionnaire/interview) or secondary (database or reports).

There were no restrictions on publication language or year.

Exclusion criteria

The exclusion criteria were as follows: studies that evaluated the relationship between alcohol or tobacco use alone and suicide ideation, studies performed in psychiatric clinics, studies with specific groups of adolescents (ethnic minorities, indigenous populations, or adolescents with mental disorders), studies that did not include adolescents in the school environment, studies performed with university students, or postmortem assessments.

Sources of information and search

Electronic searches were performed in the MedLine (*via* PubMed), Scopus, LILACS, SciELO, Embase, and Web of Science databases. OpenThesis and OpenGrey were used to capture the "gray literature" partially. The MeSH (Medical Subject Headings), DeCS (Health Sciences Descriptors), and Emtree (Embase Subject Headings) were used to select the search descriptors. Synonyms and free words were used in the search. The Boolean operators "AND" and "OR" were used to improve the research strategy with several combinations. **Table 1** lists the details of the combinations used in each database. A bibliographic search was conducted for articles published until January 2022. The results obtained in the primary databases were initially exported to the EndNote Web™ software (Thomson Reuters, Toronto, Canada) for cataloging and removing duplicates. The other results were exported to Microsoft Word (Microsoft™, Ltd, Washington, United States) for manually removing duplicates.

Study selection

Before study selection, a calibration exercise was performed. The authors discussed the eligibility criteria and applied them to a sample of 20% of the retrieved studies to determine the inter-examiner agreement. After reaching a proper level of agreement (Kappa ≥ 0.81), the reviewers performed a methodical analysis of the titles of the studies (first phase), eliminating those not pertinent

Table 1. Strategies for database search

Databases	Search Strategy
Main databases	
PubMed http://www.ncbi.nlm.nih.gov/pubmed	((("Psychotropic Drugs" OR "Psychoactive Agents" OR "Psychoactive Drugs" OR "Substance Use" OR "Narcotic" OR "Substance-Related Disorders" OR "Substance Abuse" OR "Drug Abuse") AND ("Suicide" OR "Suicides" OR "Suicidal" OR "Self-harm" OR "Self-Injurious Behavior" OR "Self Destructive Behavior" OR "Suicidal Ideation" OR "Self-Destructive Behavior" OR "Attempted Suicide") AND ("Adolescence" OR "Adolescent" OR "Student" OR "Teen" OR "Teenager" OR "Youth" OR "Young")))
Embase https://www.embase.com	(("Psychotropic Drugs" OR "Psychoactive Agents" OR "Psychoactive Drugs" OR "Substance Use" OR "Narcotic" OR "Substance-Related Disorders" OR "Substance Abuse" OR "Drug Abuse") AND ("Suicide" OR "Suicides" OR "Suicidal" OR "Self-harm" OR "Self-Injurious Behavior" OR "Self Destructive Behavior" OR "Suicidal Ideation" OR "Self-Destructive Behavior" OR "Attempted Suicide") AND ("Adolescence" OR "Adolescent" OR "Student" OR "School" OR "Teen" OR "Teenager" OR "Youth" OR "Young"))
Web of Science http://apps.webofknowledge.com/	((((("Psychotropic Drugs" OR "Psychoactive Agents" OR "Psychoactive Drugs" OR "Substance Use" OR "Narcotic" OR "Substance-Related Disorders" OR "Substance Abuse" OR "Drug Abuse") AND ("Suicide" OR "Suicides" OR "Suicidal" OR "Self-harm" OR "Self-Injurious Behavior" OR "Self Destructive Behavior" OR "Suicidal Ideation" OR "Self-Destructive Behavior" OR "Attempted Suicide") AND ("Adolescence" OR "Adolescent" OR "Student" OR "School" OR "Teen" OR "Teenager" OR "Youth" OR "Young"))))
SciELO http://www.scielo.org/	(("Psychotropic" OR "Psychoactive Agents") AND ("Suicide" OR "Suicidal") AND ("Adolescent" OR "Teenager" OR "Youth")) (("Drug Abuse" OR "Substance Abuse" OR "Narcotic") AND ("Suicide" OR "Suicidal") AND ("Adolescence" OR "Teen" OR "Young"))
LILACS http://lilacs.bvsalud.org/	(("Psychotropic" OR "Psychoactive Agents") AND ("Suicide" OR "Suicidal") AND ("Adolescent" OR "Teenager" OR "Youth")) AND (instance:" regional") AND (db:("LILACS")) tw:((("Drug Abuse" OR "Substance Abuse" OR "Narcotic") AND ("Suicide" OR "Suicidal") AND ("Adolescent" OR "Teenager" OR "Youth"))) AND (instance:" regional") AND (db:("LILACS"))
Scopus http://www.scopus.com	(("Psychotropic Drugs" OR "Psychoactive Agents" OR "Psychoactive Drugs") OR ("Suicide" OR "Suicides" OR "Suicidal" OR "Self-harm") OR ("Adolescence" OR "Adolescent" OR "Student" OR "School" OR "Teen" OR "Teenager" OR "Youth" OR "Young")) (("Narcotic" OR "Substance-Related Disorders" OR "Substance Abuse" OR "Drug Abuse") OR ("Self-Injurious Behavior" OR "Self Destructive Behavior" OR "Suicidal Ideation" OR "Self-Destructive Behavior" OR "Attempted Suicide") OR ("Adolescence" OR "Adolescent" OR "Teen" OR "Teenager"))
Gray literature	
OpenThesis https://oatd.org	(("Substance Use") AND ("Suicide") AND ("Adolescent"))
OpenGrey http://www.opengrey.eu/	(("Substance Use" OR "Drug Abuse" OR "Substance Abuse") AND ("Suicide" OR "Suicidal" OR "Self-harm" OR "Self-Injurious Behavior" OR "Self-Destructive Behavior") AND ("Adolescent" OR "Teenager" OR "Adolescence" OR "Teen" OR "Young"))

to the topic. In the second phase, the same reviewers evaluated the abstracts of the studies using the initial eligibility criteria. Titles that met the study objectives but lacking available abstracts were analyzed in the next phase. In the third phase, reviewers read the full texts of the eligible studies to confirm adherence to the eligibility criteria. Excluded studies in this phase were registered separately, accompanied by explanations for exclusion. In cases where full texts were unavailable, requests were made to library databases for bibliographic assistance, and e-mails were sent to the corresponding authors to obtain the texts. Two reviewers independently performed all phases; in cases of doubt or disagreement, a third reviewer was consulted to make the final decision.

Data collection

Before data extraction, to ensure consistency between the reviewers, a calibration exercise was performed in which data from three eligible studies were extracted together by a third reviewer. Subsequently, the two eligible reviewers extracted the following information from the studies: identification of the study (author, year, country, and study location), sample characteristics (number of patients in each study, nationality, sex, and average age), collection and processing characteristics (type of questionnaire and/or interview applied, drugs used by adolescents), and the main results (presence of suicide ideation in user and non-user adolescents, odds ratio – OR). In case of incomplete or insufficient information, the corresponding author was contacted via email.

Risk of bias assessment

The JBI Critical Appraisal Checklist for Analytical Cross-Sectional Studies was used to analyze the risk of bias and individual methodological quality of the studies selected.[25] Two reviewers independently assessed each domain regarding the potential risk of bias, as

recommended by the PRISMA statement.[23] Each study was categorized according to the rate of positive answers to the questions corresponding to the assessment tool. The risk of bias was considered high when the study obtained 49% of answers as "yes", moderate when the study obtained 50% to 69% of answers as "yes", and low when the study reached more than 70% of "yes" answers.[26]

Data synthesis and Meta-analysis

Meta-analyses were conducted to aggregate the primary findings of the eligible studies and compare the OR of suicide ideation between the exposed (drug-user adolescents) and non-exposed (non-drug-user adolescents) groups. A separate meta-analysis was conducted foreach type of substance (e.g., cannabis, cocaine, and psychotropic drug use) and user profile (e.g., dependence, current use, and use at some point in life) if at least three studies provided sufficient and comparable information. When included studies provided multiple OR estimates, the model with the highest number of adjusted variables was selected for inclusion. For longitudinal studies, the OR from the first follow-up wave was selected.

Heterogeneity among the studies was measured using three indicators: the I^2, indicating the rate of variability caused by heterogeneity among studies; H^2, denoting the level of heterogeneity ($H = 1$ indicating homogeneity); and τ^2, representing the variance among studies. All analyses were performed with random effects, considering that the high heterogeneity estimates observed in the meta-analysis models.

Funnel plots were produced to verify the publication bias in the different meta-analyses, but only for the models that included 10 or more studies.[27] Additionally, sensitivity tests were conducted, including only studies with a low risk of bias, to assess the impact of the individual risk of bias of the eligible studies in the meta-analysis. All statistical analyses were performed using the Stata 16.1 software (StataCorp LLC, College Station, TX, USA), with asignificance level set at 5 %.

Certainty of evidence

The certainty of evidence was assessed using the Grading of Recommendation, Assessment, Development, and Evaluation (GRADE) tool. The GRADE pro-GDT software (http://gdt.guidelinedevelopment.org) was used to summarize the results. The assessment was based on study design, risk of bias, inconsistency, indirect evidence, imprecision, and publication bias. The certainty of evidence can be classified as high, moderate, low, or very low.[28]

RESULTS

Study selection

During In the initial phase of study selection, 19,732 hits were identified. Following the removal of duplicates, 13,092 results underwent screening based in the title and abstract. Subsequently, 164 studies met the eligibility criteria and underwent full-text analysis. Among these, 78[17,18,29-104] were included in the qualitative synthesis of results (**Figure 1**). Supplementary **Table 1** provides details regarding the exclusion of 86 studies.

Study characteristics

The selected studies were published between 1991 and 2020, with 38 studies performed in North America, 12 in Asia, 11 in Europe, eight in Africa, four in South America, four in Oceania, and one intercontinental study in the USA and France. Regarding data collection methods, 56 studies collected their data from secondary databases, while 22 utilized questionnaires. Among the Cannabis was the most frequently studied drug in relation to suicide ideation, followed by cocaine, inhalants, injectable drugs, ecstasy, methamphetamine, glue, heroin, and crack. Among studies reporting the number of research participants, the total sample sizewas 1,122,111 answers, with 51.63% women and 48.36% men. **Table 2** and **Table 3** detail the main characteristics and outcomes of the eligible studies, respectively.

Assessment of the risk of bias of studies

Among the studies, 12 were deemed to have a moderate risk of bias, while 66 had a low risk; none were classified as having a high risk of bias. Question 1, regarding the eligibility criteria for sample selection, was answered negatively in nine studies. This answer is important because it favors sample standardization and decreases the risk of bias. Questions 5 and 6 were answered negatively in 11 studies, indicating that most studies identified confounding factors and established strategies for addressing them (**Table 4**).

Meta-analysis

Although eligible studies analyzed the likelihood of suicide ideation relative to various types of drugs, only three comparisons were suitable for meta-analysis: 1) any psychotropic recreational drug use, 2) cannabis use, and 3) cocaine use. For any psychotropic recreational drug use, the analysis included three subtypes: adolescents who used it at some point in life, those who reported currently using the drug, and those who suffered from drug dependence or abuse. Regarding cannabis, only current users were analyzed, while regarding cocaine, the analysis was performed on adolescents who used the drug at some point in their lives.

The use of any psychotropic recreational drug

Four studies[42,53,60,65] evaluated the psychotropic recreational drug use at certain points in life. The combined effect estimation was OR 1.65 (95% confidence interval, CI = 0.54; 4.99), indicating no significant association with suicide ideation for adolescents who

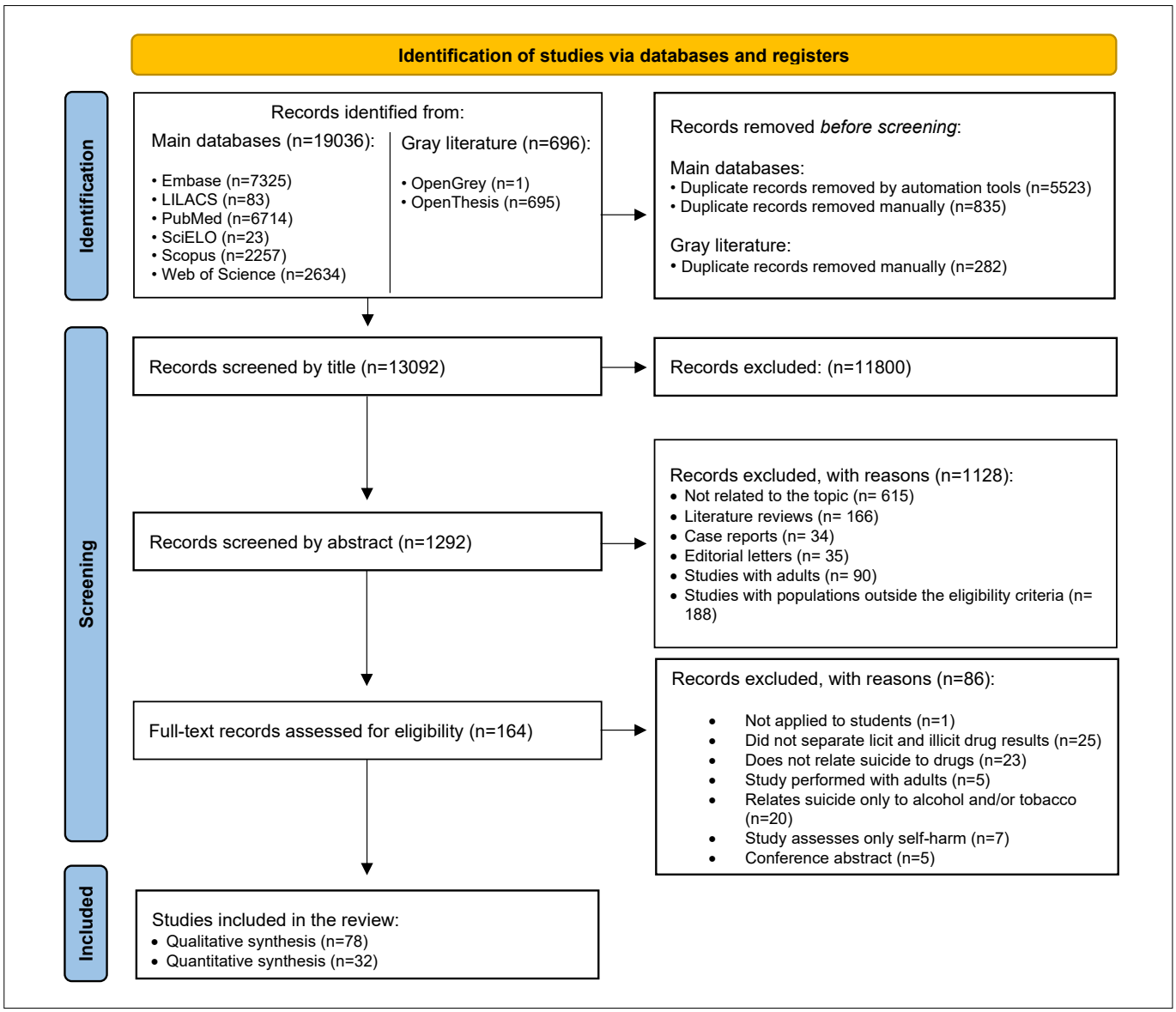

Figure 1. Flowchart depicting the study selection process (Preferred Reporting Items for Systematic Reviews and Meta-Analyses flow diagram).

used any psychotropic recreational drug at least once in their lives compared to those who never used it (**Figure 2**). A high level of heterogeneity was also observed (I^2 = 98.6%). This is justified by a study by Yip et al.[42], which indicated that drug use was a protective factor (OR < 1.00).

Regarding the recurrent use of psychotropic recreational drugs, ten studies[18,41,54,66,77,84,92,95,100,101] analyzed its association with suicidal ideation. The combined effect estimation for suicidal ideation was OR 1.96 (95%CI = 1.47; 2.61) for adolescents who reported currently using psychotropic recreational drugs compared to non-users (**Figure 3**). Heterogeneity was high (I^2 = 79.1%). The funnel plot indicates the potential risk of publication bias (**Figure 4**).

Five studies[51,60,62,99,103] analyzed the relationship between the use or abuse of any psychotropic recreational drugs and suicide ideation. The combined effect estimation for adolescents who abused drugs compared to those who did not was OR 3.32 (95%CI = 1.86; 5.93) (**Figure 5**), with considerably high heterogeneity (I^2 = 87.1%).

Cannabis use

Fifteen studies[17,18,40,50,56,67,71,73,78,85,90,91,96,97,104] provided sufficient data on the likelihood of suicide ideation related to cannabis use. Additionally, these studies provided 33 databases that were included in the meta-analysis. Overall, adolescents who reported currently using cannabis were 1.57 times more likely (95%CI = 1.34; 1.84) to present suicide ideation than non-users (**Figure 6**). The heterogeneity was considered high (I^2 = 96.6%). The funnel plot indicated a high probability of publication bias (**Figure 7**).

Table 2. Main characteristics of the eligible studies

Author, year[ref]	Country	Age (years)	Average age	n	Data source
Kandel et al., 1991[29]	USA	NR	NR	593 (NR♂;NR♀)	Self-administered questionnaire
Felts et al., 1992[30]	USA	NR	NR	3,064 (NR♂;NR♀)	North Carolina Youth Risk Behavior Survey (1990)
Garrison et al., 1993[31]	USA	NR	NR	3,674 (1,702♂;2,062♀)	South Carolina Youth Risk Behavior Survey (1990)
Vega et al., 1993[32]	USA	NR	NR	5,303 (NR♂;NR♀)	Self-administered questionnaire
Madianos et al., 1994[33]	Greece	12-17	NR	4,291(1,940♂;2,351♀)	Self-administered questionnaire
Burge et al., 1995[34]	USA	NR	NR	11,631 (5,676♂;5,955♀)	1990 Youth Risk Behavior Survey (USA)
Lopez et al., 1995[35]	Mexico	13-19	NR	3,459 (1,764♂;1,695♀)	National High-School Survey – Mexico 1992
Windle and Windle, 1997[36]	USA	n.r.	15.54 ± 0.66	975 (458♂;517♀)	Self-administered questionnaire
Simon and Crosby, 2000[37]	USA	NR	NR	16,296 (NR♂;NR♀)	1993 National School-Based Youth Risk Behavior Survey (YRBS)
Perkins and Hartless, 2002[38]	USA	12-17	NR	14,922 (NR♂;NR♀)	Self-administered questionnaire
Vermeiren et al., 2003[39]	Belgium	12-18	14.9 ± 1.9	794 (794♂;0♀)	Self-administered questionnaire
Hallfors et al., 2004[40]	USA	15-19	NR	18,922 (9,288♂;9,634♀)	Wave-I in-home contractual data set of Add Health (1994)
Wu et al., 2004[41]	USA	9-17	NR	1,458 (NR♂;NR♀)	NIMH Methods for the Epidemiology of Child and Adolescent Mental Disorders (MECA) Study and The Westchester Study
Yip et al., 2004[42]	China	15-24	15.8	2,586 (306♂;420♀)	Hong Kong Youth Sexuality Survey - 2001
Spremo and Loga, 2005[43]	Bosnia-Herzegovina	16-18	NR	202 (51♂;151♀)	Self-administered questionnaire
Ulusoy et al., 2005[44]	Turkey	17-18	NR	726 (306♂;420♀)	Self-administered questionnaire
Chabrol et al., 2008[46]	France	15-20	16.7 ± 1.3 (♂) 17 ± 1.3 (♀)	248 (76♂;172♀)	Self-administered questionnaire
Dunn et al., 2008[45]	USA	NR	12.9 ± 2.64 (♂) 12.8 ± 2.61 (♀)	10,273 (5,126♂;5,146♀)	Self-administered questionnaire
Luncheon et al., 2008[47]	USA	NR	NR	7,544 (0♂;7,544♀)	2003 Youth Risk Behavioral Surveillance System
Peltzer et al., 2008[48]	South Africa	NR	15.78 ± 1.58	1,157 (358♂;799♀)	Self-administered questionnaire
Epstein and Spirito, 2009[49]	USA	NR	NR	10,273 (5,126♂;5,146♀)	Youth Risk Behavior with probability proportional to school enroll Surveillance (United States, 2005)
Peter and Roberts, 2010[50]	Canada	15	NR	2,499 (1,222♂;1,277♀)	National Longitudinal Survey of Children and Youth – Waves 3 to 6
Pickles et al., 2009[51]	England	NR	NR	2,226 (NR♂;NR♀)	Baseline of the Isle of Wight study, an epidemiological sample of adolescents assessed in 1968
Florenzano et al., 2010[52]	Chile	NR	NR	2,322 (1,026♂;1,296♀)	Self-administered questionnaire
Page et al., 2011[53]	China and Philippines	11-17	NR	16,353 (7,450♂;8,725♀)	Global School Health Survey (2003) – Data from China and Philippines
Souza et al., 2010[54]	Brazil	11-15	NR	1,039 (501♂;538♀)	Self-administered questionnaire
Wolitzky-Taylor et al., 2010[55]	USA	12-17	NR	7,637 (NR♂;NR♀)	National Survey of Adolescents (1995/2005)
Alwan et al., 2011[56]	Seychelles	11-17	14 ± 1.4	1,432 (NR♂;NR♀)	Self-administered questionnaire
Carvalho et al. 2011[57]	Brazil	14-19	NR	4,201 (1,688♂;2,513♀)	Self-administered questionnaire
Eaton et al., 2011[58]	USA	NR	NR	6,322 (0♂;6,322♀)	Youth Risk Behavior Survey (2007)
Kim et al., 2011[59]	USA	12-17	NR	19,301 (NR♂;NR♀)	2000 National Household Survey on Drug Abuse (NHSDA)
Miller et al., 2011[60]	Mexico	12-17	NR	3,005 (NR♂;NR♀)	Mexican Adolescent Mental Health Survey (MAMHS)
Swahn et al., 2011[61]	USA and France	11-19	NR	28,323 (13,895♂;14,398♀)	2003 European School Survey Project on Alcohol and Other Drugs (France) and 2003 Youth Risk Behavior Survey (USA)
Ahmad et al., 2012[62]	Malaysia	12-17	NR	25,507 (12,498♂;13,009♀)	2012 Malaysia Global School-based Student Health Survey
Bakken and Gunter, 2012[63]	USA	NR	NR	2,548 (1,274♂;1,274♀)	Delaware High School Youth Risk Behavior Survey (YRBS-H)
Kokkevi et al., 2012[64]	17 European countries	15-16	NR	45,806 (NR♂;NR♀)	European School Survey Project on Alcohol and Other Drugs (ESPAD) - 2007

Continue...

Table 2. Continuation.

Author, year[ref]	Country	Age (years)	Average age	n	Data source
Peltzer and Pengpid, 2012[65]	Thailand	12-15	NR	2,758 (1,364♂;1,394♀)	Thailand Global School-Based Health Survey (2008)
Wilson et al., 2012[66]	Seychelles	11-17	NR	1,432 (687♂;745♀)	Global School-based Student Health Survey – Data from Seychelles
Arenliu et al., 2013[67]	Kosovo	15-16	15.65 ± 0.68 (♂) 15.63 ± 0.69 (♀)	4,709 (2,112♂;2,597♀)	2011 European School Survey Project on Alcohol and other Drugs (ESPAD)
Consoli et al. 2013[68]	France	17	NR	36,757 (18,164♂;18,593♀)	Self-administered questionnaire
Delfabbro et al., 2013[69]	Australia	14-16	15.2 ± 0.5	2,552 (1,041♂;1,485♀)	Self-administered questionnaire
Govender et al., 2013[70]	South Africa	13-17	14.7 ± 0.74	239 (112♂;127♀)	Self-administered questionnaire
Rasic et al., 2013[71]	Canada	NR	NR	976 (486♂;490♀)	Self-administered questionnaire
Shilubane et al., 2013[72]	South Africa	13-19	NR	20,646 (9,878♂;10,768♀)	2002 and 2008 South African Youth Risk Behaviour Surveys
Van Ours et al., 2013[74]	New Zealand	10-24	NR	938 (459♂;479♀)	Christchurch Health and Development Study (CHDS)
Wong et al., 2013[73]	USA	NR	NR	73,183 (37,104♂;36,079♀)	Data from the 2001 to 2009 Youth Risk Behavior Survey
Chabrol et al., 2014[75]	France	NR	17.1 ± 1.2 (♂) 16.7 ± 1 (♀)	972 (594♂;378♀)	Self-administered questionnaire
Lowry et al., 2014[76]	USA	NR	NR	14,000 (NR♂;NR♀)	11 national Youth Risk Behavior Surveys conducted biennially between 1991 and 2011
Randall et al., 2014[77]	Benin	12-16	NR	2,690 (NR♂;NR♀)	Global School-based Health Survey (2009) – Data from Benin
Zhang and Wu, 2014[78]	USA	11-21	NR	3,342 (NR♂;NR♀)	Public-use Add Health – Wave 1
Delfabbro et al., 2015[79]	Australia	14-16	15.2 ± 0.5	2,552 (1,041♂;1,485♀)	Self-administered questionnaire
Dunlavy et al., 2015[80]	Tanzania	11-16	NR	2,154 (1,034♂;1,120♀)	Global School-Based Student Health Survey (2006) – Data from Dar el Salaam
Gart and Kelly, 2015[81]	USA	NR	16 ± 1.2	15,363 (7,655♂;7,708♀)	2011 Youth Risk Behavior Survey
Lee and Choi, 2015[82]	South Korea	13-18	NR	72,435 (35,655♂;35,780♀)	2013 Online Survey of Youth Health Behavior in Korea
Peltzer and Pengpid, 2015[17]	4 countries of Oceania	13-16	NR	6,540 (2,846♂;3,534♀)	Global School-Based Health Survey (2011) – Data from Samoa, Kiribati, Salomon Island, and Vanuatu
Sampasa-Kanyinga et al., 2015[83]	Canada	11-20	14.4 ± 1.9	1,922 (883♂;3,534♀)	Ontario Student Drug Use and Health Survey (2009/2011/2013)
Sharma et al., 2015[84]	Peru	12-18	NR	916 (425♂;491♀)	Self-administered questionnaire
Dudovitz et al., 2015[85]	EUA	NR	NR	15,698 (7,656♂;8,042♀)	2011 Youth Risk Behaviors Survey
Ammerman et al., 2016[86]	USA	NR	NR	4,834 (2,315♂;2,419♀)	Longitudinal Study of Adolescent Health (2009)
DeCamp and Bakken, 2016[87]	USA	NR	NR	4,834 (2,315♂;2,419♀)	2005, 2007, and 2009 Delaware High School Youth Risk Behavior Survey (YRBS-H)
Price and Khubchandani, 2016[88]	USA	NR	NR	13,721 (NR♂;NR♀)	Youth Risk Behavior Survey (2001/03)
Weeks and Colman, 2016[90]	Canada	12-17	NR	6,788 (3,287♂;3,501♀)	National Longitudinal Survey of Children and Youth
Agrawal et al., 2017[91]	USA	12-22	NR	3,277 (NR♂;NR♀)	Baseline of the Collaborative Study of the Genetics of Alcoholism
Asante et al., 2017[92]	Ghana	NR	NR	1,973 (1,065♂;908♀)	Ghana Global School-based Student Health Survey (2012)
Janssen et al., 2017[93]	France	17	NR	22,023 (11,034♂;10,989♀)	Self-administered questionnaire
Wang and Yen, 2017[94]	Taiwan	12-19	14.75 ± 1.77	13,985 (NR♂;NR♀)	2004 Project for the Health of Adolescents in Southern Taiwan
El Kazdouh et al., 2018[95]	Morocco	14-19	NR	800 (374♂;426♀)	Self-administered questionnaire
Haskuka et al., 2018[96]	18 European countries	15	NR	105,000 (NR♂;NR♀)	European School Survey Project on Alcohol and Other Drugs (ESPAD – 2011)
Subica and Wu, 2018[97]	USA	12-18	NR	184,494 (NR♂;NR♀)	1991 – 2015 Combined National Youth Behavioral Risk Surveys

Continue...

Table 2. Continuation.

Author, year[ref]	Country	Age (years)	Average age	n	Data source
Chadi et al., 2019[98]	USA	NR	NR	26,821 (13,062♂;13,749♀)	Two waves (2015 and 2017) of the national Youth Risk Behavior Survey
Dema et al., 2019[99]	Bhutan	13-17	NR	5,809 (2,554♂;3,255♀)	Global School-Based Student Health Survey (2016) – Data from Bhutan
Georgiades et al., 2019[100]	Canada	14-17	NR	2,396 (1,189♂;1,207♀)	2014 Ontario Child Health Study
Jung et al., 2019[101]	South Korea	NR	NR	59,984 (30,384♂;29,600♀)	Korea Youth Risk Behavior Web-based Survey (2017)
Baiden et al., 2020[18]	USA	NR	NR	13,697 (6,609♂;7,088♀)	2017 Youth Risk Behavior Survey (YRBS)
Greene et al., 2020[102]	USA	NR	NR	16,390 (8,149♂;8,187♀)	2013 New Mexico Youth Risk and Resiliency Survey (NM-YRRS)
Khan et al., 2020[103]	Bangladesh	11-18	NR	2,989 (1,952♂;1,037♀)	Global School-based Student Health Survey (2014) – Data from Bangladesh
Kim et al., 2020[89]	South Korea	12-17	15.9 ± 0.02	65,528 (33,803♂;31,725♀)	2016 Korea Youth Risk Behavior Web-based Survey (KYRBS)
Sakamoto et al., 2020[104]	USA	14-18	NR	1,943 (982♂;951♀)	Youth Risk Behavior Survey (2017) – Data from the Northern Mariana Islands

♂ = Male ♀ = Female; NR = Not reported.

Table 3. Qualitative synthesis of the main results of the eligible studies

Author[ref]	Main outcomes
Kandel et al.[29]	There was a strong association between psychotropic recreational drug use and suicidal ideation in female adolescents.
Felts et al.[30]	Drug use, particularly crack and cocaine, was associated with suicidal ideation.
Garrison et al.[31]	Illicit psychotropic recreational drug use was significantly associated with suicidal ideation.
Vega et al.[32]	Illicit psychotropic recreational drug use was consistently related to higher levels of suicidal ideation.
Madianos et al.[33]	The severity and frequency of substance use influenced the prevalence of suicidal ideation in the sample analyzed.
Burge et al.[34]	Psychotropic recreational substance use showed a positive association with suicidal ideation.
Lopez et al.[35]	Psychotropic recreational drug use represented a risk factor for suicidal ideation among the students.
Windle and Windle[36]	Psychotropic recreational drug use did not show a significant relationship with suicidal ideation, but it did with suicide attempts.
Simon and Crosby[37]	There was a relationship between psychotropic recreational substance use and suicidal ideation and thoughts.
Perkins and Hartless[38]	There was a relevant association between the use of hard psychotropic recreational drugs and suicidal ideation.
Vermeiren et al.[39]	Psychotropic recreational substance use did not show a significant association with suicidal ideation.
Hallfors et al.[40]	Psychotropic recreational drug use, particularly injectable drugs, presented a significant association with suicidal ideation.
Wu et al.[41]	The association between psychotropic recreational substance use and abuse was not significant after controlling adolescent depression.
Yip et al.[42]	Illicit psychotropic recreational drug use was not considered an important risk factor for suicidal ideation.
Spremo and Loga[43]	Psychoactive substance use presented an important connection to the presence of suicidal ideation in the adolescents studied.
Ulusoy et al.[44]	There was no significant association between illicit psychotropic recreational drug use and suicidal ideation, but adolescents who smoke cigarettes were more prone to ideation.
Chabrol et al.[46]	There were significant associations between psychotropic recreational substance use and suicidal ideation among adolescents attending rural schools.
Dunn et al.[45]	Cannabis use by adolescents was significantly associated with suicidal behavior (including suicidal ideation).
Luncheon et al.[47]	Illicit psychotropic recreational drugs were seriously associated with suicidal ideation and thoughts.
Peltzer et al.[48]	The involvement with psychotropic recreational drugs was not associated with suicide risk (including suicidal ideation).
Epstein and Spirito[49]	Sniffing glue had no significant relationship with suicidal ideation, but injecting drugs showed a high association with suicidal ideation.
Peter and Roberts[50]	Cannabis use did not show a significant relationship with suicidal ideation.
Pickles et al.[51]	Problems with psychotropic recreational substance use were considered risk factors for the increase in suicidal behavior.
Florenzano et al.[52]	There was a high correlation between psychotropic recreational substance use and suicidal behavior (including suicidal ideation) and depression.
Page et al.[53]	Psychotropic recreational drug use is significantly associated with higher levels of suicidal ideation.
Souza et al.[54]	Illicit psychotropic recreational drug use was one of the factors related to suicidal ideation among adolescents.
Wolitzky-Taylor et al.[55]	Psychotropic recreational substance use was significantly associated with an increased risk of suicidal ideation in adolescents in both years of the study.
Alwan et al.[56]	Psychotropic recreational substance use was strongly associated with suicidal ideation among adolescents.
Carvalho et al.[57]	Psychotropic recreational drug use was directly associated with suicidal ideation and planning among adolescents.
Eaton et al.[58]	There was a significant association between psychotropic recreational drug use and suicidal ideation among adolescents.
Kim et al.[59]	The use of ecstasy and other psychotropic recreational drugs presented a significant association with suicidal ideation.
Miller et al.[60]	There was an association between psychotropic recreational substance use and suicidal ideation, and this relationship started with adolescents aged 13 years and increased according to age.

Continue...

Table 3. Continuation.

Author[ref]	Main outcomes
Swahn et al.[61]	The early drug initiation of adolescents showed an association with suicidal ideation in both countries analyzed.
Ahmad et al.[62]	The use of hard psychotropic recreational drugs was significantly related to suicidal ideation.
Bakken and Gunter[63]	Psychotropic recreational substance use showed a significant relationship with suicidal behavior and ideation among the adolescents participating in the study.
Kokkevi et al.[64]	Psychotropic recreational substance use was associated with suicidal ideation.
Peltzer and Pengpid[65]	Psychotropic recreational substance use did not show a strong association with suicidal ideation.
Wilson et al.[66]	There was a strong association between suicidal ideation and illicit psychotropic recreational substance use for men.
Arenliu et al.[67]	Substance use was associated with higher levels of suicide risk (including suicidal ideation), without differences between the sexes.
Consoli et al.[68]	Psychotropic recreational substance use by adolescents was potentially related to higher rates of suicidal ideation and attempts.
Delfabbro et al.[69]	Psychotropic recreational substance abuse was positively correlated with suicidal ideation.
Govender et al.[70]	Illicit psychotropic recreational drug use was significantly associated with high levels of suicidal ideation among the adolescents in the study.
Rasic et al.[71]	Psychotropic recreational substance use by the adolescents analyzed showed a significant association with suicidal ideation.
Shilubane et al.[72]	Psychotropic recreational substance abuse was a strong risk factor for suicidal ideation and behavior, and this association increases with specific illicit drugs and the concomitant use of several substances.
Van Ours et al.[74]	Intensive cannabis use led to higher levels of suicidal ideation in men.
Wong et al.[73]	The levels of suicidal ideation were significantly higher among female adolescents with mental health problems and psychotropic recreational substance use and abuse.
Chabrol et al.[75]	Cannabis use was not a significant independent predictor of suicidal ideation after adjusting the confounding factors of the total sample and subsample of cannabis users.
Lowry et al.[76]	Psychotropic recreational substance use was significantly associated with the increased risk of suicide (including suicidal ideation) among the students.
Randall et al.[77]	There was an association between illicit psychotropic recreational drug use and suicidal ideation.
Zhang and Wu[78]	Psychotropic recreational drug use did not increase the risk of suicidal ideation, but suicidal ideation increased the risk of psychotropic recreational drug use.
Delfabbro et al.[79]	Cannabis use did not show a significant association with suicidal ideation, but it was related to suicidal planning by adolescents.
Dunlavy et al.[80]	Illicit psychotropic recreational drug use was associated with suicidal ideation and planning.
Gart and Kelly[81]	Cannabis and cocaine use showed a significant relationship with suicidal ideation.
Lee and Choi[82]	The use of psychoactive recreational drugs was associated with suicidal ideation among the different sexes and age groups of adolescents.
Peltzer and Pengpid[17]	Psychotropic recreational substance use was one of the factors associated with suicidal ideation for the adolescents studied.
Sampasa-Kanyinga et al.[83]	Adolescents who reported early psychotropic recreational drug use were more likely to report suicidal ideation.
Sharma et al.[84]	Cannabis use was positively associated with suicidal ideation.
Dudovitz et al.[85]	Illicit psychotropic recreational substance use did not show a significant association with suicidal ideation.
Ammerman et al.[86]	Psychotropic recreational substance use showed a higher association with suicidal ideation than other risk behaviors.
DeCamp and Bakken[87]	Psychotropic recreational substance use (cannabis and hard drugs) is significantly correlated with suicidal ideation among female heterosexual adolescents.
Price and Khubchandani[88]	Psychotropic recreational substance abuse was associated with high levels of suicidal ideation.
Weeks and Colman[90]	There was a positive relationship between psychotropic recreational substance use and suicidal ideation.
Agrawal et al.[91]	Psychotropic recreational substance use by adolescents without a history of depression increases the risk of suicidal ideation.
Asante et al.[92]	Early psychotropic recreational substance use by adolescents was not associated with suicidal ideation.
Janssen et al. [93]	Psychotropic recreational substance use was not considered a risk factor for suicidal ideation.
Wang and Yen[94]	The regular use of psychoactive substances presented a significant association with suicidal behavior (including suicidal ideation). This relationship was measured based on the mental health of adolescents.
El Kazdouh et al.[95]	Psychotropic recreational substance use by adolescents was significantly associated with suicidal ideation, without differences between the sexes.
Haskuka et al.[96]	Psychotropic recreational substance use was significantly associated with suicidal ideation for male adolescents, and this relationship increased with age.
Subica and Wu[97]	Cannabis use presented a relationship with suicidal ideation in three of the 13 countries participating in the study.
Chadi et al.[98]	Cannabis use showed a relationship with suicidal ideation only for some of the ethnic groups studied.
Dema et al., 2019[99]	Marijuana use was associated with a higher likelihood of suicidal ideation and depressive symptoms.
Georgiades et al.[100]	Psychotropic recreational drug abuse and the impulse to consume drugs were considered risk factors for suicidal ideation.
Jung et al.[101]	The use of cannabis and other illicit substances did not show a significant association with suicidal ideation, but it did with suicide attempts.
Baiden et al.[18]	Psychotropic recreational substance use was one of the factors associated with suicidal ideation.
Greene et al.[102]	Illicit psychotropic recreational substance use was one of the factors associated with suicidal ideation, as well as a history of sexual abuse, bullying, and tobacco consumption.
Khan et al.[103]	Psychotropic recreational substance use showed a significant association with suicidal ideation for both sexes.
Kim et al.[89]	Adolescents who used psychotropic recreational drugs were more likely to present suicidal behavior (including suicidal ideation).
Sakamoto et al.[104]	The use of hard psychotropic recreational drugs by male adolescents was associated with suicidal ideation.

Table 4. Risk of bias assessed according to the Joanna Briggs Institute (JBI) Critical Appraisal Tools for use in JBI Critical Appraisal Checklist for Analytical Cross-Sectional Studies

Author[ref]	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	% Yes	Risk
Kandel et al.[29]	--	√	--	√	√	√	--	√	62,5	Moderate
Felts et al.[30]	√	√	√	√	--	--	√	√	75	Moderate
Garrison et al.[31]	√	√	√	√	√	√	√	√	100	Low
Vega et al.[32]	√	√	√	√	--	--	√	√	75	Moderate
Madianos et al.[33]	√	√	√	√	√	√	--	√	87,5	Low
Burge et al.[34]	√	√	√	√	--	--	√	√	75	Moderate
Lopez et al.[35]	√	√	--	√	√	√	√	√	87,5	Low
Windle and Windle[36]	√	√	√	√	--	--	√	√	75	Moderate
Simon and Crosby[37]	√	√	√	√	√	√	√	√	100	Low
Perkins and Hartless[38]	--	√	√	√	√	√	√	√	87,5	Low
Vermeiren et al.[39]	√	√	√	√	√	√	√	√	100	Low
Hallfors et al.[40]	√	--	√	√	√	√	√	√	87,5	Low
Wu et al.[41]	√	√	√	√	√	√	√	√	100	Low
Yip et al.[42]	√	√	√	√	√	√	√	√	100	Low
Spremo and Loga[43]	--	√	√	√	--	--	√	√	62,5	Moderate
Ulusoy et al.[44]	√	√	√	√	--	--	√	√	75	Moderate
Chabrol et al.[46]	--	√	√	√	√	√	√	√	87,5	Low
Dunn et al.[45]	--	√	√	√	--	--	√	√	62,5	Moderate
Luncheon et al.[47]	√	√	√	√	√	√	√	√	100	Low
Peltzer et al.[48]	√	√	√	√	--	--	√	√	75	Moderate
Epstein and Spirito[49]	√	√	√	√	√	√	√	√	100	Low
Peter and Roberts[50]	√	√	√	√	√	√	√	√	100	Low
Pickles et al.[51]	√	√	√	√	√	√	√	√	100	Low
Florenzano et al.[52]	--	√	√	√	--	--	√	√	62,5	Moderate
Page et al.[53]	√	√	√	√	√	√	√	√	100	Low
Souza et al.[54]	√	√	√	√	√	√	√	√	100	Low
Wolitzky-Taylor et al.[55]	√	√	√	√	√	√	√	√	100	Low
Alwan et al.[56]	√	√	√	√	√	√	√	√	100	Low
Carvalho et al.[57]	√	√	√	√	√	√	√	√	100	Low
Eaton et al.[58]	√	√	√	√	√	√	√	√	100	Low
Kim et al.[59]	--	√	√	√	√	√	√	√	87,5	Low
Miller et al.[60]	√	√	√	√	√	√	√	√	100	Low
Swahn et al.[61]	√	√	√	√	√	√	√	√	100	Low
Ahmad et al.[62]	√	√	√	√	√	√	--	√	87,5	Low
Bakken and Gunter[63]	√	√	√	√	√	√	--	√	87,5	Low
Kokkevi et al.[64]	√	√	√	√	√	√	√	√	100	Low
Peltzer and Pengpid[65]	√	√	√	√	√	√	√	√	100	Low
Wilson et al.[66]	√	√	√	√	√	√	√	√	100	Low
Arenliu et al.[67]	√	√	√	√	√	√	√	√	100	Low
Consoli et al.[68]	√	√	√	√	--	--	√	√	75	Moderate
Delfabbro et al.[69]	√	√	√	√	√	√	√	√	100	Low
Govender et al.[70]	--	√	√	√	√	√	√	√	87,5	Low
Rasic et al.[71]	√	√	√	√	√	√	√	√	100	Low
Shilubane et al.[72]	√	√	√	√	√	√	√	√	100	Low
Van Ours et al.[74]	√	√	√	√	√	√	√	√	100	Low
Wong et al.[73]	√	√	√	√	√	√	√	√	100	Low
Chabrol et al.[75]	--	√	√	√	√	√	√	√	87,5	Low
Lowry et al.[76]	√	√	√	√	√	√	√	√	100	Low
Randall et al.[77]	√	√	√	√	√	√	√	√	100	Low
Zhang and Wu[78]	√	√	√	√	√	√	√	√	100	Low
Delfabbro et al.[79]	√	√	√	√	√	√	√	√	100	Low
Dunlavy et al.[80]	√	√	√	√	√	√	√	√	100	Low
Gart and Kelly[81]	√	√	√	√	√	√	√	√	100	Low

Continue...

Table 4. Continuation.

Author[ref]	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	% Yes	Risk
Lee and Choi[82]	√	√	√	√	√	√	√	√	100	Low
Peltzer and Pengpid[17]	√	√	√	√	√	√	--	√	87,5	Low
Sampasa-Kanyinga et al.[83]	√	√	√	√	√	√	√	√	100	Low
Sharma et al.[84]	√	√	√	√	√	√	√	√	100	Low
Dudovitz et al.[85]	√	√	√	√	√	√	√	√	100	Low
Ammerman et al.[86]	√	√	√	√	√	√	√	√	100	Low
DeCamp and Bakken[87]	√	√	√	√	--	--	√	√	75	Moderate
Price and Khubchandani[88]	√	√	√	√	√	√	√	√	100	Low
Weeks and Colman[90]	√	√	√	√	√	√	√	√	100	Low
Agrawal et al.[91]	√	√	√	√	√	√	√	√	100	Low
Asante et al.[92]	√	√	√	√	√	√	√	√	100	Low
Janssen et al[93]	√	√	√	√	√	√	√	√	100	Low
Wang and Yen[94]	√	√	√	√	√	√	√	√	100	Low
El Kazdouh et al.[95]	√	√	√	√	√	√	√	√	100	Low
Haskuka et al.[96]	√	√	√	√	√	√	√	√	100	Low
Subica and Wu[97]	√	√	√	√	√	√	√	√	100	Low
Chadi et al.[98]	√	√	√	√	√	√	√	√	100	Low
Dema et al., 2019[99]	√	√	√	√	√	√	√	√	100	Low
Georgiades et al.[100]	√	√	√	√	√	√	√	√	100	Low
Jung et al.[101]	√	√	√	√	√	√	√	√	100	Low
Baiden et al.[18]	√	√	√	√	√	√	√	√	100	Low
Greene et al.[102]	√	√	√	√	√	√	√	√	100	Low
Khan et al.[103]	√	√	√	√	√	√	√	√	100	Low
Kim et al.[89]	√	√	√	√	√	√	√	√	100	Low
Sakamoto et al.[104]	√	√	√	√	√	√	√	√	100	Low

Q1 = Were the inclusion criteria in the sample clearly defined?; Q2 = Were the study subjects and the setting described in detail?; Q3 = Was exposure measured validly and reliably?; Q4 = Were objective and standard criteria used for measuring the condition?; Q5 = Were confounding factors identified?; Q6 = Were strategies to deal with confounding factors stated?; Q7 = Were the outcomes measured validly and reliably?; Q8 = Was appropriate statistical analysis used?; √ = Yes; -- = No; NA = Not Applicable; U = Unclear.

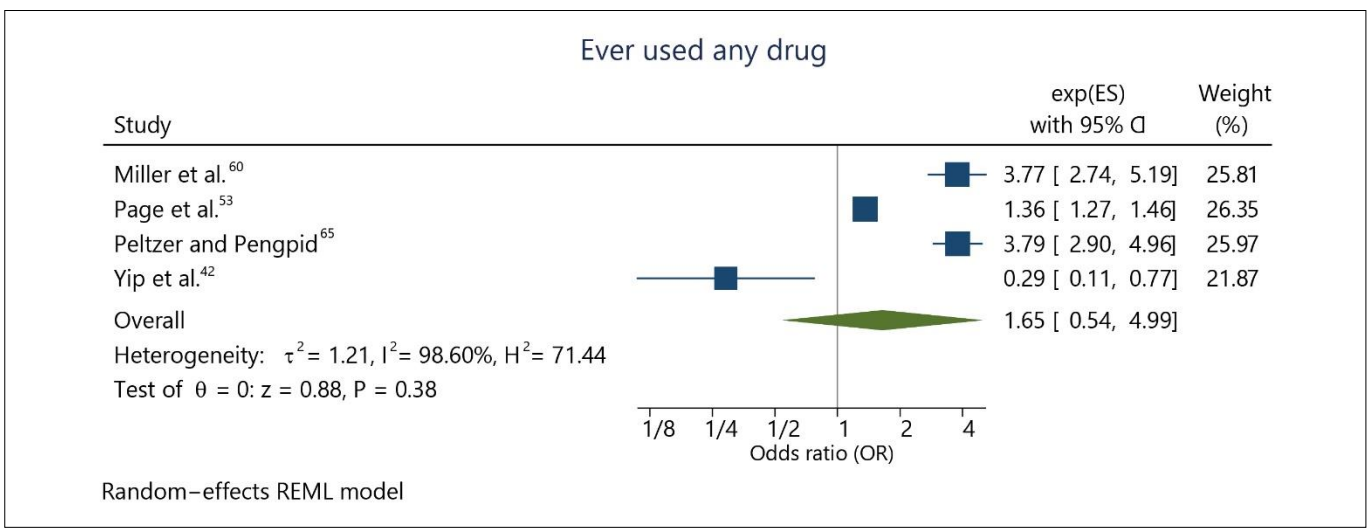

Figure 2. Meta-analysis assessing the use of any illicit drug at some point in life and suicide ideation.

Cocaine use

Only three studies[47,73,88] analyzed the likelihood of suicide ideation relative to cocaine use. Suicide ideation was 2.57 times more likely (95%CI = 1.47; 4.50; I^2 = 96.0%) for adolescents who used cocaine at some point in their lives than for those who never used the drug (**Figure 8**).

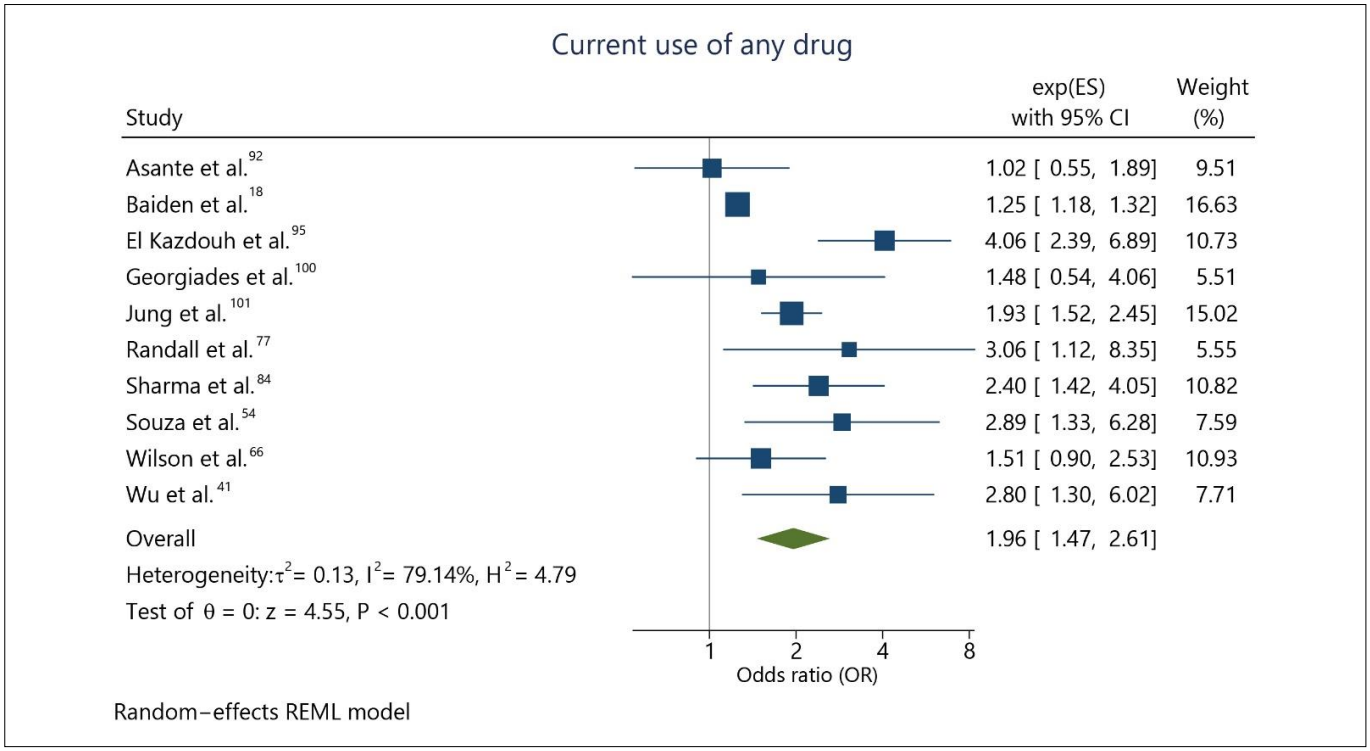

Figure 3. Meta-analysis assessing the recurrent use of any illicit drug and suicidal ideation.

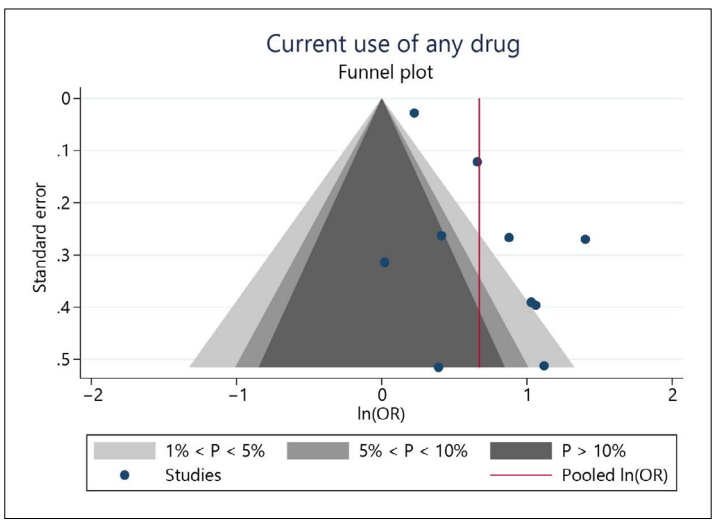

Figure 4. Funnel plot indicating a potential risk of publication bias in the recurrent use of any illicit drugs analysis.

Certainty of evidence

All outcomes indicated a very low certainty of evidence (**Table 5**), downgraded due to inconsistency, imprecision, or potential publication bias.

DISCUSSION

This systematic review and meta-analysis aimed to investigate the association between suicide ideation and psychotropic recreational drug use among adolescents. The findings indicate that drug use is associated with higher odds of suicide ideation in adolescents.

Suicide ideation among adolescents is a prevalent social problem globally[20,105], with varying prevalence rates observed across studies conducted in different countries. For instance, a Korean study conducted between 2018 and 2019 found that approximately 4.0% of adolescents reported having contemplated suicide, with about 3.0% attempting suicide, and substance use was identified as a contributing risk factor.[105] Similarly, in China, the prevalence of suicide ideation among the young population was reported at 26.4%, with higher rates observed among those with a smoking habit.[106]Canadian adolescents experienced a surge in suicidal ideation, reaching 44% during the coronavirus disease 2019 (COVID-19) pandemic.[107] A multicenter study comprising 77 countries indicated a weighted prevalence of 18% for suicide ideation among adolescents.[108] Thus, suicide ideation can be considered a frequent event among youth, warranting urgent attention, especially considering that adolescents with suicide ideation are reportedly 12 times more likely to attempt suicide by the age of 30.[109]

While some studies suggest a higher prevalence among female adolescents,[29,62,87,110] others demonstrated a higher prevalence among male adolescents.[67,74,95,111,112] Studies that did not verify differences between the sexes were also identified.[94,102] This lack of consensus may arise from cultural factors[113] or may signify the absence of inherent differences between the sexes.

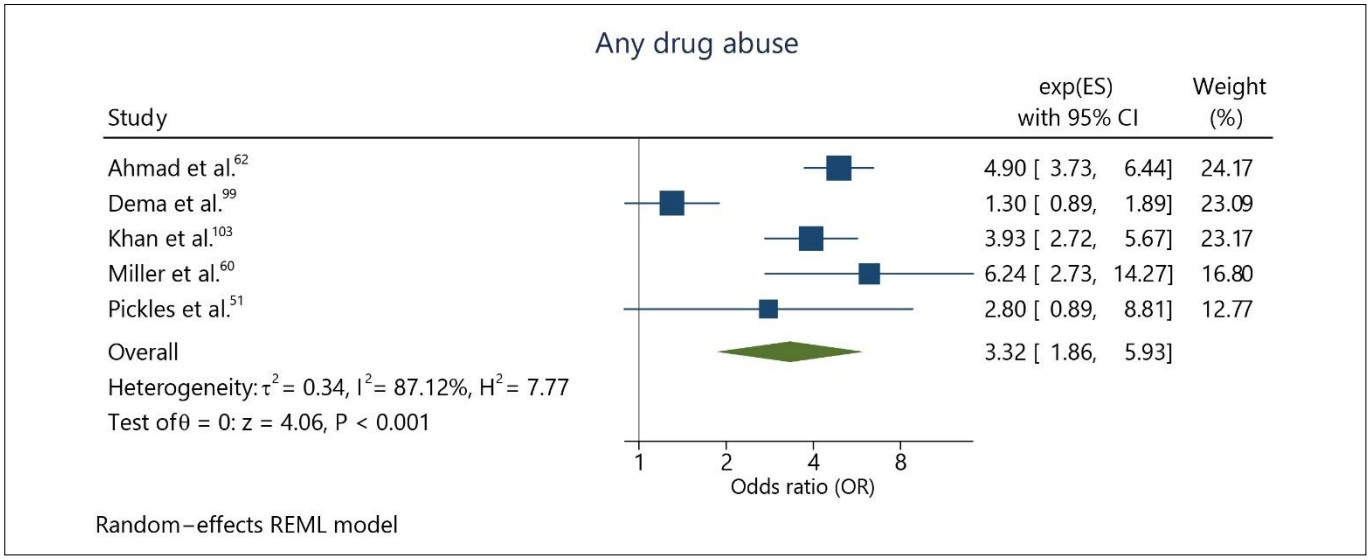

Figure 5. Meta-analysis assessing the abuse of any illicit drug and suicidal ideation.

Overall, the analyses conducted in this review indicated that adolescents who engage in psychotropic recreational drug use are more susceptible to experiencing suicide ideation. Furthermore, the meta-analysis results suggest that the higher the frequency of substance use, the higher the likelihood of suicidal ideation. This correlation can be attributed to various factors associated with adolescent drug use, including depression, stress throughout life, familial conflicts, and exposure to bullying and moral abuse in school settings, all of which act as risk factors for suicide ideation. However, two studies included in the meta-analysis did not identify statistically significant differences in suicide ideation between adolescents who did or did not use psychotropic recreational drugs .[66,92] Notably, these two studies were performed in African countries and indicated the possibility of data underreporting, considering the low number of positive answers to psychotropic recreational drug use.

Additionally, the meta-analysis revealed that cannabis was linked to an increased likelihood of developing suicidal ideation among adolescents. Chronic cannabis use during adolescence has been associated with the onset of mental disorders,[114,] and including psychosis, schizophrenia, schizoaffective disorders, and a high risk of mood disorders.[115] Cannabis use before the age of 15 years and frequent recent use further increase the risk of suicidal ideation.[114] Moreover, cannabis use was associated with a higher prevalence of suicidal ideation and deliberate self-harm among Canadian adolescents evaluated during the COVID-19 pandemic.[107]

The meta-analysis results potentially indicate that cocaine use is associated with suicide ideation. However, the high heterogeneity and low number of studies assessing this outcome suggest that further studies are required to confirm this association. Exposure to cocaine starting in adolescence increases vulnerability to developing drug dependence and decreases individuals' likelihood of seeking treatment.[116] Moreover, cannabis and cocaine use are associated with a higher prevalence of the first psychotic episode and the incidence of the first prodromal or psychotic symptom earlier than individuals who only use tobacco.[117]

The analysis could not fully explore the relationship between suicide ideation and other drugs, such as LSD, heroin, or methamphetamine, due to the small number of studies specifying the type of substance used. Further studies using detailed questionnaires to differentiate between the types of drugs used are warranted.

In the public health context, developing robust surveillance systems for suicide prevention is imperative.[118] Various policies, including firearm access restrictions, awareness of communication media on the importance of addressing the topic correctly, identification of people at risk, [13] and the provision of psychotherapeutic, pharmacological, or neuromodulatory treatment, and training of health professionals to identify people at risk for suicide and offer proper care, are essential for effective suicide prevention efforts.[119]

Programs for drug use prevention targeting adolescent drug use should incorporate discussions on bullying (both practicing and being a victim)[9] and strategies for addressing associated problems, such as interpersonal conflicts, anxiety, loneliness, an intimidating environment, a lack of support from parents and close friends, sedentary behavior,[108] and drug use, as evidenced in the present systematic review. A systematic review and meta-analysis concluded that the development of interpersonal skills, emotional regulation, and alcohol and drug education significantly impacted mental health[120]. Therefore, this intervention should

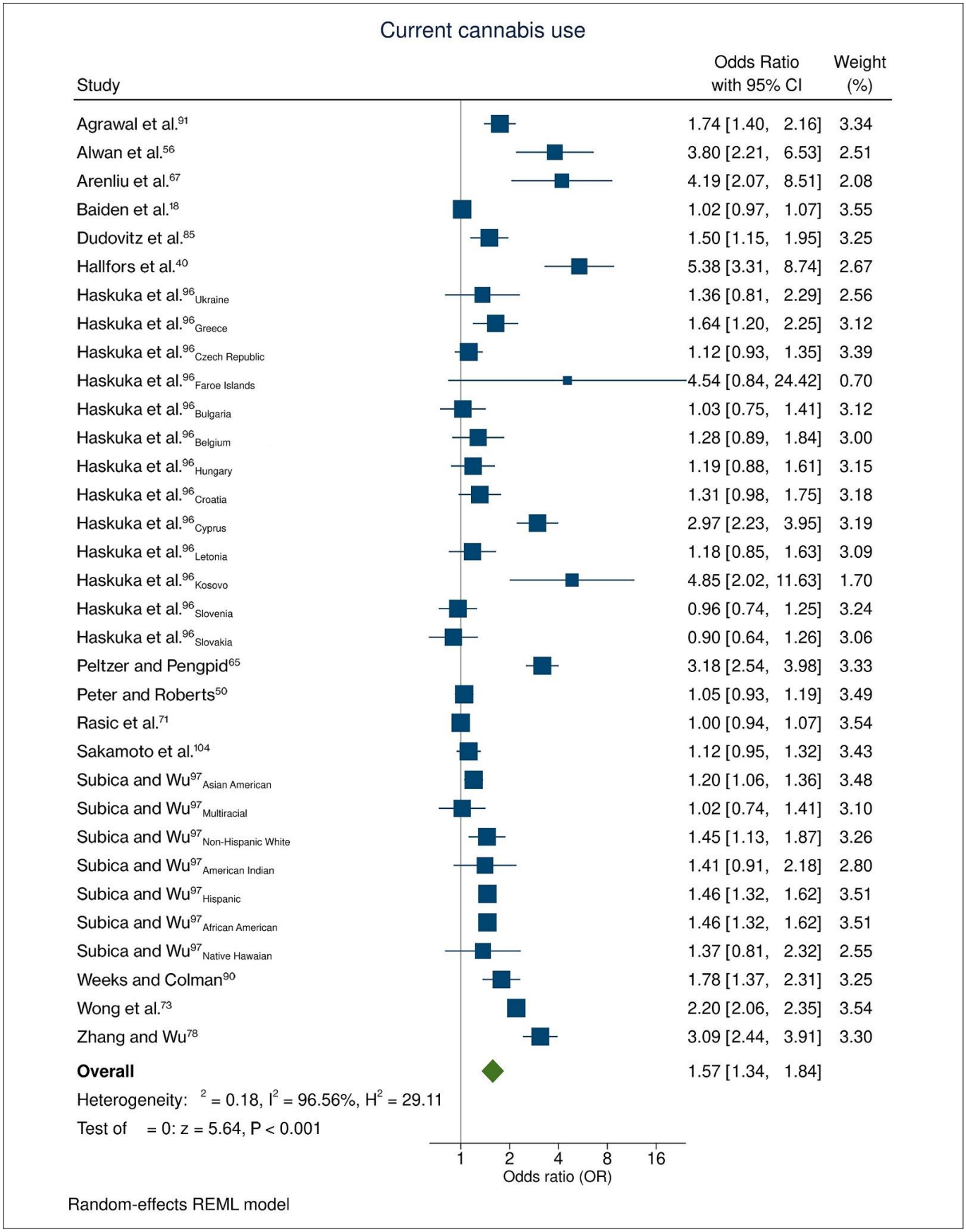

Figure 6. Meta-analysis assessing the recurrent use of cannabis and suicidal ideation.

start in childhood to increase an individual's ability to deal with adverse and unwanted situations.

School-based programs, like the 'Unplugged' program, comprising 12 one-hour interactive sessions by trained teachers to address social and personal skills and attitudes towards drugs, were implemented in Europe (Italy, Greece, Spain, Austria, Belgium, Germany, and Sweden) in 2003, presenting promising results in decreasing tobacco and cannabis use among adolescents.[121] However, adaptations of such programs, like the "#tamojunto' project in Brazilian schools, did not present a difference in substance use between the experimental and the control group.[122]

A potential limitation of this study could be the presence of social desirability bias, in which participants may have been inclined to provide socially acceptable answers, potentially distorting the accuracy of their answers to conform to societal norms.[123] Consequently, there may have been an underreporting of adolescents acknowledging drug use. Moreover, most the majority of eligible studies did not specify whether adolescents were screened for substance use, potentially excluding individuals who had previously used psychoactive substances from the analysis. Another factor that may undermine the generalizability of our results is the predominance of the eligible studies conducted in high-income countries. Given the multifactorial origins of suicidal ideation and drug use, further research is needed to comprehensively elucidate these factors, particularly within diverse socioeconomic contexts[124].

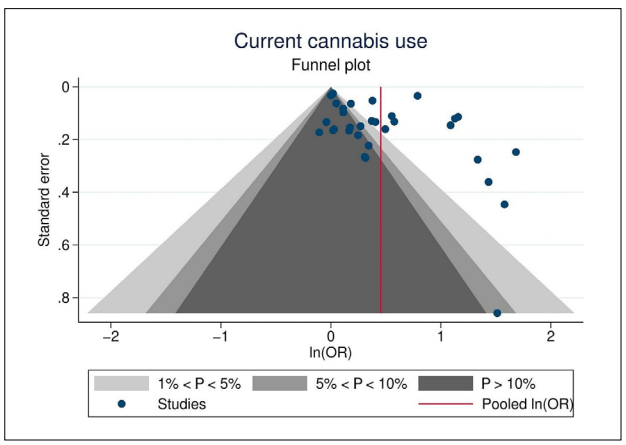

Figure 7. Funnel plot indicating a potential risk of publication bias in the recurrent use of cannabis analysis.

Despite these limitations, our results are relevant because this is the first systematic review investigating the relationship between psychotropic recreational drug use and suicide ideation among adolescents. Additionally, we should emphasize the large number of eligible studies,[78] including gray literature, and the assessment of results from studies performed worldwide. Further studies in developing countries are suggested, especially in Africa and Latin America, for better analysis and extrapolation of results, and more specific details regarding the use and specific types of drugs.

CONCLUSIONS

This systematic review confirmed the association between psychotropic recreational drug use and suicide ideation among adolescents, irrespective of their current or previous use, abuse, or specific type of substance used. Adolescents who currently use cannabis or cocaine exhibit a higher likelihood of experiencing suicide ideation than those who do not engage in psychotropic recreational drug use. Policymakers and health professionals should be aware that suicidal behavior is multifaceted and not solely attributed to substance use or abuse. Furthermore, both suicide ideation and psychotropic recreational drug use share common predictors, underscoring the interwoven nature of these phenomena.

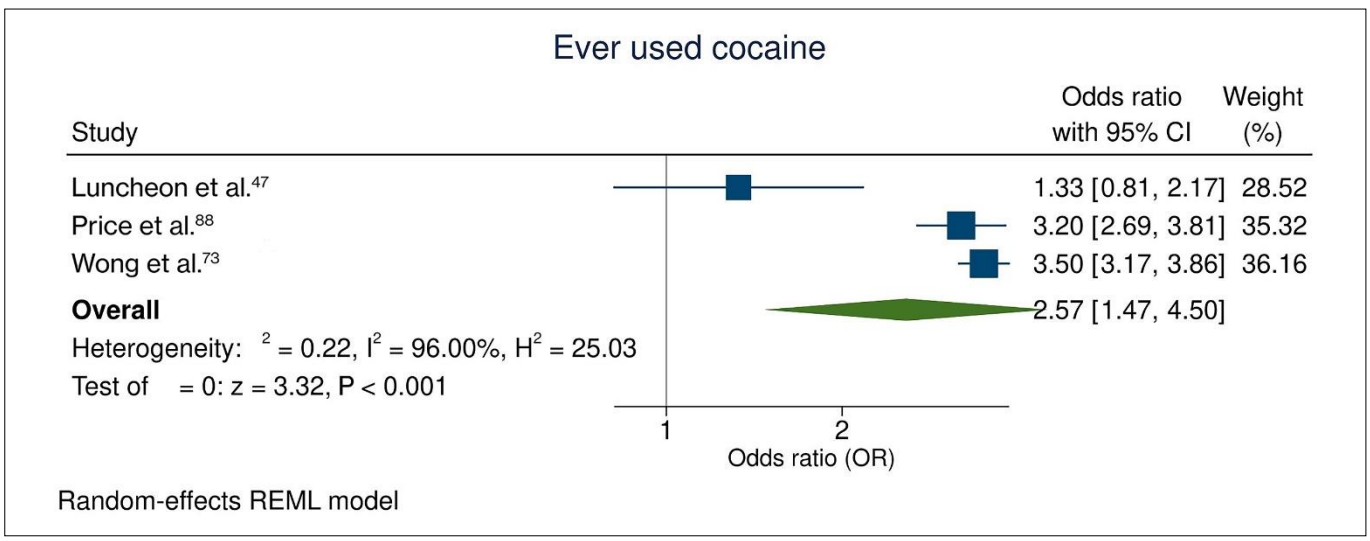

Figure 8. Meta-analysis assessing the use of cocaine and suicide ideation.

Table 5. Grading of Recommendations Assessment, Development, and Evaluation (GRADE) Summary of Findings Table for the Outcomes of the Systematic Review

Certainty assessment							Effect	Certainty
Number of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Relative (95% CI)	
Current use of any illicit drug vs. no use								
10	observational studies	not serious	serious[a]	not serious	not serious	Publication bias detected	OR 1.96 (1.47 to 2.61)	⊕◯◯◯ VERY LOW
Ever used any illicit drug vs. no use								
4	observational studies	not serious	serious[a]	not serious	very serious[b]	none[d]	OR 1.65 (0.54 to 4.99)	⊕◯◯◯ VERY LOW
Any illicit drug abuse vs. no use								
5	observational studies	not serious	serious[a]	not serious	serious[c]	none[d]	OR 3.32 (1.86 to 5.93)	⊕◯◯◯ VERY LOW
Current cannabis use vs. no use								
15	observational studies	not serious	serious[a]	not serious	not serious	Publication bias detected	OR 1.57 (1.34 to 1.84)	⊕◯◯◯ VERY LOW
Ever used cocaine vs. no use								
3	observational studies	not serious	serious[a]	not serious	serious[c]	none[d]	OR 2.57 (1.47 to 4.50)	⊕◯◯◯ VERY LOW

CI = confidence interval; OR = odds ratio, [a] high unexplained statistical heterogeneity ($I^2 > 50\%$) and/or no overlapping of effect estimates – Rated down by one level; [b] confidence interval suggests trivial no association in one extreme and strong association in another – Rated down by two levels; [c] confidence interval suggests trivial association in one extreme and strong association in another – Rated down by one level; [d] publication bias was not assessed due to the low number of studies.

GRADE Working Group grades of evidence

High certainty: Very confident that the true effect is close to the effect estimate.

Moderate certainty: Moderately confident in the effect estimate: The true effect is likely close to the effect estimate but may be substantially different.

Low certainty: The confidence in the effect estimate is limited: The true effect may differ substantially from the effect estimate.

Very low certainty: Very little confidence in the effect estimate: The true effect may differ substantially from the effect estimate.

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Case report of scrub typhus complicated by hypokalemia and multiple organ dysfunction syndrome

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KEYWORDS (MeSH terms):
Scrub typhus.
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Hypokalemia.

AUTHORS' KEYWORDS:
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Hypokalemia.
Tsutsugamushi disease.

ABSTRACT

CONTEXT: Scrub typhus, caused by *Orientia tsutsugamushi*, has a wide range of clinical manifestations, including meningoencephalitis, acute renal failure, pneumonitis, myocarditis, and septic shock. However, there are no documented cases of scrub typhus with hypokalemia. In this report, we present a case of scrub typhus with hypokalemia and multiple organ failure syndrome, highlighting the importance of electrolyte imbalance in patients with scrub typhus.

CASE REPORT: A 59-year-old woman presented to the emergency department with abdominal pain that had been present for 1 day. On admission, the physical examination and laboratory test results indicated that the patient had renal, liver, and circulatory failure, and hypokalemia. She developed meningitis and disseminated intravascular coagulation during hospitalization. She recovered with appropriate management, and was discharged on day 17.

CONCLUSION: This report highlights the potential for atypical presentations of scrub typhus, including a previously undocumented association with hypokalemia. Although the contribution of hypokalemia to the patient's clinical course remains uncertain, this case underscores the importance of considering electrolyte imbalance in the management of patients with scrub typhus. Further research is warranted to better understand the relationship between scrub typhus and electrolyte imbalance.

INTRODUCTION

Scrub typhus, caused by *Orientia tsutsugamushi*, is a significant public health concern in the Asia-Pacific region.[1] Patients may present with various signs of infection 5 to 14 days after a bite from an infected vector.[1] Severe scrub typhus can result in a range of complications, including jaundice, acute renal failure, pneumonitis, acute respiratory distress syndrome, myocarditis, septic shock, meningoencephalitis, pericarditis, and disseminated intravascular coagulation (DIC).[2] Despite the effectiveness of tetracycline, delayed therapy and complications are associated with a 30% mortality rate.[3]

In clinical practice, hypokalemia is a common electrolyte imbalance is a typical clinical manifestation, characterized by weakness.[4] Severe hypokalemia can lead to life-threatening complications such as malignant arrhythmia and respiratory muscle paralysis.[4] However, to our knowledge, the occurrence of hypokalemia in the context of scrub typhus and its association with multiple organ dysfunction syndrome has not been previously reported. Herein, we present a case of scrub typhus complicated by hypokalemia and multiple organ dysfunction syndrome.

CASE REPORT

This study was approved by the Institutional Review Board of the Guangdong Provincial Hospital of Chinese Medicine on May 22, 2023 (G2023-13). Written informed consent was obtained from the patient for the publication of this case report and the accompanying images.

The patient, a 59-year-old woman with a history of hypertension presented to the emergency department of a traditional Chinese medicine hospital in Guangdong Province on June 26, 2022 (day 0) with mild abdominal pain, which had been present for 1 day. On admission, her vital signs included a blood pressure of 75/53 mmHg, a heart rate of 85 beats per minute, a body temperature of 36.6°C, and a respiratory rate of 22 breaths per minute. Laboratory test results revealed a

platelet count of 71×10^9 platelets/L and a white blood cell count of 8.92×10^9 cells/L. The levels of C-reactive protein (239.10 mg/)L, pro-calcitonin (3.97 ng/mL), and blood lactic acid (4.96 mmol/L) were elevated. Liver function tests showed elevated levels of alanine aminotransferase (ALT) (178 U/L), aspartate aminotransferase (AST) (197 U/L), total bilirubin (23.4 µmol/L, and direct bilirubin at 21.3 µmol /L, and decreased levels of total protein (60.0 g/L), and albumin (33.4 g/L). Electrolyte imbalances were also noted, with decreased blood potassium and sodium levels of 2.62 mmol/L and 128 mmol/L, respectively. Creatinine was elevated at 272 µmol/L, and urea was 10.40 mmol/L. Coagulation parameters showed a prolonged prothrombin time of 14.3 s, an elevated activated partial thromboplastin time of 36.8 s, an international normalized ratio of 1.26, and an elevated D-dimer level of 12.64 mg/L. Computed tomography of the chest and abdomen revealed a renal calculus on the left side. Based on the clinical history, laboratory test results, and imaging findings, preliminary diagnoses of septic shock and multiple organ dysfunction syndrome (MODS) involving the liver, kidney, circulation, blood, acute liver failure, acute kidney failure, hypertension, and electrolyte disorders (hypokalemia and hyponatremia) were made. The patient was admitted to the Emergency Intensive Care Unit for further management.

On physical examination, the patient's body was found to be covered with small purple skin lesions, and an eschar was present on her left buttock (**Figure 1**). Given the strong suspicion of scrub typhus, a blood sample was sent for metagenomic next-generation sequencing (mNGS) and quantitative polymerase chain reaction (qPCR) to confirm the diagnosis. Due to worsening hypoxemia and oliguria on day 1, ventilator support and continuous renal replacement therapy were initiated. Antibiotic treatment was modified to doxycycline 100 mg twice daily, considering the patient's severe condition with MODS and suspected scrub typhus. Supportive measures, including platelet transfusion, liver protection, acid inhibition, and maintenance of homeostasis, were also provided.

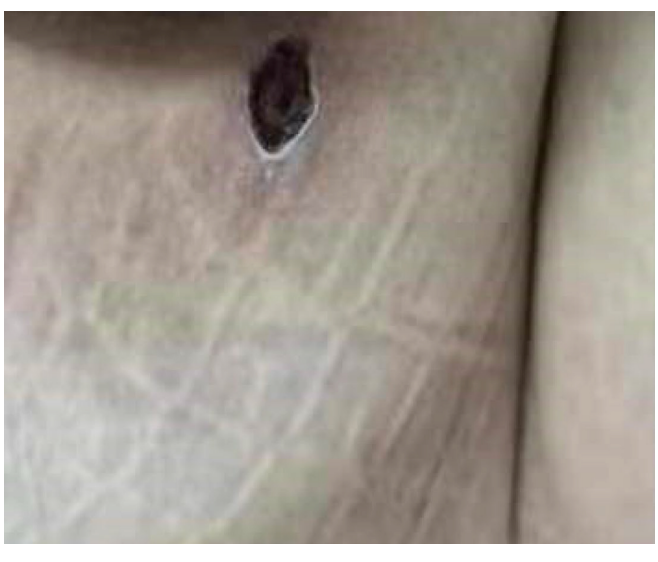

Figure 1. Eschar on the left buttock on day 0.

The patient's condition deteriorated on day 2, with worsening of her level of consciousness, disappearance of physiological reflexes, limb convulsions, swelling of the face and limbs, and worsening ecchymosis in the lower limbs. Laboratory test results showed a decreasing platelet count, an increasing white blood cell count, and abnormal liver function tests with decreasing ALT levels and increasing AST, total bilirubin, direct bilirubin, total protein, and D-dimer levels. The mNGS and qPCR results (**Figure 2**) confirmed the presence of *O. tsutsugamushi*, suggesting that scrub typhus was the underlying cause.

The patient received platelet transfusions and appropriate management, and by day 8, she had started to recover with an improved level of consciousness (Glasgow Coma Scale: E4VTM5). She developed blisters and worsening ecchymoses on the lower legs, scattered bruises, and minor skin lesions all over her body (**Figure 3**). On day 17, she was transferred to the Rehabilitation Department for further rehabilitation and was discharged 2 days later.

DISCUSSION

The patient was diagnosed with scrub typhus and experienced multiple complications including renal failure, liver failure, circulatory failure, pneumonia, encephalitis, and DIC. Hypokalemia, was present on admission. The prevalence of hypokalemia in patients with scrub typhus varies in different research reports, ranging from 10% in South India[5] to 22.9% in South Korea[6]; however, to our knowledge, hypokalemia has not previously been reported in patients in China. The exact cause of hypokalemia in patients with scrub typhus is not well understood; however, several factors could potentially contribute to its development.

First, the patient had a history of hypertension and was taking blood pressure medication; however, specific details of the medication were not available. Some of the diuretics commonly prescribed to manage hypertension can cause hypokalemia by increasing the excretion of potassium in the urine. This could explain the hypokalemia observed in this patient.

Second, the patient had a history of mild abdominal pain and reduced appetite, which could have resulted in a decreased intake of potassium-rich foods. Additionally, the patient received fluid replacement therapy to address shock, which potentially resulting in fluid dilution and subsequently contributing to the development of hypokalemia.

Third, the renal tubular acidosis and the effects of scrub typhus on ion channels that may have disrupted the serum potassium balance. The patient's serum potassium levels recovered, so no further investigations were conducted to determine the cause of the hypokalemia.

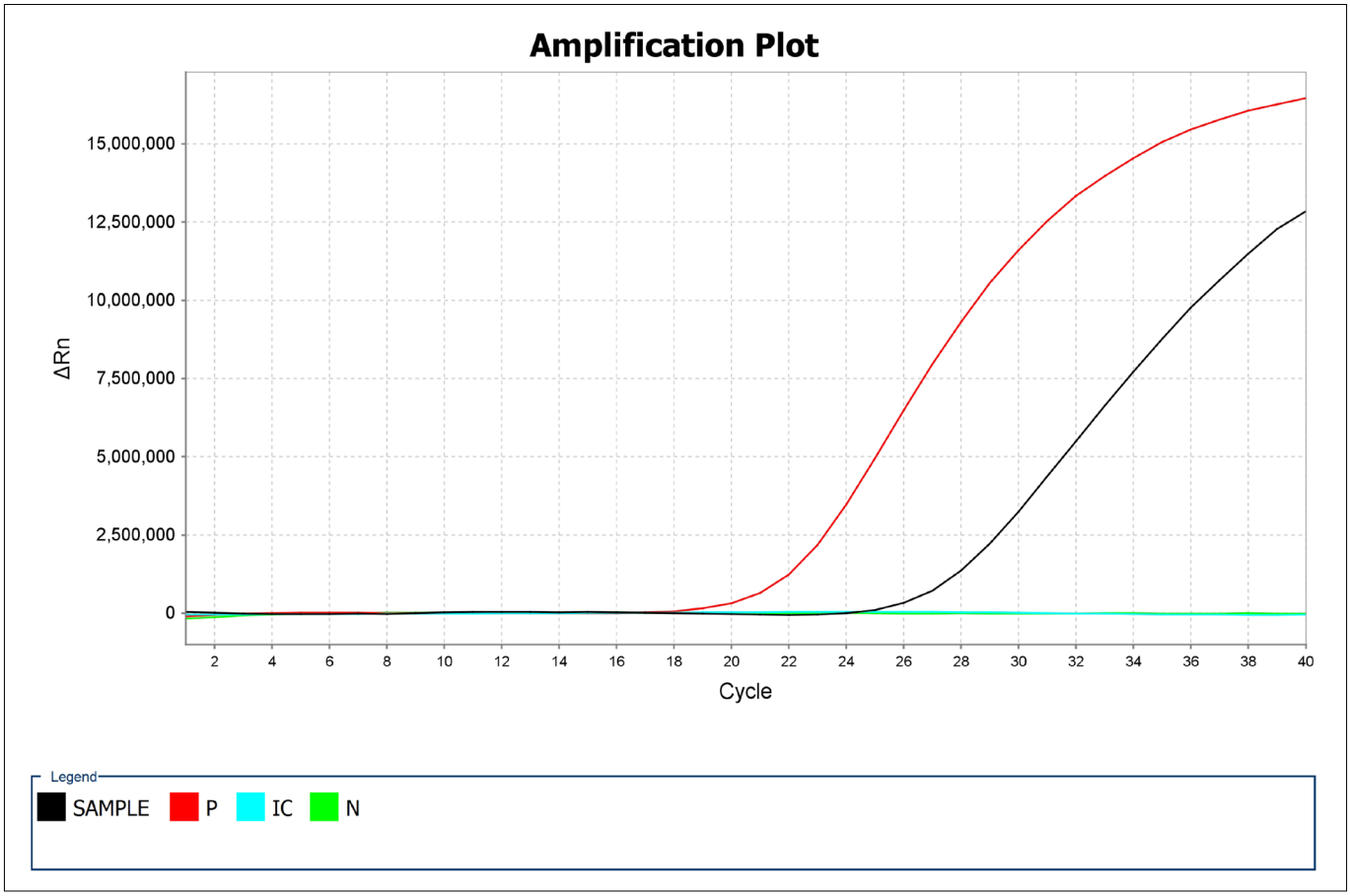

Figure 2. The quantitative polymerase chain reaction (qPCR) test results: The black line shows the amplification of the *Orientia tsutsugamushi* DNA in the test sample obtained from the patient's plasma; the red line shows the positive control sample; and the green line shows the negative control sample.

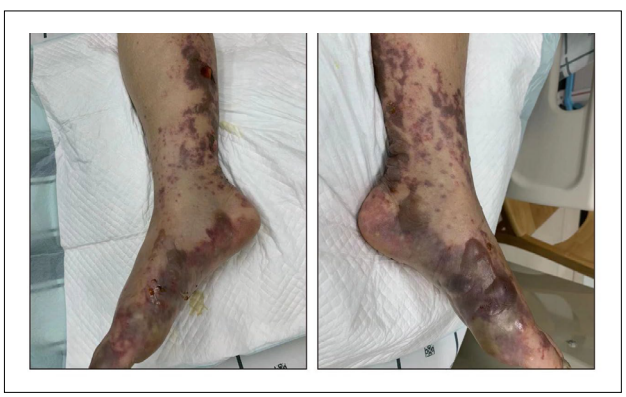

Figure 3. Blisters and ecchymoses on the patient's lower legs on day 8.

Hypokalemia can cause weakness and other symptoms, and can be life-threatening if left untreated.[7] Hypokalemia has also been reported in other infectious diseases, including COVID-19).[4]

In summary, although the exact cause of hypokalemia in this patient with scrub typhus remains unclear, it is likely multifactorial and may be related to factors such as fluid balance, medication use, renal tubular acidosis, and disease mechanisms. Further research and investigations are warranted to better understand the pathophysiology of hypokalemia in patients with scrub typhus and to guide appropriate management strategies. This report highlights the importance of monitoring and managing electrolyte abnormalities in patients with scrub typhus and other critical conditions.

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Utilization of dapsone and hemoglobin in the epithelial skin regeneration therapy of cutaneous loxoscelism: A case report and integrative literature review

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ABSTRACT

BACKGROUND: *Loxosceles spp* are arthropods found worldwide. Its bite may produce cutaneous loxoscelism (necrotic or edematous) or cutaneous-visceral loxoscelism. Depending on their severity and location, cutaneous forms are managed with local cold application and systemic administration of antihistamines, corticosteroids, antibiotics, polymorphonuclear inhibitors, and analgesics.

OBJECTIVE: This study aimed to report a case of cutaneous loxoscelism and to identify the main dermatological manifestations associated with the *Loxosceles spp* bite.

DESIGN AND SETTING: This case report and literature review was conducted in a Mexican university.

METHODS: A detailed report on the medical management of a patient with cutaneous loxoscelism treated at the emergency department of a public hospital was published. Scopus, PubMed, Web of Science, and Google Scholar databases were searched to identify articles reporting cutaneous loxoscelism. The following keywords were used during the database search: "loxoscelism" OR "spider bite," OR "loxosceles" OR "loxosceles species" OR "loxosceles venom" OR "loxoscelism case report" AND "cutaneous" OR "dermonecrotic arachnidism."

RESULTS: A 62-year-old female patient with cutaneous loxoscelism was treated with systemic dapsone and local heparin spray. Eighteen studies with 22 clinical cases were included in this systematic review. Of the 22 patients, 12 (54.5%) were men. *L. rufescens* was the predominant spider species.

CONCLUSIONS: The administration of dapsone and heparin for the management of cutaneous loxoscelism demonstrated success in this case, with no sequelae observed. In general, the literature review indicated favorable outcomes in patients treated with antimicrobials and corticosteroids, with continuous healing of skin lesions.

SYSTEMATIC REVIEW REGISTRATION: PROSPERO ID CRD42023422424 (https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42023422424).

INTRODUCTION

Currently, more than 40,000 species of the order Araneae (grouped into approximately 4,205 genera and 128 families) have been identified. Spiders are distributed worldwide and cohabit with humans.[1,2] Despite thousands of identified spider species, only a few are of clinical interest. Although some spider bites can cause severe or fatal poisoning in humans, most cause minor skin lesions. Only two spider bite syndromes are of clinical importance with a global distribution: latrodectism (caused by *Latrodectus* spp.) and loxoscelism (caused by *Loxosceles* spp.).[1-3]

Loxoscelism is the result of poisoning due to the injection of venom from *Loxosceles* spiders (solitary, recluse, fiddle-back, or brown spiders). Loxoscelism is classified as cutaneous, systemic, or viscerocutaneous.[1,4] Cutaneous loxoscelism is the most prevalent form (85%), while viscerocutaneous is less common. Cutaneous loxoscelism can manifest as a flat erythematous plaque at the bite site and a sunken necrotic lesion of variable depth with benign evolution. Approximately 2–3 hours following a bite by *Loxosceles* spider, soft pain, erythema, and cyanosis may develop, with blisters or vesicles appearing on the skin. After several hours, the lesion became hemorrhagic and painful, accompanied by edema, erythema, ischemia, and thrombosis. An irregular area of ecchymosis or livedoid plaque with characteristic coloration (often resembling a bull's eye or displaying red, white, and blue signs) emerged. Several days after the bite, the lesion may progress into a deep necrotic area, forming a dry necrotic eschar with sharp borders. Necrotic tissue sloughs off after

a few weeks, leaving an ulcer with granulation tissue, which can take weeks or months to heal depending on the depth and extent of the lesion. Generally, no secondary infection occurs, and the lesion completely heals, leaving only a scar with variable characteristics.[1,4,5] Patients with cutaneous loxoscelism may not present any systemic alterations or symptoms; however, generalized pruritus, arthralgia, headache, nausea, vomiting, and low-grade fever may occur.[1,3-5] Edematous loxoscelism (5%), classified as a cutaneous type, is the most benign form. In such cases, the bite usually on the face results in extensive edema, erythema, and scant necrosis. By contrast, systemic loxoscelism (10%), in addition to the local dermonecrotic lesions with hemolysis, is accompanied by metabolic alterations, acute renal, pulmonary, and hematological damage (hemolytic anemia and coagulation disorders). It is lethal in approximately 15% of patients, primarily due to acute renal failure and disseminated intravascular coagulation.[1,3-5]

Loxoscelism is caused by the venom of *Loxosceles* spiders, with more than 100 species distributed across all continents. According to the findings of clinical case reports examining spider bites, *L. laeta, L. deserta, L. reclusa, L. gaucho, and L. intermedia* are the most important species.[1,3-6] The venom of *Loxosceles* spiders contains sphingomyelinase-D (the main dermonecrotic and hemolytic factor), hyaluronidase, metalloproteases, 5 nucleotidases, collagenase, esterase, phospholipases, 5' ribonucleotide phosphohydrolase, and alkaline phosphatase.[7,8] The occurrence of hemolysis is mediated by complement activation and cytokine release, resulting in a clinical presentation resembling endotoxic shock. Inoculation with *Loxosceles* venom increases the concentrations of tumor necrosis factor, interleukins (ILs) 6 and 10, granulocyte-macrophage colony-stimulating factor, and nitric oxide.[3,7,8] Edema, vascular endothelium thinning, inflammatory cell accumulation, vasodilation, coagulation, vascular wall degeneration, and hemorrhage occur in the bite area. These features are associated with vasculitis and contribute to tissue necrosis. Ceramides in the skin released by sphingomyelinase promote platelet adhesion and thrombus formation, which cause further alterations in the microcirculation. The inflammatory process and vasculitis with thrombus formation are the primary causes of local necrotic lesions. This leads to intravascular coagulation and areas of ischemia interspersed with hemorrhage, resulting in the distinctive marbled or livedoid plaque lesion characteristic of loxoscelism.[3,5,7,8]

The standard treatment of loxoscelism has not been established, and the approach depends on the type (cutaneous or systemic), time of the bite, visit to health services, clinical evolution of the patient, and probable complications. In cutaneous cases, local cold application, rest, elevation of the extremity if possible, and systemic pharmacotherapy with polymorphonuclear inhibitors (such as dapsone), and analgesics are recommended.[4,5,9] The application of anti-loxosceles serum and hyperbaric oxygen may be indicated.[4,5,9] In cases of systemic loxoscelism, it is crucial to closely monitor the renal and hepatic functions, correct severe hemolysis with blood products, administer bicarbonates to manage hemoglobinuria, and perform dialysis in the event of renal failure.[4,5,9,10]

CASE REPORT

A 62-year-old female patient with a history of systemic arterial hypertension, managed pharmacologically for the past 5 years, received losartan 50 mg and metoprolol 50 mg twice daily. The patient experienced a myocardial infarction 4 years ago and a transient cerebrovascular event a month earlier, without significant sequelae from either event. The patient was treated with acetylsalicylic acid 100 mg and atorvastatin 40 mg once a day.

The patient arrived at the emergency department of our hospital with a spider bite on the inner region of the left thigh and severe burning pain. Upon examining a photograph of the spider, it was identified as an arachnid from the Sicariidae family and the genus *Loxosceles*.

A physical examination of the patient revealed a blood pressure of 113/74 mmHg, a heart rate of 90 beats per minute, a respiratory rate of 18 per minute, and a temperature of 36.2°C. Neurological assessment revealed the absence of alterations in the sensory or motor responses and showed no signs of focalization or frontalization. The laboratory tests yielded normal results according to the patient's characteristics.

Sixteen hours after the bite, the patient presented with an erythematous lesion measuring 2 cm in diameter with an indurated erythematous perilesional area or a bull's-eye measuring 16 cm in diameter, with irregular edges and considerable pain upon palpation (**Figure 1A**). Treatment was initiated with dapsone 1 mg/kg

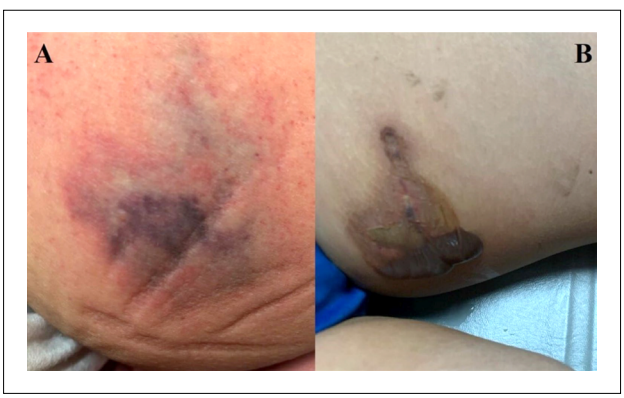

Figure 1. Skin lesions resulting from the bite of the *Loxosceles* spider. A: Lesion located in the inner region of the left thigh, measuring approximately 4 cm in diameter, exhibiting an undefined appearance with the presence of a livedoid plaque characterized by areas of erythema, ischemia, and necrosis, from the periphery to the center. B: Formation of vesicles with serohematic content, with irregular borders, delimited by an area of hyperpigmentation

body weight once a day, clindamycin 600 mg intravenously every 8 hours, and buprenorphine 150 µg IV twice daily. After 24 hours, a blister approximately 12 cm in diameter developed on the lesion (**Figure 1B**).

Twelve hours after the initiation of pharmacological treatment, the lesion became painless. The necrotic area diameter did not increase, while the perilesional induration decreased due to blister rupture. Additionally, a livedoid spot appeared (**Figure 2**).

Considering the favorable prognosis, the patient was discharged after 40 h of hospital stay. Dapsone 1 mg/kg body weight once daily for 7 days was prescribed as home treatment, and the patient was scheduled for follow-up at 7, 14, 21, and 28 days after the spider bite.

During the 28-day appointment, debridement of the lesion was performed, followed by ulcer washing and topical application of Granulox® (hemoglobin spray) (**Figure 3A**). Granulox® was prescribed for at-home topical treatment every 8 hours for 7 days. Subsequently, the topical application of Granulox® was indicated every 12 hours for 21 days and then every 24 hours for another 14 days. The patient returned for a follow-up examination of the lesion (**Figure 3B-3D**).

On day 70 following the spider bite, the patient underwent examination, revealing cutaneous epithelial regeneration that persisted until the lesion disappeared, leaving a hyperpigmented scar (**Figure 4**).

METHODS

This systematic review was conducted in accordance with the guidelines of the Report of Paper for Systematic Reviews and Meta-Analyses (**Figure 5**).[11] The study was developed and submitted to the International Prospective Register of Systematic Reviews. The Scopus, PubMed, Web of Science, and Google Scholar databases were searched to find articles reporting loxoscelism in Mexico. The following keywords were used during the database search: "loxoscelism" OR "spider bite," OR "loxosceles" OR "loxosceles species" OR "loxosceles venom" OR "loxoscelism case report" AND "cutaneous" OR "dermonecrotic arachnidism." A literature search was independently conducted by three reviewers until December 2022. The reference sections of relevant articles were manually searched to identify additional manuscripts. After the search, the manuscripts were imported into Mendeley Desktop 1.17.11 software (Glyph & Cog, LLC, London, UK) to eliminate duplicates.

Three independent reviewers evaluated the titles and abstracts of all publications. The following eligibility criteria were considered: (a) Systematic reviews, meta-analyses, case reports, and case series of patients of any sex and age presenting with local dermatologic manifestations derived from spider bites of the *Loxosceles* genus;

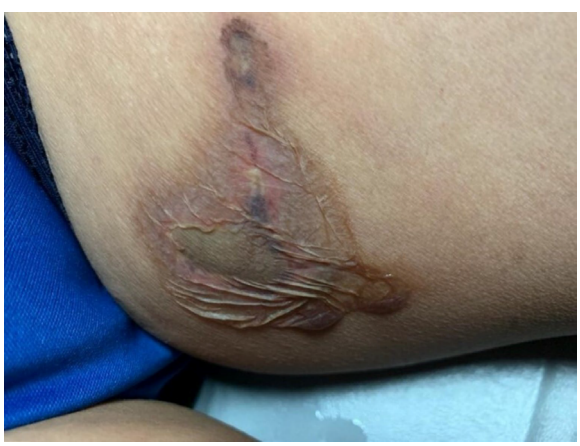

Figure 2. Lesion with irregular borders, necrotic background, delimited by an area of hyperpigmentation and erythema, showing exudate coming out of the vesicles

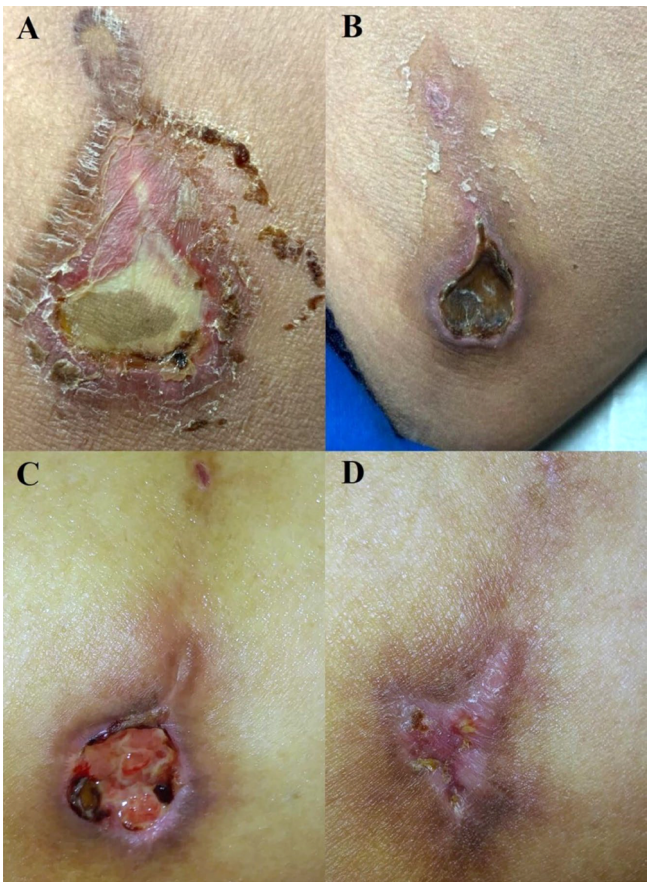

Figure 3. Lesion with irregular borders, delimited by a hyperpigmented area with scaly changes, erythema, and an area of central ischemia. A: Erythematous livedoid plaques with fine scaling and central necrotic eschar with accompanying scaling changes. B: Wound in the granulation phase, delimited by an area of hyperpigmentation. C and D: Lesions in the remodeling phase, with perilesional hyperpigmented area

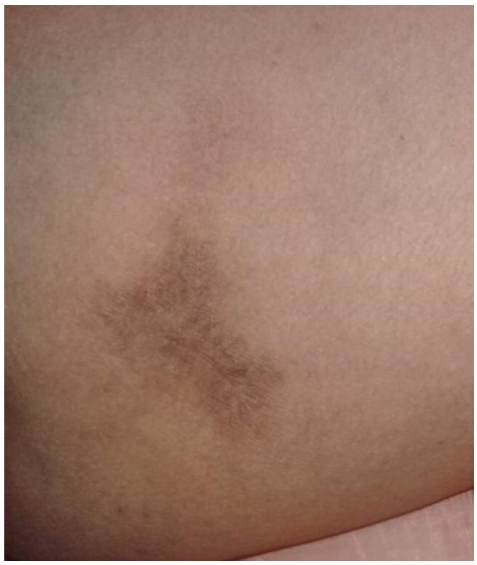

Figure 4. Complete remission of the lesion, discoloration, or formation of mild scar tissue

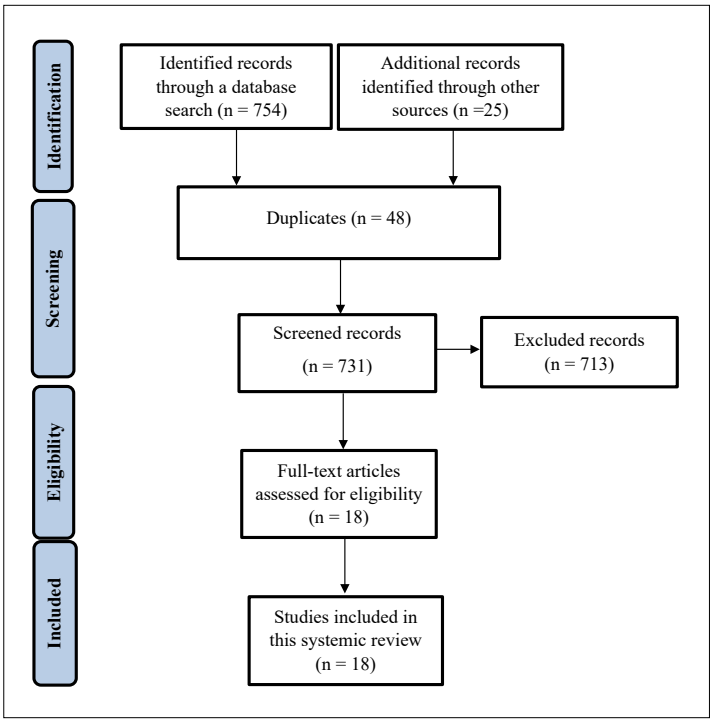

Figure 5. Preferred Reporting Item Guidelines for Systematic Reviews method included in this study

(b) studies published in English, French, Portuguese, and Spanish languages; and (c) studies published between 1980 and 2022 were included in the analysis. Meanwhile, case reports involving patients with systemic signs or symptoms, focusing on cutaneous-visceral loxoscelism, with no available full text, and with insufficient data for the analysis were excluded. Articles that met the inclusion criteria were submitted for full-text evaluation. Any discrepancies in the decision-making process concerning the suitability of the included articles were resolved by consulting a fourth reviewer. Only publications that satisfied all the eligibility criteria were considered in the assessment.

The manuscript was systematically evaluated, and data were obtained from the studies after exhaustive evaluation. The following variables of interest were recorded on standardized worksheets: author, patient age, sex, country, location of the spider bite, size and type of lesion, local effects, time course of signs, spider species, and medical treatment. The Mix Methods Appraisal Tool (MMAT) was used for quality determination.[12] The studies were analyzed by three reviewers in the following domains: clear research questions, adequate data collection, appropriate quantitative approach, appropriate methods to obtain the data, validation and interpretation of recorded data, appropriate statistical analysis, and interpretation. The fourth reviewer resolved any conflicts of interest. Using the MMAT, a score of 1 point was assigned for each quality marker, with a maximum total score of seven.

The results are summarized in **Table 1**. Qualitative and quantitative results were analyzed. Data analysis was performed using descriptive statistics and frequency measures (mean, standard deviation, minimum, median, maximum, frequency, and percentage).

The Declaration of Helsinki was used to protect the privacy and confidentiality of the study patients. Formal written consent was sought from the patient for the utilization of photographic images depicting the evolution of the lesion resulting from the spider bite. Likewise, permission to publish the clinical case was obtained from the authorities of the hospital where the patient was treated.

RESULTS

After the initial evaluation of the publications, 754 documents were obtained from the databases, and 25 additional records were identified by manual search. After removing the duplicates, 731 publications underwent screening based on their titles and abstracts. Of these, 713 did not meet the inclusion criteria and were excluded from the full-text review. A total of 18 articles were then subjected to a full-text review, all of which had sufficient data availability.[13-30] For this systematic review, 18 full-text articles (2 investigating three cases and 16 reporting one clinical case) were included in the statistical analysis according to the inclusion, exclusion, and quality criteria (**Figure 5**).[13-30] The selected studies were published between 1986 and 2022 and had cross-sectional designs. The quality ratings of the 18 case reports ranged from 6 to 7 according to the MMAT.[12] Among these studies, 3 (16.7%) obtained a score of 6, while 15 (83.3%) obtained a score of 7.

Table 1. Summary of findings from the literature search, detailing location, size and type of lesion, local effects, time course of signs, species involved, treatment, age, gender, and country

Reference	Age in years	Sex	Country	spider species	Location of the spider bite	Size of the injury (cm^2)	Kind of injury and local effects	Evolution time in weeks	Treatment and drugs
Farace et al.[13] Case 1	75	M	Italy	Ru	Face	U	Necrotic ulcer, erythema, bull's-eye (red, white, and blue sign), blister or vesicles, itchiness, and bleeding	5	Antibiotic, debridement and skin grafting, others
Farace et al.[13] Case 2	50	M	Italy	Ru	Face	U	Necrotic ulcer, erythema, bull's-eye (red, white, and blue sign), blister, and vesicles.	5	Antibiotic, debridement and skin grafting, others
Farace et al.[13] Case 3	55	F	Italy	Ru	Thigh	60	Necrotic ulcer, erythema, bull's-eye (red, white, and blue sign), pain, blister or vesicles, and bleeding	2	Antibiotic, corticosteroid, debridement and skin grafting, others
Hadanny et al.[14] Case 1	30	M	Israel	Spp	Leg	2.3	Necrotic ulcer, erythema, swelling, and others	15	Antibiotic, hyperbaric chamber, others
Hadanny et al.[14] Case 2	42	F	Israel	Spp	Hip	20	Necrotic ulcer, swelling, and others	14	Corticosteroid, hyperbaric chamber
Hadanny et al.[14] Case 3	72	F	Israel	Spp	Medial malleolus	6.3	Necrotic ulcer, swelling, and others	12	Antibiotic, corticosteroid, hyperbaric chamber
Hillis et al.[15]	8	M	USA	Re	Upper and lower extremities	U	Erythema, bull's-eye (red, white, and blue sign), blister or vesicles, and bleeding	4	Others
Miller et al.[16]	53	M	USA	Re	Arm	10	Necrotic ulcer, swelling, bull's-eye (red, white, and blue sign), and pain	10	Antibiotic, hyperbaric chamber, debridement and skin grafting, others
Laack et al.[17]	46	M	USA	Re	Back	U	Erythema, bull's-eye (red, white, and blue sign), and pain	3	Antibiotic, others
de Entrambasaguas et al.[18]	27	F	Spain	Ru	Face	U	Swelling, pain, and others	1	Antibiotic, corticosteroid, others
Bajin et al.[19]	69	F	Turkey	Ru	Face	U	Necrotic ulcer, erythema, and swelling	8	Antibiotic, corticosteroid
Yi et al.[20]	70	M	USA	Re	Leg	U	Necrotic ulcer, pain, and itchiness	7	Antibiotics, others
Hubiche et al.[21]	80	M	France	Ru	Arm	U	Erythema, bull's-eye (red, white, and blue sign), and pain	1.8	NSAID
Molgó et al.[22]	30	M	Chile	La	Leg	U	Necrotic ulcer, erythema, and swelling	U	Debridement and skin grafting
Morales-Moreno et al.[23]	53	M	Spain	Ru	Thigh	20	Swelling, blister or vesicles, others, and itchiness	12	Antibiotic, NSAID
Guglielmetti et al.[24]	20	F	Chile	Spp	Thigh	50	Necrotic ulcer, erythema, bull's-eye (red, white, and blue sign), blister, or vesicles	8	Antihistamine, corticosteroid, antibiotic, debridement and skin grafting, others
Trave et al.[25]	40	F	Italy	Ru	Thigh	U	Necrotic ulcer, erythema, bull's-eye (red, white, and blue sign), blister, or vesicles	12	Antibiotic, corticosteroid, others

Continue...

Table 1. Continuation

Reference	Age in years	Sex	Country	spider species	Location of the spider bite	Size of the injury (cm^2)	Kind of injury and local effects	Evolution time in weeks	Treatment and drugs
Esteban and Malmierca[26]	69	F	Spain	Ru	Face	4	Necrotic ulcer, swelling, bull's-eye (red, white, and blue sign), pain, and fang marks	1	Antibiotic, corticosteroid, NSAID
Combi et al.[27]	79	F	Italy	Re	Breast	10	Necrotic ulcer, erythema, swelling, and itchiness	U	Antibiotic, hyperbaric chamber, others
Simões et al.[28]	23	M	Brazil	Spp	Genital organs	7	Necrotic ulcer, erythema, and pain	U	Antibiotic, NSAID, debridement and skin grafting, others
Luna-Muñoz et al.[29]	4	F	Peru	Spp	Face	U	Swelling and others	1.4	Antihistamine, Antibiotic, corticosteroid, antiloxosceles serum, others
Morales-Avalos et al.[30]	53	M	Mexico	Re	Face	U	Necrotic ulcer, erythema, swelling, and itchiness	3	Antibiotic, corticosteroid, debridement and skin grafting, others

F = female; M = male; U = undetermined; Re = *reclusa*; Ru = *rufescens*; La = laeta; Spp = species; NSAID = nonsteroidal anti-inflammatory drug

In the analysis of 18 manuscripts included in the systematic review, 22 patients with cutaneous loxoscelism were reported to have no systemic or general involvement. Five (22.7%) case reports were conducted in Italy, 4 (18.2%) in the United States, 3 (13.6%) in Israel, 3 (13.6%) in Spain, and 7 in various countries (**Table 1**).[13-30] A total of 22 patients were included in the analyses. Of the total patients, 12 (54.5%) were men. The patients had a mean (M) age (± standard deviation: SD) of 47.6 (22.8) years, with a minimum age of 4 years and a maximum age of 80 years.

According to previous reports, *Loxosceles rufescens* was involved in nine cases (40.9%), *Loxosceles reclusa* in six cases (27.3%), and *Loxosceles laeta* in one case (4.5%) (**Table 1**).[13-30] In relation to the location of the spider bite, seven patients (31.8%) had a bite in the face, four (18.2%) in the thigh, three (13.6%) in the arm, three (13.6%) in the legs, and six in other regions of the body (**Table 1**).[13-30] The primary types of lesions or local damages reported at the spider bite site were necrotic ulcer (n = 16), erythema (n = 14), inflammation (n = 12), bull's-eye sign (n = 10), and blisters (n = 7) (**Table 1**). The primary treatments used were antibiotics (n = 18), corticosteroids (n = 10), analgesics (n = 4), and debridement (**Table 1**). The evolution time in weeks and the size of the injury in cm^2 are listed in **Table 1**.[13-30]

DISCUSSION

This case report focused on the clinical characteristics, treatment, and evolution of cutaneous loxoscelism. Here, we present the case of a 62-year-old female patient who was admitted to our emergency department due to cutaneous loxoscelism. In this case, comprehensive management of the patient involved systemic administration of dapsone for 7 days and local administration of hemoglobin for 45 days, achieving satisfactory improvement until remission.

In cutaneous loxoscelism, the intensity of the reaction depends on the amount of poison inoculated, the susceptibility of the patient to the components, and the time at which treatment is started. As an initial treatment, the application of a cold compress and the elevation of the affected part are recommended to minimize the spread of the inoculated venom and mitigate the inflammatory and metabolic cascades triggered by the venom at the lesion site.[1,4,5,9,31] Likewise, systemic treatment with dapsone is recommended. It inhibits the release of the enzyme myeloperoxidase, blocks neutrophil adherence, and decreases the production of IL-8, prostaglandins, tumor necrosis factor-α, and histamine.[32] Due to its antineutrophilic effect, dapsone is an effective treatment for skin lesions caused by the bite of the *Loxosceles* spider.[1,4,5,9,31,32] Other lesion treatments have been used, such as silver sulfadiazine, chlorhexidine gluconate, sucrose therapy, surgical debridement, skin grafting, hyperbaric oxygen, and vacuum therapy.[5,9,33] In our case report, a hemoglobin solution (Granulox® spray) was applied to the wound for several days until complete recovery, leaving only an acceptable scar and no sequelae. Granulox is a highly purified porcine hemoglobin fraction modified by carboxylation. It has been used as an auxiliary agent in the treatment of chronic wounds. The layer of hemoglobin applied to the wound transports and diffuses oxygen to the hypoxic surface tissues and helps maintain moisture in the wound to facilitate optimal healing.[6] To our knowledge, this case report is the first to document the use of a hemoglobin spray for the successful treatment of a *Loxosceles* spider bite wound.

Our literature review revealed a scarcity of studies and case reports on cutaneous loxoscelism without systemic symptoms. Loxoscelism can be classified as cutaneous or dermonecrotic and systemic or cutaneous-visceral.[5] Another classification system stratifies it according to severity: mild or grade 1 (erythema, punctum, no necrosis, and pruritus), moderate or grade 2 (erythema, mild edema, vesicle, pain, and necrosis ≤1 cm^2), severe or grade 3 (erythema, edema, hemorrhagic bullae, pain, ulcer, and necrosis >1 cm^2), and systemic (skin lesions grade I, II, or II, plus rash, fever, hemolysis, thrombocytopenia, myalgia, headache, and disseminated intravascular coagulation).[31] In a systematic review of 120 case reports of loxoscelism, 89 (74%) cases were classified as cutaneous, of which 22 (25%) only had local symptoms.[5]

Several studies have explored the correlation between the presence and severity of loxoscelism and the patient's characteristics, such as age, sex, the site of the bite, comorbidities, and the immune system status.[3-5,31,34] Some reports have suggested an elevated risk of more severe loxoscelism in children, adolescents, and older adults.[34] However, other authors have not identified a specific age with a higher prevalence.[3-5,31,34] Our review aligns with the latter. Although the average age of the 22 patients was 40 years, loxoscelism was reported in all age groups. Our results in terms of age are consistent with the data reported from 200 patients with loxoscelism in Chile.[10] In terms of sex, some studies reported a higher prevalence in women, primarily due to their increased involvement in domestic activities.[5] However, other studies did not observe a significant difference in the prevalence of loxoscelism between men and women,[36] which was also observed in 54.5% of men in our study. According to literature reports, the upper or lower extremities, face, neck, and thorax are the most common sites of *Loxosceles* spider bites.[10] This greater predilection of bite sites occurs accidentally or randomly. In the present study, the main sites of the lesions were the face and extremities. Consequently, individuals should inspect the objects that they come into contact with, shake clothes and beddings before use, and constantly clean and rearrange the furniture.

Globally, 143 species of *Loxosceles* spiders have been documented, with approximately 122 species found in America. Among these, only the *L. reclusa, L. deserta, L. arizonica, L. rufescens, L. laeta, L. Gaucho,* and *L. intermedia* species are of great clinical interest.[8,37,38] In Mexico (which is divided into 32 federal entities or states), 28% of the 143 identified species are found. Noteworthy species with broad distribution and medical importance in Mexico were *L. deserta, L. boneti, L. reclusa*, *and L. arizonica*. In the state of Hidalgo (one of the 32 states of Mexico and where the patient in the case report resides), four *Loxosceles* species (*L. jaca, L. nahuana, L. Tenango, and L. tolantongo*) have been identified.[2,35] In terms of the toxicity level of *Loxosceles* venom, variations in enzymatic constituents and substrate preferences contribute to different lethal effects. For instance, the lethal dose required by each species is higher for *L. laeta* (1.45 mg/kg) and lower for *L. similis* (0.32 mg/kg). Additionally, female spiders produce a greater amount of venom owing to the greater weight and size of their bodies, enhancing the venom's impact.[39-41] In the literature review of our study, the main species identified were *L. rufescens* and *L. reclusa*. In our case report, the specific specie of *Loxosceles* spiders was not identified. The identification of the spider species is crucial for generating accurate statistics, since our country lacks precise records of poisonings related to loxoscelism (accounting for only 5% of cases). Consequently, many cases of loxoscelism may be overlooked, leading to misdiagnosis. The lesions are often mistaken for other conditions, including necrotizing fasciitis, deep vein thrombosis, ulcers due to diabetes, infections of bacterial origin, cutaneous anthrax, erythema multiform, and lymphomas.[42]

With regard to the presentation of skin lesions in the 22 patients included in our literature review, erythema was observed in 63%, aligning with the findings of other systematic reviews reporting percentages between 68% and 72%.[43,44] Another investigation reported a lower occurrence, with only 17% of patients presenting with erythema, of whom 8.5% were specifically associated with cutaneous loxoscelism.[10] This variation could be attributed to factors such as the amount of venom inoculated, the species of the spider, and the individual immune response of the patients.[10] In more than half of the patients in our sample, their skin lesion progressed into a necrotic lesion, a prevalent characteristic consistent with other reviews reporting percentages equal to or exceeding 50%.[43-45] The formation of vesicles, inflammation, and the characteristic bull's-eye sign were also frequently observed in the patients included in our review, consistent with the findings of other studies.[3,5,31,43,45]

As previously reported, the initial treatment of cutaneous loxoscelism centers on decreasing the distribution of venom through local cold application and elevation of the affected site, with the goal of limiting skin injury for prompt and effective recovery. The use of dapsone is recommended to reduce inflammatory reactions and skin injury.[4,5,9] Other systemic treatments include antivenom, corticosteroids, hyperbaric oxygen, and antihistamines. In our literature review, the primary treatments administered were antibiotics and corticosteroids (**Table 1**),[13-30] and only one patient received dapsone.[24] In this particular case, the necrotic process was stabilized with dapsone. However, ulcer healing was delayed.[24] On the contrary, surgical debridement and skin grafting were also performed in eight (36.4%) patients in our review (**Table 1**). This latter treatment is rarely recommended due to the potential for increased damage.[4,5,9]

The present study has several limitations. One limitation is the scarcity of information and statistics available on case reports of cutaneous loxoscelism without systemic manifestations. Many of these reports, being incomplete or of poor quality, lack the necessary

data to construct a comprehensive clinical description, affecting the formulation of the conclusions. Variations in the type and severity of the lesion are influenced by the amount of venom inoculated, the patient's time of arrival for medical evaluation, and the initiation of medical treatment post-bite. Currently, no method has been established to measure the amount of venom inoculated, which creates another limitation in published studies.[44] In only 5% of patients, it is possible to identify the arachnids species involved. In our clinical case, the spider specie could not be identified.[4,5,9] This limitation hinders the generation of accurate statistics and the provision of an appropriate diagnosis.[42] Currently, no systematic reviews have evaluated patients with cutaneous loxoscelism without local manifestations. Therefore, healthcare professionals must identify the signs and symptoms to facilitate the correct classification of cases.

CONCLUSION

The management of patients with cutaneous loxoscelism using dapsone and heparin proved favorable, with no sequelae. Reports on cutaneous loxoscelism without systemic manifestations in the global literature remain scarce. Generally, cases of cutaneous loxoscelism without systemic involvement, as reported in the literature, have shown favorable outcomes with the administration of antimicrobials and corticosteroids, facilitating the continuous healing of the skin lesion.

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INSTRUCTIONS FOR AUTHORS

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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);

7. Each author should present his/her ORCID identification number (as obtained from HYPERLINK "http://www.orcid.org/" www.orcid.org);
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;
- Design and setting – Declare the study design correctly, and the setting (type of institution or center and geographical location);
- Methods – Describe the methods briefly. It is not necessary to give all the details on statistics in the abstract;
- Results – Report the primary results;
- Conclusions – Make a succinct statement about data interpretation, answering the research question presented previously. Check that this is concordant with the conclusions in the main text of the article;
- 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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