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ORIGINAL ARTICLE

Oral leukoplakia—epidemiological survey and histochemical analysis of 107 cases in Brazil

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Abstract

Objective To perform an epidemiological survey comparing the cell proliferative activity of 107 cases of oral leukoplakia with their clinical and histopathological characteristics.

Materials and methods A cross-sectional, observational, and histological-histochemical study. The cases came from the Histopathological Diagnostic Service of UPF/RS and the School of Dentistry of Araçatuba FOA/UNESP/SP (1986–2016). The histopathological sections were stained using the silver staining (AgNOR) technique and the nuclei of 100 epithelial cells selected randomly were recorded to count the number of nucleolar organizer regions (NORs). The mean NORs per lesion were correlated to clinical and histological characteristics using ANOVA, at 5% significance.

Results Most of the cases included men (62.62%), white (64.49%), and with an average age of 53.94 years. The most probable etiological factors were smoking (44.7%) and alcohol consumption (9.85%). The evolution time of most lesions was fast (33.65%), manifesting mainly in the form of plaques (70.37%) and without symptoms (58.88%). They were located mainly in the cheek mucosa (26.62%) and presented white color (66.35%), well-defined edges (59.81%), firm consistency (47.5%), and keratinized surface (49.53%). Etiological factor ($p = 0.003$), evolution time ($p = 0.006$), symptoms ($p = 0.029$), location ($p = 0.020$), consistency ($p = 0.047$), histopathological characteristics ($p = 0.004$), and superficial keratinization ($p = 0.001$) were statistically significant regarding the mean NORs of the leukoplakias studied.

Clinical relevance Oral leukoplakias caused by alcohol consumption and/or tobacco use, considering an evolution time of fewer than 12 months, asymptomatic, located in the lower lip or tongue, and with a firm consistency and increased superficial keratinization should be treated more aggressively by the clinician to avoid cancerization.

Keywords Leukoplakia · Histological classification · Prognosis · AgNOR

Introduction

Oral squamous cell carcinoma (OSCC) is common malignant neoplasia with significant morbidity and mortality rates [1–3]. The survival rate of patients within 5 years after the diagnosis of OSCC is around 50% to 60% [4, 5]. Therefore, the prevention and early diagnosis of this disease are the best alternatives so far [1, 3, 4, 6, 7].

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Usually, OSCC develops after the appearance and evolution of precancerous lesions, which are characterized by the presence of a morphologically changed tissue more likely to evolve to malignant neoplasia than the normal adjacent tissues [8–10]. Among lesions with this characteristic, leukoplakia, erythroplakia, and erosive lichen planus stand out [6, 11].

Currently, the World Health Organization (WHO) has added pre-malignant lesions and conditions under a single group of disorders, classified as potentially malignant disorders. Thus, oral leukoplakias have become a potentially malignant disorder that can affect the oral mucosa. Leukoplakias are the most commonly diagnosed precancerous lesions in the oral cavity [5, 7, 8, 11]. The definition of "leukoplakia" has changed over the past few decades and the latest revision was established by the WHO in 2007. Currently, the term is used to define "white plaques of questionable risk, having excluded other known diseases or disorders that do not have an increased risk of cancerization" [12].

The pooled prevalence estimated for leukoplakia of the oral cavity is between 1.5 and 2.6%, and this lesion has a malignant transformation rate that varies from 0.13 to 17.9% [1, 6, 11, 13, 14]. Petti (2003) analyzed 23 primary studies from all over the world published between 1986 and 2002 and affirmed that the estimates for leukoplakias were between 1.49 and 2.60%. This author also reports that leukoplakia was significantly more prevalent among men (prevalence ratio of 3.22) but no difference was found among geographical areas and between younger and older adults. Considering these data, the crude annual oral cancer incidence rate attributable to leukoplakia would be between 6.2 and 29.1 per 100,000, thus suggesting that the global number of oral cancer cases is probably underreported [15]. The malignancy potential of the lesion varies from study to study and it is associated with factors specific to the patient, the sample analyzed, and the lesion itself. Thus, it is believed that about 16% to 62% of oral carcinoma cases evolve from leukoplakias [6].

Smoking and alcohol consumption are considered the main etiological factors of leukoplakia. However, the age and sex of the patients affected as well as the anatomical location of the lesion and the presence of epithelial dysplasia (mainly of high degree) are also linked to malignancy in different studies [1, 13, 16].

The tissue silver staining (AgNOR) technique can assist the diagnosis and identification of aspects that predispose leukoplakia malignancy [17]. This technique allows counting the number of nucleolar organizer regions (NORs) expressed in the nucleus of the lesion cells. The NORs are regions that show the genes responsible for producing ribosomes, which are present in the translation of proteins. Thus, the high number of NORs may indicate a tumor [17–20].

Cell proliferation is vital to all living organisms for growth and maintenance of tissue homeostasis, and the AgNOR number is directly proportional to the speed of the cell cycle. The nucleolus is not a constant structure, for it dissolves before mitotic cell division and reorganizes later. Nucleolar organizer regions are a vital part of the nucleolus machinery. This process is dysregulated in cancer. Dysregulated proliferation is considered a key characteristic of malignancies, and the interphase AgNOR numbers are closely associated with cell proliferative activity, suggesting that this parameter has a diagnostic significance. The number of AgNORs in the nucleus may reflect the activation state and the malignancy degree of the lesion involved [21, 22].

However, despite the significant progress achieved regarding leukoplakias so far, their diagnosis and treatment remain a challenge [1, 11]. Determining the characteristics that indicate "high risk of malignancy" would allow the clinician to define more aggressive and effective programs, seeking to stop the transformation of lesions into oral carcinoma [6].

Hence, this study aimed to perform an epidemiological survey of a series of 107 cases of leukoplakia applying the AgNOR technique to compare the cell proliferative activity of the lesions with their clinical characteristics, allowing dentists to establish more suitable conducts in the prognosis and treatment of such lesion.

Materials and methods

Ethical considerations

The Research Ethics Committee of the University of Passo Fundo approved this study (No. 172/2011).

Study design and data collection

A cross-sectional, observational, histochemical-laboratory, and epidemiological study was performed. A survey was performed on leukoplakia cases filed at the Histopathological Diagnostic Service of the Institute of Biological Sciences of the University of Passo Fundo and the School of Dentistry of Araçatuba-UNESP, from 1986 to 2016. The study included histopathologically diagnosed cases such as acanthosis, hyperkeratosis, hyperorthokeratosis, and/or hyperparakeratosis, with or without dysplasia.

Sample selection

Initially, all clinical data were collected. The following information was selected from the histopathological records: ethnicity, age, and sex of the patients, as well as the data of the lesion such as size, main lesion, anatomical location, symptomatology, consistency, edges, color, evolution time, probable etiological factor, and histological characteristics.

Inclusion criteria

The study included all cases diagnosed histopathologically, between 1986 and 2016, as hyperparakeratosis, hyperorthokeratosis, with or without acanthosis, and with or without epithelial cell dysplasia. Besides the histopathological characteristics described previously, the cases included in the study should be represented clinically by a white or grayish keratotic spot or plaque with acceptable diagnostic quality, intraoral or labial location, and they should have been referred to a histopathological examination by dentists, including the clinical reference information about the patient and the respective lesion.

Exclusion criteria

The sample excluded cases diagnosed outside the study period, with insufficient histopathological material for analysis, and with incomplete or insufficient clinical and histopathological information to establish the diagnosis of leukoplakia.

Training and examinations

Two examiners were calibrated and trained to assess the lesions from both institutions (kappa 0.90). In this stage of the study, a single dentist specialized in oral pathology received calibration training to diagnose and classify precancerous oral lesions compatible with leukoplakia. For this stage, a set of training glass slides applied to the lesions was used.

Histopathological classification

The diagnoses of leukoplakia were confirmed by analyzing histological sections of 4 μm stained with hematoxylin and eosin (H&E) by the examiners previously described. The criterion for diagnosing leukoplakia was the presence of epithelial changes such as hyperparakeratosis, hyperorthokeratosis, with or without acanthosis, with or without epithelial cell dysplasia in the mucosa, and lamina propria, as well as the presence of an inflammatory infiltrate ranging from mild to severe. Dysplasia (when present) was classified according to the criteria proposed by the World Health Organization [23].

Histochemical analysis

The AgNOR technique was performed in histological sections of the same cases stained by H&E, with a thickness of 4 μm, from tissue samples embedded in paraffin and fixed in formalin. Such sections were dewaxed in xylol and solutions with decreasing concentrations of ethanol. Then, tissue silver staining was performed, according to Ploton et al. [24]. The microscopic areas of interest were selected on slides stained by H&E and printed at a magnification of × 1000 (immersion). In such images, NORs were counted in each case, considering 100 epithelial cell nuclei selected randomly. The data obtained were tabulated in an Excel™ spreadsheet.

Statistical analysis

The epidemiological data on patients and their respective lesions were subjected to statistical analysis using the SSPS software, version 23.0 (Chicago, USA). The means of the number of NORs obtained in 100 epithelial nuclei in each case were compared with the clinical and histopathological characteristics using ANOVA, at a significance level of 5%.

Results

From the analysis, 107 cases of leukoplakia were obtained over a period of 30 years. Most of the patients affected by the lesions were men (62.62%) and white (64.49%). Age varied between 26 and 84 years (mean of 53.94 years), and the age group of 46–60 years was the most affected (Table 1). Missing information in the histopathological records about sex, age, and ethnicity of patients, as well as the characteristics of the lesions, are described in the "not included" item in each table.

The habit of smoking was found in most cases (44.7%) and it was considered the main probable etiological factor of leukoplakia, followed by idiopathic factors (28.03%) and alcohol consumption (9.85%). Some patients had two or more associated factors (Fig. 1).

Table 1 Distribution of cases of oral leukoplakia according to sex, age, and ethnicity of the patients (ICB/UPF and FOA/UNESP, 1986–2016)

Characteristic	Incidence (n = 107)	%
Sex		
Male	67	62.62
Female	39	36.45
Not included	1	0.93
Age		
25 to 45 years	30	28.04
46 to 60 years	40	37.38
61 to 80 years	33	30.84
80+ years	2	1.87
Not included	2	1.87
Ethnicity		
White	69	64.49
Brown	21	19.63
Black	14	13.08
Yellow	1	0.93
Not included	2	1.87

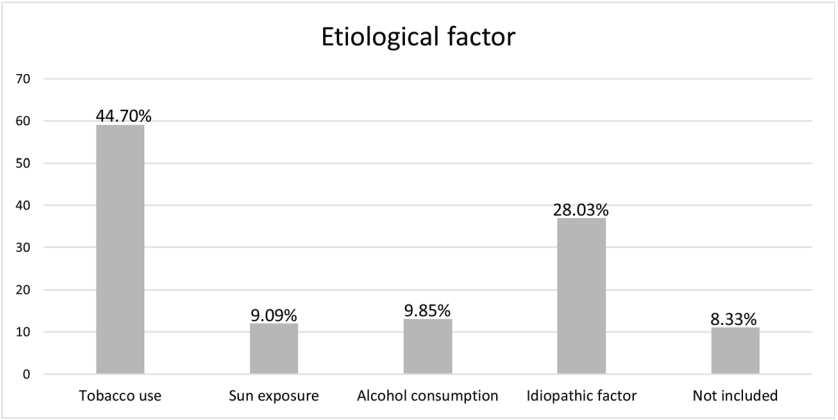

Fig. 1 Distribution of cases of oral leukoplakia regarding the probable etiological factor of the lesion. The item "Sun exposure" is associated with lip lesions (ICB/UPF and FOA/UNESP, 1986–2016)

Most of the lesions evolved rapidly, developing in less than 12 months (33.65%). Lesions with a slower evolution, over 36 months, manifested in only two cases (1.87%) (Fig. 2).

Plaque-shaped lesions were seen in 70.37% of the cases and the second most common type of the main lesion was spots (16.67%). There was an association between plaque and spot lesions in the same individual. Nodule and ulcer were found in 2.78% and 1.85% of cases, respectively (Fig. 3). Regarding size, there were leukoplakias ≤ 20 mm (49.53%) and lesions ≥ 20 mm (38.32%) (Fig. 4).

In 58.88% of the leukoplakia cases studied, the patients did not show any symptoms. The cases with symptomatology reported pain, pain and burning, or itching. The cheek mucosa, lip, and tongue were the anatomical sites most affected, representing 26.62%, 23.02%, and 16.55%, respectively. The lesion manifested in more than one anatomical site concomitantly in some patients. The lesions had a firm consistency (47.5%) and a flat surface (49.07%) in most cases. The manifestation of more than one type of consistency was observed in some cases and one lesion had a verrucous surface with grooves. Defined edges around the lesions were observed in 59.81% of the cases and the white color was predominant for leukoplakia (66.35%). Regarding the superficial keratinization of the lesions, none was removable (Table 2).

The histological evaluation showed that hyperkeratosis and acanthosis were present in most lesions (39.46% and 35.87%, respectively). Epithelial dysplasia (ED) was observed in 37 cases (16.59%), with 10.76% of mild ED, 4.48% of moderate ED, and 1.35% of severe ED. In 4.05% of the reports, no information was obtained (Table 3).

When relating the variables studied to the mean number of NORs obtained in the respective lesions by the ANOVA statistical test (significance level of 5%), a statistically significant value ($p \leq 0.05$) was observed for the following variables: etiological factor, evolution time, symptoms, location, consistency, histopathological characteristics, and superficial keratinization (Table 4).

Discussion

Leukoplakia is the most common precancerous lesion found in the oral cavity [2, 11]. Thus, the present study aimed to perform an epidemiological survey of a series of 107 cases of leukoplakia, as well as to correlate the mean number of NORs obtained in 100 epithelial cell nuclei of such lesions with their respective clinical and histopathological characteristics to facilitate the establishment of therapeutic and prognostic approaches.

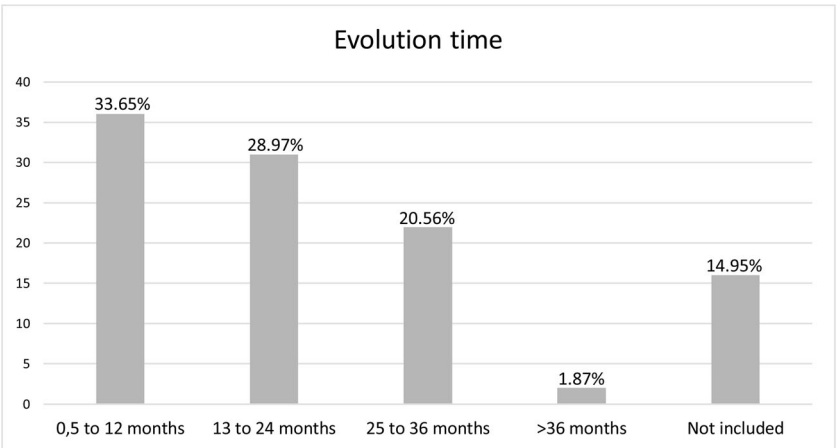

Fig. 2 Distribution of cases of oral leukoplakia according to the evolution time of the lesion (ICB/UPF and FOA/UNESP, 1986–2016)

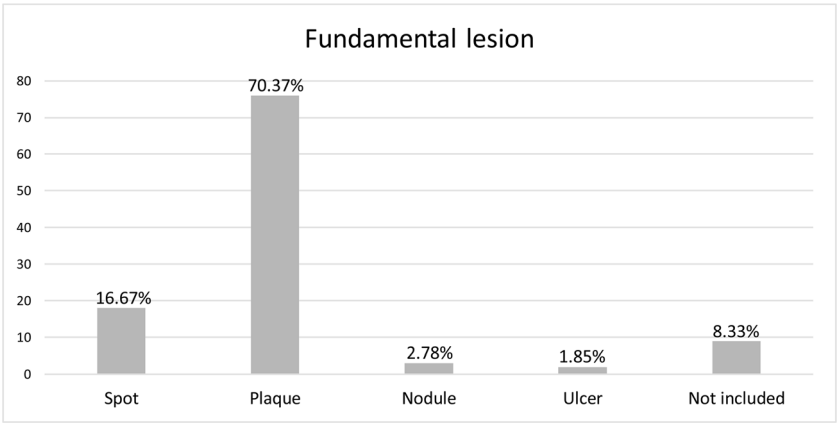

Fig. 3 Distribution of cases of oral leukoplakias regarding the main lesion (ICB/UPF and FOA/UNESP, 1986–2016)

The gradual increase in the mean AgNOR count indicates that the number of AgNOR was proportional to the cell proliferative activity, from normal to premalignant to malignant lesions, and it is related to the abnormalities in their proliferation [21]. The results of the present study allow verifying that certain clinical characteristics of oral leukoplakias (etiological factor, evolution time, symptoms, location, consistency, histopathological characteristics, and superficial keratinization) were statistically significant regarding the mean NORs of the lesions. That is, when treating a case of oral leukoplakia, the clinician must carefully observe the characteristics mentioned previously and consider them when establishing the prognosis (cancerization potential) of the lesion. This analysis suggests that oral leukoplakias caused by alcohol consumption and/or tobacco use, considering an evolution time of fewer than 12 months, asymptomatic, located on the lower lip or tongue, with a firm consistency and increased superficial keratinization, and histopathologically composed of hyperkeratosis, acanthosis, and some degree of epithelial dysplasia should be treated more aggressively by the clinician to avoid cancerization.

Attempting to overcome disadvantages of the techniques commonly used and discover newer non-invasive and less expensive measures to detect early changes of malignancy, the AgNOR technique may be used as a complement before the routine histopathological study, especially in lesions with potential malignant transformation, because numerical variations of NORs may indicate important cellular changes, minimizing potential diagnostic mistakes [22]. This statement justifies the use of the AgNOR method in the present study and emphasizes its importance as an auxiliary tool in the clinical determination of the prognosis/treatment of oral leukoplakias.

As in the present study, the prevalence of oral leukoplakias in men was observed in previous research [13, 25]. Although there is no consensus regarding the prevalence of leukoplakia in men, this lesion has a strong relationship with habits such as smoking and alcohol consumption, which are more frequent in men [5, 16, 25, 26]. Additionally, the increased estrogen level in women seems to be a potentially protective factor against the appearance of leukoplakia in women [27].

The appearance of leukoplakia increases in patients aged between 40 and 80 years, who are highly susceptible to the development of oral keratotic lesions [25, 28]. According to some studies [5, 25], increasing age is related to the potential for evolving from leukoplakia to carcinoma, which is more common in groups from the fourth decade of life onwards. Such statements corroborate the results obtained in the present study, considering that most leukoplakias were diagnosed in individuals aged 46 to 60 years.

Although the present study showed that leukoplakia was more prevalent in white patients, there is no evidence to

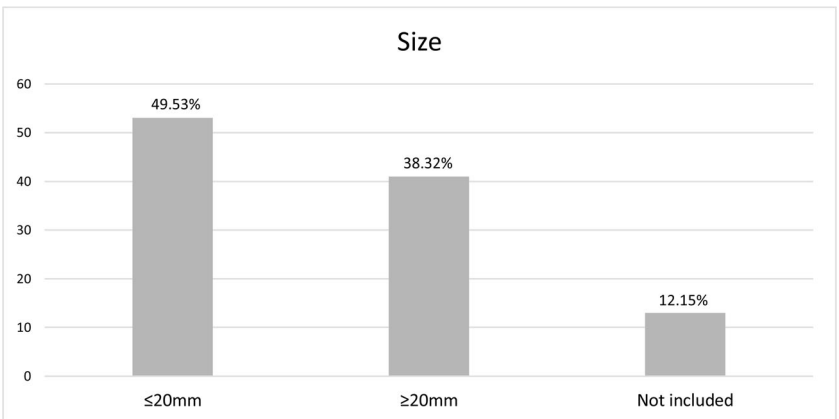

Fig. 4 Distribution of cases of oral leukoplakias according to the size of the lesions (ICB/UPF and FOA/UNESP, 1986–2016)

Table 2 Distribution of cases of oral leukoplakia in terms of symptoms, location, consistency, surface, edges, color, and superficial keratinization of the lesions (ICB/UPF and FOA/UNESP, 1986–2016)

Characteristic	Incidence (n = 107)	%
Symptomatology		
Asymptomatic	63	58.88
Pain	9	8.42
Pain and burning	12	11.21
Itching	1	0.93
Not included	22	20.56
Location		
Alveolar mucosa	2	1.44
Alveolar ridge	19	13.67
Tongue	23	16.55
Lip	32	23.02
Retromolar	8	5.75
Soft palate	7	5.04
Hard palate	9	6.47
Cheek mucosa	37	26.62
Tuber region	1	0.72
Not included	1	0.72
Consistency		
Firm	57	47.5
Soft	32	26.67
Elastic	9	7.5
Fibrous	6	5.0
Not included	16	13.33
Surface		
Rough	28	25.93
Flat	53	49.07
Flat and ulcerated	9	8.33
Rough and ulcerated	7	6.48
Ulcerated	3	2.78
Verrucous	2	1.85
Grooves	1	0.93
Not included	5	4.63
Edges		
Defined	64	59.81
Undefined	25	23.37
Not included	18	16.82
Color		
White	71	66.35
Dark	10	9.35
Reddish-white	22	20.56
Grayish	2	1.87
Not included	2	1.87
Superficial keratinization		
Not removable	53	49.53
Removable	0	0.0
Not included	54	50.47

Table 3 Distribution of cases of oral leukoplakia according to histological characteristics (ICB/UPF and FOA/UNESP, 1986–2016)

Histopathological characteristic	Incidence (n = 107)	%
Hyperkeratosis	88	39.46
Acanthosis	80	35.87
Hyperorthokeratosis	4	1.79
Hyperparakeratosis	5	2.24
Epithelial dysplasia		
Mild ED	24	10.76
Moderate ED	10	4.48
Severe ED	3	1.35
Not included	9	4.05

support the fact that these lesions affect more white individuals than black ones. A previous study reports that the low frequency of lesions in black individuals is linked to the fact that this group seeks medical care only when the lesion is already in an advanced stage of oral carcinoma, underestimating the leukoplakia stage [16].

The appearance of leukoplakia seems to be associated with certain factors and habits expressed by patients, more specifically smoking or alcohol consumption [25, 29]. Such statements agree with the findings of the present study, in which smoking and alcohol consumption represented the main etiological factors of leukoplakia and were statistically related to the proliferative activity of the lesions. Moreover, smoking has been related to the appearance and development of leukoplakia in the oral mucosa in several other studies [16, 29–31] and quitting smoking results in improvement or total cure of the lesions [32], which proves the participation of tobacco in the genesis of leukoplakias [29].

The present study showed that the size of leukoplakias is not closely linked to their cell proliferative activity. This result contradicts statements from a previous study, which affirms that larger lesions are more predisposed to malignant transformation [11, 33].

The results obtained in the present study report that the anatomical sites most affected by leukoplakia are the cheek mucosa, lip, and tongue. There was also a statistically significant relationship between the anatomical location of the lesions and their cell proliferative activity. The cheek mucosa is also considered one of the main sites affected by leukoplakias [34, 35]. For Nagarkar et al. (2019), the tongue and floor of the mouth are the anatomical sites that present the highest malignancy rate [10]. It is worth noting that the lower lip and the lateral edge of the tongue are among the anatomical sites most affected by OSCC [23], which corroborates the findings of the present study that leukoplakias in such locations have a statistically higher cell proliferative activity detected by the AgNOR method.

Table 4 Clinical or histopathological characteristics of the 107 leukoplakia lesions studied and the respective statistical significance with the mean number of NORs, obtained by the mean NORs of 100 cell nuclei of each lesion. (p) value was significant when ≤ 0.05

Clinical and histopathological characteristic	Statistical significance obtained when related to the mean number of NORs (p) (ANOVA)
Age	0.067
Ethnicity	0.425
Gender	0.096
Etiological factor	0.003
Evolution time	0.006
Main lesion	0.653
Size	0.097
Symptomatology	0.029
Location	0.020
Consistency	0.047
Histopathological characteristics	0.004
Surface	0.263
Edges	0.964
Color	0.646
Superficial keratinization	0.001

In this study, the histopathological characteristics showed a statistically significant relationship with the cell proliferative activity of the lesions. The prevalence of hyperkeratosis agrees with that in Silveira et al. [34]. Acanthosis and epithelial dysplasia have also been found frequently; hence, a significant difference is observed between the levels of epithelial dysplasia, with severe dysplasia increasing the risk of malignant transformation by approximately 4.57 times relative to the mild degree [16, 35].

The results hereby presented indicate that leukoplakia with a shorter evolution time had a higher mean NORs per epithelial cell nucleus, theoretically configuring more aggressive lesions or with a greater potential for cancerization. Thus, it is believed that lesions with a shorter evolution time have greater cell proliferative activity and, therefore, are more likely to evolve to a malignant stage [11].

As for symptomatology, the results showed that asymptomatic lesions had a statistically higher mean number of NORs when compared with symptomatic lesions. Thus, it is assumed that, for not manifesting clinical signs, most patients end up seeking medical care later. Thus, leukoplakias would present themselves in an advanced stage when diagnosed and might turn into cancer in a short period.

The multicenter sample analyzed in this research (107 lesions) is of considerable size, having been accumulated over three decades. The limitation of the present study was the lack of some clinical information in the histopathological records analyzed. However, the missing information did not harm the diagnosis of "oral leukoplakia" of the lesions evaluated.

Conclusion

In the present study, the etiological factor, evolution time, symptoms, location, consistency, histopathological characteristics, and superficial keratinization of oral leukoplakias were statistically significant regarding the mean NORs of the lesions. Considering that the increase in the number of NORs may indicate an increase in cell proliferative activity, oral leukoplakias caused by alcohol consumption and/or tobacco use, with an evolution time of fewer than 12 months, asymptomatic, located on the lower lip or tongue, and with a firm consistency and increased superficial keratinization should be treated more aggressively by the clinician to avoid cancerization.

Author contributions Conceptualization: Marcelo Macedo Crivelini, João Paulo De Carli; methodology: Letícia Copatti Dogenski, Sara de Figueiredo Ribeiro, Diego José Gambin, Patrícia Canova Maso; formal analysis and investigation: Sara de Figueiredo Ribeiro, Diego José Gambin, Patrícia Canova Maso; writing—original draft preparation: Letícia Copatti Dogenski; writing—review and editing: Letícia Copatti Dogenski, João Paulo De Carli; supervision: Maria Salete Sandini Linden, Micheline Sandini Trentin, Marcelo Macedo Crivelini, João Paulo De Carli.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants agreed with the ethical standards of the institutional and/or national research committee and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. Approval was obtained from the ethics committee of University of Passo Fundo (No. 172/2011).

Informed consent For this type of study, formal consent is not required.

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