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P53 overexpression in epidermoid carcinoma of the head and neck

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The theory of field cancerization in tumors of the head and neck reflects the complex oncogenesis that occurs in this region. The mechanisms that control cell proliferation at the molecular level in epidermoid carcinomas (ECs) of the upper aerodigestive tract are still unclear. Mutations in p53 are the genetic alterations most often detected in ECs of the head and neck and seem to contribute actively to the carcinogenic process triggered by p53 as a tumor-suppressor gene and to its association with tobacco. The objective of the present study was to investigate the expression of p53 protein in epidermoid head and neck carcinomas by immunohistochemistry and its immunohistochemical correlation with other prognostic factors. The study was conducted on 63 consecutive ECs cases not submitted to previous treatment. Specimens of the tumor and of the normal adjacent mucosa were collected during surgery and submitted to immunohistochemical reaction for the determination of the expression of anti-protein p53 antibody (M7001 DAKO A/S, Denmark). Anatomo-clinical and demographic data were not significantly correlated with the presence of lymph node metastases or p53 expression in the tumor or in the adjacent normal mucosa. Tumor localization in the larynx was significantly correlated with p53 expression. Histological grading as grades I, II, III and IV was correlated with significant p53 expression ($p = 0.025$). Conclusions: 1) in the studied material obtained from 63 cases of head and neck ECs, we detected a 48 percent rate of immunohistochemically detectable p53 overexpression; 2) we did not detect a relationship between demographic patient data and p53 expression in the tumor or in the normal adjacent mucosa; 3) p53 overexpression was significantly more frequent in ECs material from the larynx; and 4) The presence of 12 cases with p53 overexpression in the normal adjacent mucosa and with a p53-negative tumor is in agreement with the theory of field cancerization. Follow-up of this patient series for a longer period of time will permit a better analysis of these values.

UNITERMS: P53, squamous cell carcinoma, head and neck cancer, immunohistochemistry

INTRODUCTION

Epidermoid carcinomas (ECs) represent more than 90 percent of the neoplasias of the upper aerodigestive tract[6] and their incidence increases among subjects who smoke cigarettes and consume alcoholic beverages.[12] ECs correspond to the sixth most frequent form of cancer in the world.[9]

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Patients with head and neck ECs in the same stage may respond differently to different forms of treatment.[2] Currently recognized prognostic factors are not sufficiently objective or standardized, and do not correspond to the biological behavior of the tumor at the cellular level.

The theory of field cancerization in head and neck neoplasias reflects the complex oncogenesis that occurs in this region.[33] The frequent occurrence of multiple primary tumors in the upper aerodigestive tract may be explained by continuous exposure of the mucosa to various carcinogens. However, the sequential stages of this complex carcinogenesis have not been fully elucidated and the molecular mechanisms that control cell proliferation and the aberrant behavior of ECs in the upper aerodigestive tract are still unknown.

Genes involved in carcinogenesis may be classified as proto-oncogenes and tumor-suppressor genes according to their action mechanism.[23,35] Tumor-suppressor genes code for proteins with functions related to the control and suppression of cell growth and proliferation by transactivation or transrepression of specific genes. These genes actively affect the cell cycle, regulating it in a repressive manner.[11]

The role of gene p53 has been the subject of several studies on solid human tumors.[5,6,15,16,18,19,25,26,30] P53 mutations are the genetic alterations most frequently detected in head and neck ECs,[28,34] specifically in the larynx, and seem to actively contribute to the carcinogenic process of the gene[31] as a tumor suppressor and to its association with tobacco.[4,10,17]

The accumulation of mutation of gene p53 associated with head and neck ECs has been reported in the literature, although it has not been correlated with other prognostic factors.[1,2,7,13] A better understanding of the role and mechanism of action of gene p53 in the various stages of carcinogenesis may be of help in terms of specific therapeutic approaches, and may identify new tumor markers for early diagnosis.

The objective of the present study was to investigate the expression of p53 protein in head and neck ECs by immunohistochemistry and to determine its relation to other prognostic factors.

MATERIAL AND METHODS

The study was conducted on 63 consecutive ECs cases not submitted to previous treatment registered at the Department of Head and Neck Surgery of the A.C. Camargo Hospital in 1994 and 1995. Tumor and normal adjacent mucosa specimens were collected during surgery and fixed in Carnoy solution. After fixation, the material was embedded in paraffin and 4 μm thick sections were obtained and stained with HE. The material was then submitted to immunohistochemical reaction for the determination of the expression of anti-protein p53 antibody (anti-human p53 protein clone DO7, M7001 DAKO, A/S, Denmark).

Immunohistochemistry

The material was deparaffinized and immersed in a bath containing decreasing concentrations of xylol and ethyl alcohol. The antigen was recovered by treatment in a microwave oven under immersion in a nitric citrate solution, with 4 baths of 5 min each at maximum potency. Endogenous peroxidase (H_2O_2) was blocked and the material was incubated 1:100 with primary antibody overnight at 4°C, followed by incubation with 1:200 biotinylated secondary antibody for 30 min at 37°C. After incubation with 1:800 StrepAB complex:HRP for 30 min at 37°C (DAKO StrepAB complex/GRP Duet, mouse/rabbit kit), the material was immersed in 60 mg percent DAB (2,3 diaminobenzidine tetrahydrochloride, Sigma Chemical Co., D5637) plus 1 percent dimethyl sulfoxide PA (Labsynth) for 5 min at 37°C and counterstained with hematoxylin.

When more than 20 percent of the tumor cell nuclei stained dark brown, the sample was considered positive. The same criterion was used for the evaluation of normal adjacent mucosa. Positive and negative controls were systematically performed for each reaction.

Table 1
Correlation between p53 expression and demographic data

Variables	Categories	p53 expression (+)	(-)	
Sex	male	26	25	p=o.51
	female	4	7	
Race	White	28	30	p=1.00
	Asian	1	0	
	Black	1	1	
Age	< 45	4	5	p=0.89
	46-60	13	12	
	>60	13	15	
Family History	present	24	23	p=0.45
	absent	6	9	
Smoking habit	present	26	25	p=0.424
	absent	2		
alcoholism	present	6	8	p=0.485
	absent	22	19	

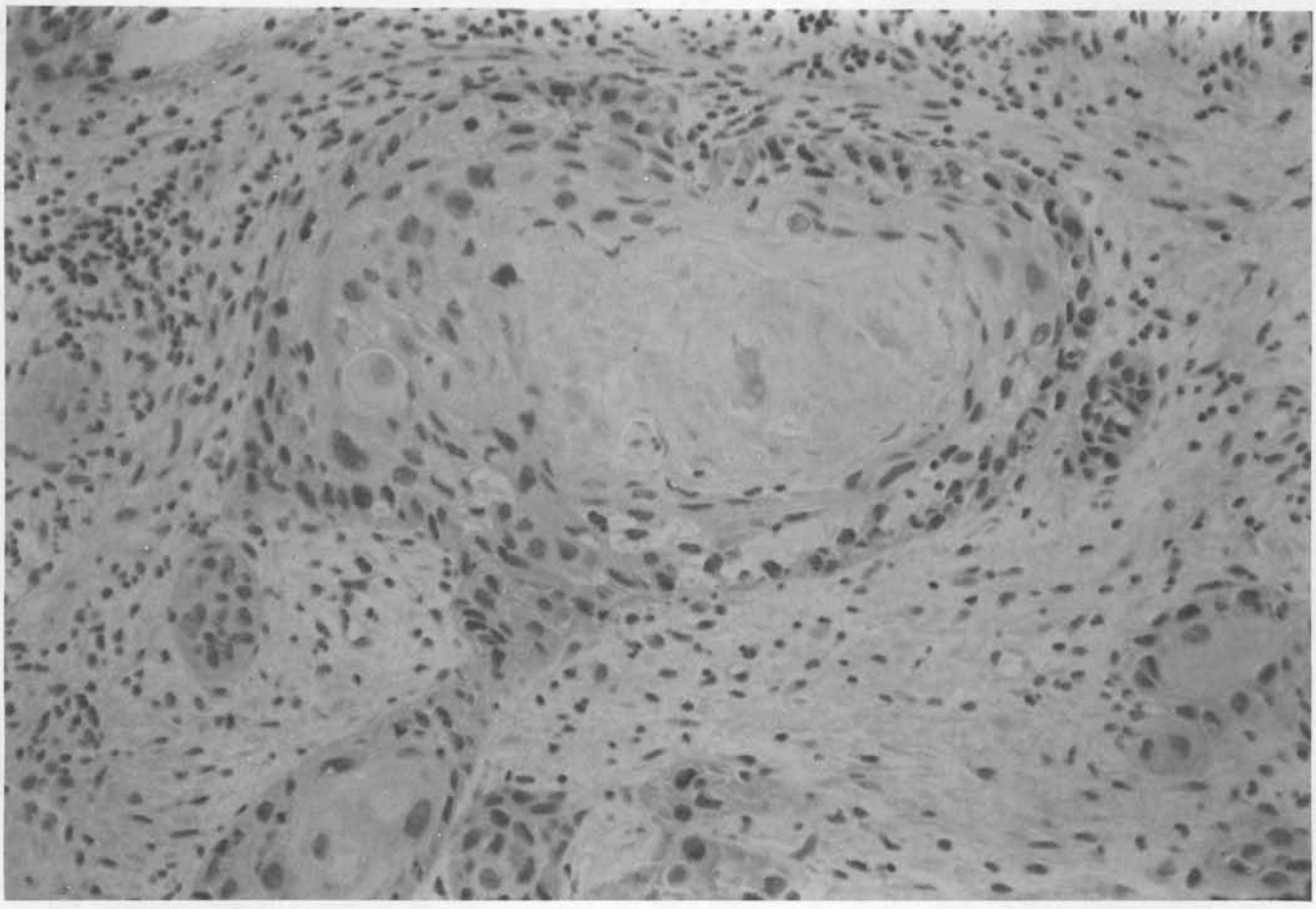

Figure 1 - p53 expression in grade II epidermoid carcinoma of the tongue.

Statistical analysis

Double-entry convergence tables were constructed for the analysis of correlations between the clinical-pathologic variables studied and the immunohistochemical expression of mutated p53 protein. Statistical significance was calculated by the chi-square test or by the exact Fisher test when applicable.

RESULTS

Expression of mutated p53 protein was considered to be positive in 30 of the 63 specimens of primary tumors (48 percent). The study did not show a significant correlation between protein p53 overexpression and patient demographic variables (family history of cancer, cigarette smoking or alcohol consumption) (Table 1). Cases for whom demographic data were not available were excluded from the table.

Figure 1 shows a grade II EC of the tongue with p53 overexpression.

To facilitate data analysis, the tumors were schematically assigned to the three following topographies: pharynx, including the cervical esophagus; oral cavity, including palate, mouth floor, tongue and gingiva; and larynx. Table 2 shows the correlation between p53 expression and anatomical location, histologic grade and lymph node status.

Epidermoid carcinoma of the larynx was significantly correlated with p53 expression which did not occur in other locations.

Histological grading was schematically divided into grades II. III and IV. Usually, grades III and IV have the best prognosis. Correlation with p53 expression was significant ($p = 0.025$). No correlation was observed between p53 expression and presence of lymph node metastases.

Table 2
Association between p53 expression and tumor topography, histological grade and lymph node involvement

		p53 expression		p=0.0225
Tumor topography		(+)	(-)	
Oral cavity		15	18	
Pharynx		6	12	
Larynx		10	2	
Histological grade				p=0.025
I + II		27	28	
III + IV		3	4	
Lymph node	negative	17	14	p=0.20
metastases	positive	13	19	

Data concerning p53 expression in the normal adjacent mucosa and in the tumor of the same patient are presented in Table 3. The results show that p53 expression at these two sites was not coincident in 29 cases. The tumor was p53+ and the mucosa p53- in 17 cases, and 12 cases presented opposite results (a p53- tumor and a p53+ adjacent mucosa).

Figure 2 shows an area of the normal adjacent mucosa with moderate epithelial dysplasia and strongly p53 positive. Figure 3 shows an area of normal adjacent mucosa that is negative for p53 overexpression.

Although the follow-up time of the present series is still short, three patients who presented recurrences had p53+ tumors, whereas only one case had a recurrence among patients without p53 expression. This small number of recurrences prevents statistical analysis.

DISCUSSION

The hypothesis of the participation of gene p53 in the process of carcinogenesis of head and neck tumors has been discussed in several studies.[7-9,13,14,21,24,27,29,32,34,37] After the discovery of anti-protein p53 antibodies, several retrospective studies have been published, with variable results, i.e., rates of p53 positivity of 30 to 67 percent. Thirty to 65 percent of the carcinomas have characteristic mutations in gene p53, with an allele usually having a point mutation and producing a mis-sense p53 protein that accumulates at high levels in cancer cells. The second allele was lost by conversion and/or genetic deletion.[22]

Cell lines from ECs of the upper aerodigestive tract have revealed increased p53 expression in vitro.[1,36] Mutations, deletions and amplification of the undeleted allele of gene p53 are commonly found in these lines.[37]

Considering the multicentric etiology of tumors of the aerodigestive tract, several investigators have also examined the normal prelesional adjacent mucosa of some cases and have detected an irregular pattern of p53 expression, although they did not detect a correlation between p53 expression in the normal mucosa adjacent to the tumor and the appearance of a second neoplasia or recurrence.[14,20,27,29,32]

Brachman et al.[2] investigated 31 specimens of head and neck ECs and observed mutations of gene p53 in 53 percent of cases, demonstrated by polymorphism of single strand conformation. The same investigators later detected mutations in gene p53 by the polymerase chain reaction in 63 percent of the cell lines studied. Of the 31 cases of head and neck ECs studied by Chung et al.,[7] 21 presented at least one mutation of gene p53, analyzed by the single chain polymorphism conformation method. Field et al.,[10] in a study of 73 ECs cases, observed the presence of protein p53 overexpression in 67 percent of them by immunohistochemistry. In another immunohistochemical study, Frank et al.[13] detected p53 overexpression in 37 percent of cases of ECs of the hypopharynx. Shin et al.[32] obtained a 43 percent index in an immunohistochemical study of head and neck ECs. The presence of p53+ in 48 percent of the 63 head and neck ECs cases positive for p53 in our study is compatible with literature data.

In vitro studies carried out by Yin et al.,[37] Somers et al.[34] and Weinberg et al.[36] showed abnormalities of gene p53 in cell lines from head and neck carcinomas, such as overexpression of protein p53 demonstrated by immunohistochemistry and mutations and deletions of the gene detected by sequential analysis by the polymerase chain reaction.

Maestro et al.[24] investigated 58 cases of larynx tumors and found p53 overexpression in 60 percent using an immunohistochemical method and molecular analysis by the polymerase chain reaction and in situ hybridization. The authors concluded that this is the genetic alteration most frequently occurring in these tumors. Of 89 cases of ECs of the larynx studied by Nadal et al.,[27] 64 percent presented immunohistochemically-detected p53

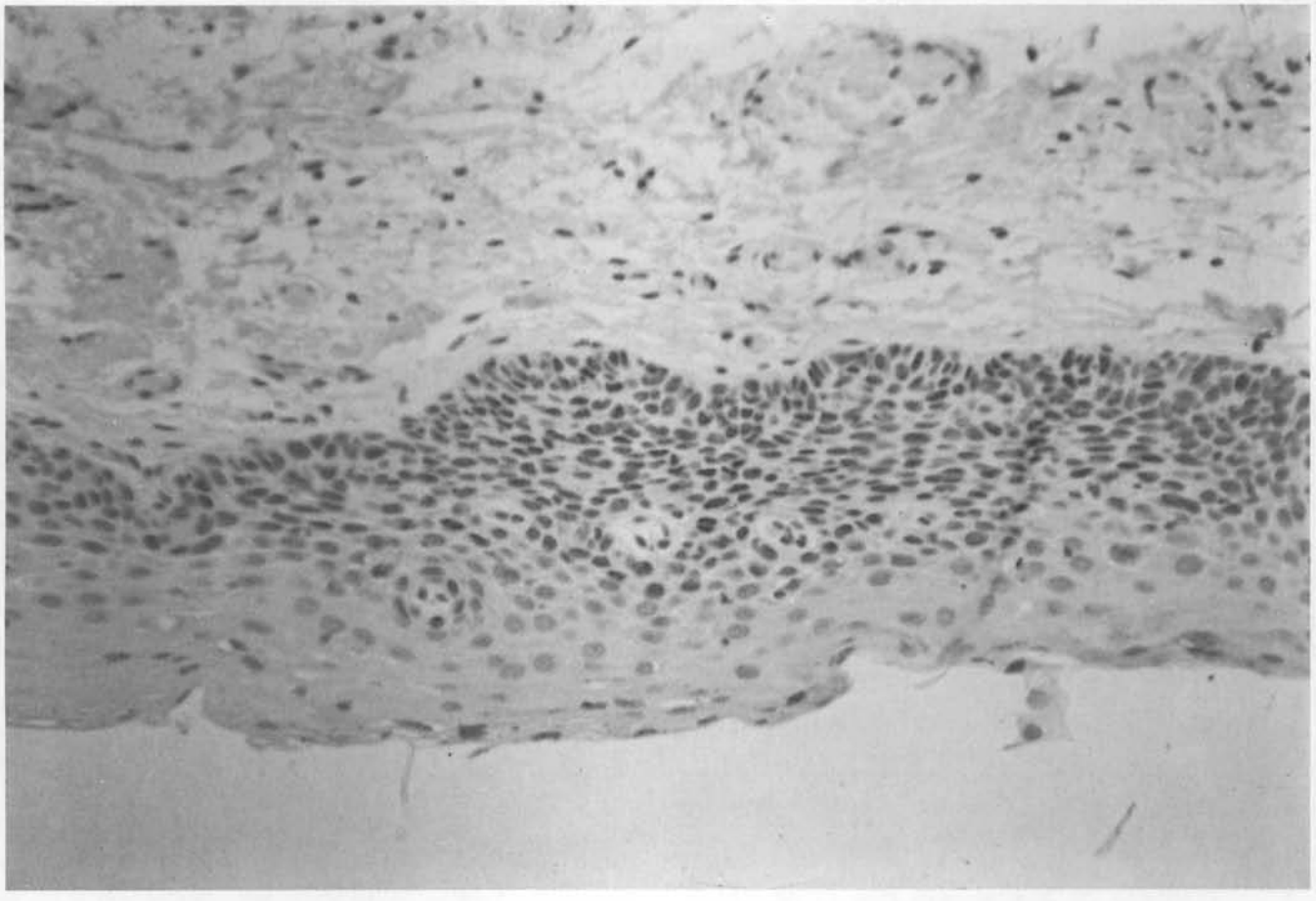

Figure 2 - Normal adjacent mucosa with moderate epithelial dysplasia and strongly p53 positive.

overexpression. The specific localization for these cases of cancer of the larynx was statistically significant in terms of p53 expression, a fact not previously reported.

The p53 overexpression observed in 10 of the 12 cases of cancer of the larynx studied here was more frequent than in the study discussed above. Nees et al. (1993) found p53 overexpression in only 44 percent of their cases of ECs of the larynx and in 80 percent of the cases of cancer of the oropharynx. We are currently expanding our study to determine p53 expression in the various topographies of the head and neck.

Prospective studies with appropriate material for analysis of p53 have only permitted a simple statistical correlation with some isolated factors such as smoking[4,10] and human papillomavirus.[3] The absence of a correlation between smoking and p53 gene expression in the present study disagrees with the data reported by others.[2,4]

With a longer follow-up time, few studies were consistent in showing the absence of a correlation between the prognostic factors normally used and p53 expression in head and neck tumors. Frank et al.[13] did not detect a correlation between p53 expression in ECs of the hypopharynx and other data such as incidence of secondary tumors, survival, ploidy or histological grade. Nadal et al.[27] did not find a prognostic significance of p53 expression in neoplastic and dysplastic lesions of the larynx in a study of 89 cases, and concluded that the presence of mutations of gene p53 detected by the polymerase chain reaction is not correlated with radiosensitivity in these tumors.

Taking into account the theory of field cancerization, Nees et al.[29] identified mutations of gene p53 in tumors and in the normal adjacent mucosa and suggested the participation of p53 in the carcinogenesis of multiple tumors. Shin et al.[32] and Gusterson et al.[14] studied premalignant lesions of the squamous epithelium, including dysplasia, hyperplasia, Bowen disease, and the normal adjacent mucosa and found 21 to 45 percent and 15 to 16.6 percent p53 positivity by immunochemistry in their respective series. The scarcity of data concerning

survival prevented these authors from correlating their results with prognostic factors. However, there is no objective evidence of a correlation between p53 expression and prognostic factors for head and neck carcinomas.[27]

The absence of a correlation between the expression of p53 in the normal adjacent mucosa and in the tumor area of the same patients detected here agrees with literature data. Nees et al.[29] found p53 overexpression in the normal adjacent mucosa and absence of p53 protein in patients with head and neck ECs This apparent discrepancy was observed in 12 of our cases. The presence of p53 expression in the adjacent mucosa may indicate the possible origin of multiple tumors or the local recurrence in this area through different carcinogenic processes, involving or not mutation of the p53 gene. The specific localization in cancer of the larynx was statistically significant in terms of p53 expression, a fact that is not uncommon in literature reports. Our series must be expanded for a better analysis of the topographic distribution of tumors according to p53 expression.

Table 3
Expression of p53 in the primary tumor and in the adjacent mucosa.

	p53(+) expression	p53(-) expression
	in the tumor	
p53(+) expression	13	12
	in mucosa	
p53(-) expression	17	20

CONCLUSIONS

1. In material obtained from 63 cases of head and neck ECs, we observed 48 percent immunohistochemically-detectable p53 overexpression, a rate compatible with literature data.
2. We did not observe a relation between patient demographic data and p53 expression in the tumor or in the normal adjacent mucosa.
3. p53 overexpression was significantly more frequent in material from ECs of the larynx.
4. The presence of 12 cases with p53 overexpression in the normal adjacent mucosa and a p53-negative tumor agrees with the theory of field cancerization. This finding may be a factor for the early detection of patients at high risk to develop other p53-positive tumors.

A longer follow-up of this series will permit a better analysis of these values in terms of recurrence and the possible appearance of other tumors, which is about 6 percent (unpublished data).

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RESUMO

A teoria de campo de cancerização em neoplasias de cabeça e pescoço (CP) reflete a complexa oncogênese que ocorre nesta região. Os mecanismos a nível molecular que controlam a proliferação celular em Carcinomas epidermóides (CEC) de vias aerodigestivas superiores ainda são pouco conhecidos. Mutações no p53 são as alterações genéticas mais encontradas em CEC de CP e parecem contribuir ativamente em seu processo carcinogênico como gene supressor de tumor e a sua associação com tabaco. O objetivo deste trabalho é analisar a expressão da proteína p53 em carcinoma epidermóide de cabeça e pescoço por meio de reação imunohistoquimica e sua correlação com outros fatores prognósticos. O estudo inclui 63 casos consecutivos de CEC, não tratados previamente. Espécimes de tumor e de mucosa normal adjacente foram colhidos durante o ato cirúrgico e submetidos a reação imunohistoquímica para avaliação da expressão do anticorpo anti-proteína p53 (M7001 DAKO A/S, Denmark Inc.) Não houve significância entre os dados anatomo-clínicos e demográficos, presença de metástases linfonodais e a expressão de p53 no tumor ou na mucosa adjacente normal. A localização do tumor na laringe apresentou correlação estatistica significativa com a expressão da p53 . A graduação histológica, separada em graus I e II, e III e IV apresentou correlação com a expressão de p53 significante (p=0,025). Conclusões: 1-No material estudado proveniente de 63 casos de CEC de cabeça e pescoço encontramos 48% de superexpressão de p53 imunohistoquimicamente detectável, índice compatível com os demais relatados na literatura; 2-Não encontramos relação entre os dados demográficos do paciente e a expressão de p53 no tumor e na mucosa normal adjacente; 3- O achado de superexpressão de p53 mais freqüente em material de CEC de laringe foi estatisticamente significativo. 4-A presença de 12 casos com superexpressão de p53 na mucosa normal adjacente e com tumor p53 negativo está de acordo com a teoria de cancerização de campo. O seguimento desta série por maior tempo possibilitará uma melhor análise destes valores.

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