Original Article

Archives & Clinical Experimental Surgery

Increased of Langerhans Cells in Smokeless Tobacco-Associated Oral Mucosal Lesions

Érica Dorigatti de Ávila[1], Rafael Scaf de Molon[2], Melaine de Almeida Lawall[1], Renata Bianco Consolaro[1], Alberto Consolaro[1]

Abstract

Objective: To evaluate the changes in the number of Langerhans Cells (LC) observed in the epithelium of smokeless tobacco (SLT-induced) lesions.

Methods: Microscopic sections from biopsies carried out in the buccal mucosa of twenty patients, who were chronic users of smokeless tobacco (SLT), were utilized. For the control group, twenty non-SLT users of SLT with normal mucosa were selected. The sections were studied with routine coloring and were immunostained for S-100, CD1a, Ki-67 and p63. These data were statistically analyzed by the Student's t-test to investigate the differences in the expression of immune markers in normal mucosa and in SLT-induced leukoplakia lesions.

Results: There was a significant difference in the immunolabeling of all markers between normal mucosa and SLT-induced lesions ($p<0.001$). The leukoplakia lesions in chronic SLT users demonstrated a significant increase in the number of Langerhans cells and in the absence of epithelial dysplasia.

Conclusion: The increase in the number of these cells represents the initial stage of leukoplakia.

Key words: *Smokeless tobacco, leukoplakic lesions, cancer, langerhans cells, chewing tobacco.*

[1]*Bauru Dental School*
University of São Paulo
Bauru–SP, Brazil

[2]*Araraquara Dental School*
São Paulo State University
Araraquara-SP, Brazil

Received: February 05, 2012
Accepted: February 29, 2012
Arch Clin Exp Surg 2012; 1: 85-93
DOI: 10.5455/aces.20120229052919

Corresponding author
Érica Dorigatti de Avila
Departamento de Estomatologia
da Faculdade
de Odontologia de Bauru
Universidade de São Paulo (USP)
Avenida Alameda Octávio
Pinheiro Brizola, 9-75, 17012-901
Bauru–SP, Brasil
erica.fobusp@yahoo.com.br

Introduction

Among tobacco users, there is a false belief that SLT is safe because it is not burned, which leads many people to quit cigarettes and start using SLT [1]. However, SLT contains higher concentrations of nicotine than cigarettes and, in addition, nearly 30 carcinogenic substances, such as tobacco-specific N-nitrosamines (TSNA), which is formed during the aging process of the tobacco, [2-4] and which presents high carcinogenic potential. Moreover, because the tobacco has direct contact with the oral mucosa and creates a more alkaline environment, its products may even be more aggressive to tissue [5]. The percentage of SLT users is lower compared to cigarette users; however, usage is increasing among young individuals and it is therefore a significant and disturbing danger [6,7].

Initial studies on the effects of SLT on the oral mucosa demonstrated the formation of white lesions induced by chronic exposure to tobacco, characterized by epithelial thickening, increased vascularization, collagen alterations, as well as coagulation necrosis [8,9]. Several oral lesions are associated with SLT use, of which oral cancer is the most prominent. Its use can be addictive, leading to oral

leukoplakias and gingival recession, and it may play a contributory role in the development of cardiovascular disease, peripheral vascular disease, hypertension and fetal morbidity and mortality. Another epithelial alteration observed in SLT users was a variation in the number of Langerhans cells (LC) in the oral epithelium.

Langerhans cells (LC) are antigen-presenting cells that reside in the epidermis of the skin and traffic to lymph nodes. The general role of these cells in skin immune responses is not clear because distinct models of LC depletion resulted in opposite conclusions about their role in contact hypersensitivity responses. While some authors [10] conclude an increased number of LC and reduced mitotic activity at the basal layer, others [11] noticed a reduced number of Langerhans cells. However, it is possible to suppose that the participation of Langerhans cells in antitumor immunity indicates that the behavior of these cells can be directly related to disease progression [12,13].

To improve understanding in regards to the significance of the variation in the number of Langerhans cells, the present study was designed to evaluate the alterations in the distribution and number of Langerhans cells as well as the occurrence of epithelial dysplasia in SLT-induced leukoplakia lesions.

Material And Methods

Patients

This protocol (#81/2005) was reviewed by the Ethics Committee of Bauru Dental School, University of São Paulo and each participant in the project signed a detailed informed consent form. Two groups were formed: 20 chronic SLT users and 20 non-users of SLT (control group). The chronic SLT users with oral leukoplakia were selected from venues related to horseracing, rodeos and agriculture exhibitions, and were referred to the post-graduation clinic of Bauru Dental School. A careful medical history was taken of each participant and the following data was tabulated: age; gender; ethnicity; location, size and evolution of the lesion; frequency and duration of SLT use; preferred placement of SLT in the oral cavity; type of tobacco used and the present symptomatology. The non-SLT users were selected from the Pathological Anatomy Service of the Discipline of Pathology of Bauru Dental School, University of São Paulo. Thirteen cases were composed of oral mucosa submitted with pericoronal material after extraction of unerupted teeth, which received the final diagnosis of a pericoronal follicle. The seven remaining cases were comprised of oral mucosa adjacent to removed mucoceles, with the final diagnosis of a mucus extravasation phenomenon.

Sample preparation and staining

Incisional biopsies of the leukoplakias were performed. The specimens presented average sizes of 10 mm x 7 mm x 5 mm at their widest points and were sectioned into two fragments along the largest axis for embedding in paraffin and sectioning.

The specimens were sectioned at 4-μm thick sections. Two sections were stained with Harris and Lison's hematoxylin-eosin and six were mounted on silanized glass slabs for immunohistochemistry. The immunohistochemical technique was applied using the streptavidin-biotin method. Antigenic recovery was performed with a citrate buffer at 0.01 M and pH 6.0 in a microwave oven, in 3 cycles with 5 minutes each, at a power of 750W, using antibodies for protein S-100 (polyclonal, 1:5,000, Dakocytomation), CD1a (O10, 1:20, Immunotech), MIB (MIB-1, 1:4,800, Dakocytomation) and p63 (4A4, 1:300, Neomarkers). Counterstaining was performed with Meyer's hematoxylin. The primary antibody was omitted in the negative control, and the positive controls were obtained using antibodies for S-100 and CD1a for tissue components of the oral mucosa and submucosa, and for Ki-67 and p63 for sections of epidermoid carcinoma.

All glass slabs were individually analyzed by two professionals using a binocular microscope. The epithelial changes were evaluated in accordance with World

Health Organization (WHO) criteria: increased keratin layer on the epithelial surface (hyperparakeratosis/hyperorthokeratosis) and/or increased squamous layer (acanthosis); presence of dysplastic changes in the epithelial cells, including enlarged and pleomorphic nuclei and cells, large and prominent nucleoli, increased nucleus/cytoplasm ratio, hyperchromatic nuclei, diskeratosis, increased number of mitoses and atypical mitoses [14]. These phenomena defined the occurrence or absence of epithelial dysplasia, as well as if it was mild, moderate or severe.

The immunohistochemical labeling was characterized as present according to the brown staining of cells, or absent when this was not observed. The cells labeled by S-100, CD1a, Ki67 and p63 were counted on 20 randomly selected fields for each section, at 40x magnification, by two pathologists.

Statistical Analyses

These data were statistically analyzed by the Student's t-test to investigate the differences in the expression of immune markers CD1, S-100, Ki67 and p63 in normal mucosa and in SLT-induced leukoplakia lesions.

Results

All 20 patients in the group of normal mucosa were males aged 15 to 26 years (mean age 22.1 years), who were not tobacco or alcohol users. The group composed of chronic users of SLT presenting leukoplakia lesions included 20 male patients aged 15 to 26 years (mean age 23.4 years), all of whom were students and practiced sports or activities related to horseracing, rodeos and agriculture exhibitions.

The type of SLT most widely employed (18 patients) was humid and sold in cans. The tobacco was placed in the mouth by moistening the fingers and placing it in the space between the cheek/lip and the gingiva or on the sublingual space, between the tongue and the teeth. The users varied the site of placement of the SLT in the mouth. Two patients used canned SLT packaged into small pouches, which were individually placed in the mouth at the aforementioned sites. The time of use of the SLT ranged from 30 minutes to 4 hours, with a mean of 1.2 hours.

The leukoplakia lesions on the 20 SLT users were clinically characterized as blurred white lesions without defined contours, with sizes ranging from 1 to 2.5 cm. They were located at the buccal sulcus and extended to the upper region, either to the cheek (12 patients) or to the lip (8 patients). The lesions were asymptomatic and had slightly irregular surfaces, forming small undulations when observed in detail.

The thickness of the epithelium of the normal oral mucosa in all specimens exhibited ranged from 15 to 30 layers. The superficial layers predominantly exhibited a parakeratinization process. The cells on the superficial and squamous layers presented eosinophilic cytoplasm, yet some regions occasionally exhibited clear cytoplasm and mildly pyknotic nuclei. The epithelial stratification and horizontalization were typical, as observed in the normal oral mucosa. In the lamina propria and submucosa, the pattern of distribution of cells and collagen fibers was considered normal in all specimens.

In the SLT-induced lesions, the epithelium in all specimens displayed moderate hyperplasia with areas of acanthosis (Figure 1). The superficial layers presented intensive parakeratinization. The cells on the superficial and squamous layers exhibited clear cytoplasm, small nuclei and chromatin condensation. Narrow and dense zones of parakeratinization were occasionally observed among the cells in the superficial layers.

The epithelial stratification and horizontalization were similar to that observed in normal oral mucosa. No specimen exhibited morphological signs of epithelial dysplasia. In the lamina propria and submucosa, the pattern of distribution of cells and collagen fibers was normal in all specimens. There was no inflammatory infiltrate or edema on the lamina propria and submucosa.

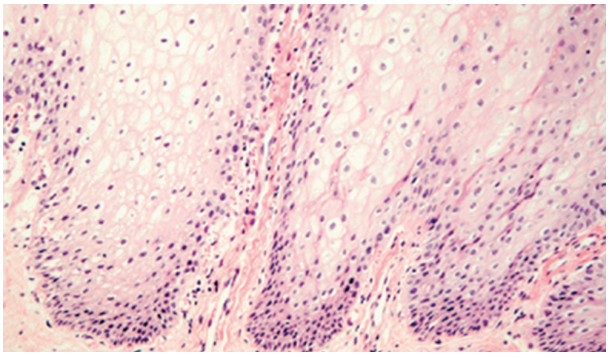

Figure 1. Vacuolated cells with a clear cytoplasm and mildly pyknotic nuclei on the superficial and squamous layers. (Hematoxylen & Eosina, x25).

In the cases of normal oral mucosa, the Langerhans cells labeled by the protein S-100 were homogeneously distributed along the superficial and squamous layers. Those located exclusively on the basal layer, which are probably melanocytes also sensitive to labeling with this antibody, were not considered. These star-shaped, dendritic cells had larger and clearer nuclei, compared to the adjacent cells, and were permeated between the keratinocytes. In SLT-induced lesions, the distribution was very similar (Figure 2a, b).

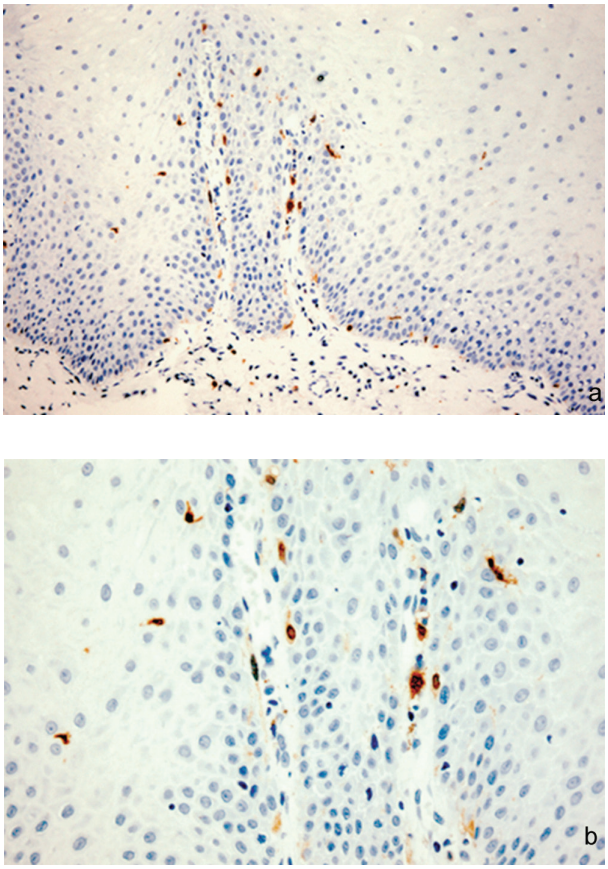

Figure 2. Microscopic features of SLT-induced leukoplakia. Langerhans star-shaped cells presented immunoreaction for S-100 and were homogeneously distributed along the squamous layer. The immunoreactive cells located exclusively on the basal layer are probably melanocytes. (S100, A: X10, B: X25).

The CD1a immunolabeled Langerhans cells present in the normal mucosa were homogeneously distributed along the squamous and superficial layers. These cells had a star-shaped and dendritic appearance, had large and clear nuclei and permeated the spaces between the keratinocytes. Also, in SLT-induced leukoplakia lesions, the distribution of Langerhans cells labeled by CD1a was homogeneous along the squamous and superficial layers (Figure 3). Morphologically, they were more exuberant compared to those immunolabeled by the antibody S-100.

Immunolabeling by Ki-67 observed in the cases of normal oral mucosa was more evident in parabasal cells, while cells in the basal layer were occasionally immunolabeled. As in the cases of normal oral mucosa, the basal cells and especially the parabasal cells were labeled and homogeneously distributed along the epithelium of SLT-induced leukoplakia lesions (Figure 4) [2].

The immunolabeling by p63 observed in cases of normal oral mucosa highlighted the distribution of basal and parabasal cells in the normal epithelium of the oral

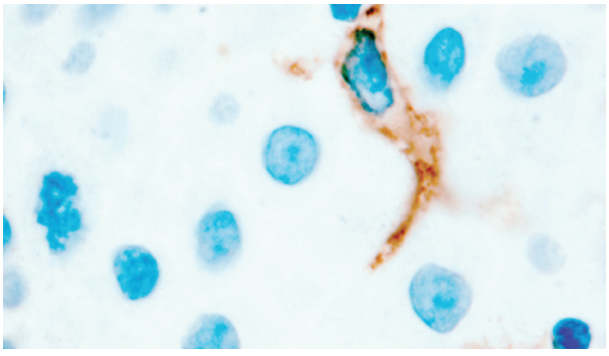

Figure 3. Microscopic features of SLT-induced leukoplakia. Star-shaped Langerhans cells presented immunoreaction for CD1a and were homogeneously distributed along the squamous and basal layers. (CD1a, X40).

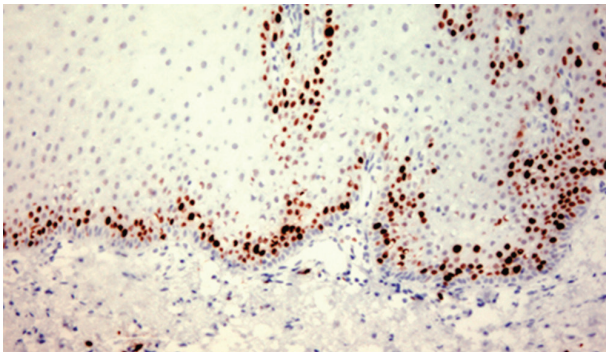

Figure 4. Microscopic features of SLT-induced leukoplakia. Immunoreaction for Ki-67 observed was more evident in parabasal cells, while cells in the basal layer were occasionally immunoreactive. (Ki-67, X10).

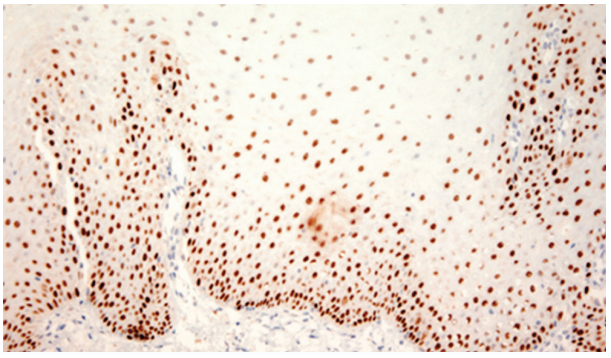

Figure 5. Microscopic features of SLT-induced leukoplakia. Immunoreaction for p63 was widely observed in the basal and parabasal cells of the oral mucosa, characterized by homogeneous distribution juxtaposed to the basal membrane. (p63, X10).

mucosa, characterized by homogeneous distribution when juxtaposed with the basal membrane. However, some occasional basal and parabasal cells were not immunolabeled.

The distribution of basal and parabasal cells in the epithelium of SLT-induced leukoplakia lesions immunolabeled by p63 was similar to that observed on the normal mucosa, i.e. it was characterized by homogeneous distribution close to the basal membrane (Figure 5).

In each specimen analyzed, the Langerhans cells were counted on 20 randomly selected fields in the linear direction of the lining epithelium, at 40x magnification. The mean number of these cells was statistically analyzed by the Student's t-test for each immune marker, S-100 and CD1a, at a significance level of 5%. The means and standard deviations obtained for each immune marker are presented in Table 1.

There was a significant difference in the number of cells immunolabeled by CD1a in the normal mucosa and SLT-induced leukoplakia lesions ($p<0.001$). Similarly, there was a significant difference between the number of cells immunolabeled by S-100 in the normal mucosa and in SLT-induced leukoplakia lesions ($p<0.001$). In order to evaluate the higher specificity of cell labeling presented by CD1a, compared to the protein S-100, the Pearson test and the paired Student's t-test at a significance level of 5% were applied. There was a high correlation between the expression of CD1a and S-100 (0.78); however, a significant difference was observed between their expressions ($p<0.001$). The means and 10 standard deviations obtained for each immune marker are demonstrated in Table 2.

Table 2. Means and standard deviations CD1a and S-100

Proteins	Mean ± SD	P value
CD1a	5.3 (±1.3)	p<0.001
S-100	4.4 (±1.0)	

Table 1. Means and standard deviations control group and SLT-induced lesion.

	Mean ± SD		
	Controls	**SLT-induced lesion**	**P value**
S100	3.8 (± 0.9)	5.0 (± 0.7)	p<0.001
CD1a	4.5 (± 1.0)	6,1 (± 1,1)	p<0.001
Ki-67	33.3 (± 3.4)	44.8 (± 3.4)	p=0.000
p63	68.4 (± 7.2)	81.7 (± 6.4)	p=0.000

The quantification of cells expressing the markers Ki67 and p63 was also assessed on 20 randomly selected fields in the linear direction of the lining epithelium, at 40x magnification. The mean number of immunolabeled cells was then submitted to statistical analysis by the Student's t-test for each immune marker, Ki67 e p63, at a significance level of 5%. The means and standard deviations obtained for each immune marker are presented in Table I. The Student's t-test revealed a significant difference in the number of cells immunolabeled by Ki67 and p63 between the normal mucosa and SLT-induced leukoplakia lesions ($p<0.001$).

Discussion

The results of this study showed a significant increase in the number and concentration of Langerhans cells in leukoplakia lesions of chronic SLT users.

Initially, the immunolabeling of Langerhans cells was planned with polyclonal antibodies to protein S-100. The S-100 represents a multigenic family of proteins binding to the Ca2+ of low molecular weight and with 19 members expressed in a wide spectrum of cell types. Members of the S-100 family are associated with Ca2+-dependent regulation in a variety of intracellular activities, such as phosphorylation of proteins, cell proliferation and differentiation, including neoplastic transformation [15]. The protein S-100 has been identified in many cell types, such as Schwann cells, chondrocytes, Langerhans cells and other cells of neural origin. The expression of this protein was also observed in several types of neoplasias, especially of neural origin, salivary gland neoplasias, granular cell neoplasias, muscular neoplasias, chondrosarcomas, in nearly 95% of melanomas, and also in Langerhans cells found in the Langerhans cell disease [16,17].

Since 1991, El-Hakim et al. have proposed that some products of arachidonic acid cascade are involved in the development and growth of oral cancer. Lipid peroxidation products can be released, for example, as a consequence of inflammatory reactions of leukocytes or macrophages directed against tumor development. These products may interact directly with DNA and may mediate oxidative DNA damage. Lipid peroxidation products arising from the cyclooxygenase or the lipoxygenase pathways may be involved in the processes of tumor metastasis or tumor promotion [18]. In 1992, Arenberger et al. also presented the hypothesis that lipoxygenase products are chemotactic for LC [19]. Two years later, Metzger et al. noted that the concentration of the main lipoxygenase product, 12-HETE, is twice as high in saliva of patients with squamous cell carcinoma, when compared to healthy control [20].

When Ahmed et al. (2007) evaluated the variation in the number and localization of CD1a-positive Langerhans cells, they found that the number and pattern of distribution were different in chronic hyperplastic candidiasis, leukoplakia, and healthy control tissue study groups [21]. The results of this study showed a higher number of LC in leukoplakia lesions with dysplasia.

These observations continued in subsequent years. Wenghoufer et al. (2010) analyzed the leukoplakia of the oral mucosa by gene expression of a subset of genes involved in inflammation and they confirmed the presence of Cox-1 and Cox-2 in leukoplakia lesions [22].

Therefore, these findings suggest that the lipoxygenase products released during the inflammatory process caused by TSF may stimulate the proliferation of Langerhans cells [20].

In this study, there was a statistically significant difference in the expression of CD1a and S-100 antibodies in Langerhans cells between the specimens of normal mucosa and specimens of SLT-induced leukoplakia lesions. The present results also confirm the findings of Shklar et al. (1985), who observed an increase in the Langerhans cells in leukoplakia lesions and attributed this increase as an attempt at epithelial protection against the substances present in SLT.

However, the present data disagree with the report of Daniels et al. (1992), who observed a reduction in

the number of Langerhans cells in SLT-induced lesions. These authors believed that this reduction is a result of changes caused by ST, and is one factor in the long-term pathogenesis of ST-associated oral carcinoma [11]. Benharroch et al. (2010) investigated the implications of LC excess in lymphomas and concluded that an excess of LC in Hodgkin lymphoma patients is not correlated with a worse outcome [23].

It is interesting to note that the literature shows significant expression in the number of LC in lesions classified as malignant lesions. In 1999, Sokolova et al. presented a rare case of a tumor arising from Langerhans cells in the tongue and neck area in a 37-year-old man [24]. In this case, despite the electron microscopy that identified granules of Langerhans, no expression of protein S-100 was found. The antigen Ki-67 is present in normal and proliferating tumor cells and may be used as a marker to determine the growth fraction of a cell population. Thus, antibodies against the protein Ki-67 have been used as diagnostic tools for the different types of neoplasias and preneoplastic lesions [25].

The protein p63 has been detected in several normal human tissues, in proliferating cells and also as a marker of stem cell keratinocytes. In the normal and hyperplastic epithelia, the nuclear labeling is strong and may be observed on the basal and suprabasal layers [26]. The increased expression of p63 in epithelial tumors of the head and neck suggests that it may play a role in the oncogenesis of these tumors [27, 28].

There was a statistically significant increase in the expression of Ki67 and p63 in SLT-induced leukoplakia lesions, compared to normal mucosa. The distribution and intensity of labeling were similar in the normal oral mucosa and in SLT-induced leukoplakia lesions. These findings may be considered in the context of a hyperplastic epithelium with intensive parakeratinization, which indicate an adaptation of oral mucosa cells to constant aggression, either physical or chemical. For Arredondo et al. (2001), nicotine and its byproducts can alter the normal balance of cell growth and differentiation, which accelerates squamatization and increases the risk for malignant transformation [29]. Possibly, the increased epithelial desquamation with a consequent increase in the rate of cell proliferation can explain a larger number of cells immunolabeled by Ki-67 and p63 observed in SLT-induced leukoplakia lesions. Nevertheless, the absence of dysplastic alterations did not allow us to suggest that SLT-induced leukoplakias have a higher potential of developing into pre-cancerous lesions and oral malignancy than other leukoplakias.

For Merne et al. (2002), keratinization and thickening of the epithelium have been regarded as a protective response, helping to impede the delivery of tobacco constituents to the cell and the nucleus [30]. However, the direct application of SLT on the mucosa promotes a local attrition of the tissue and allows a direct release of chemical products and byproducts present in SLT into the oral environment, which gradually reaches the connective tissue and consequently the blood circulation. These two types of aggression to the oral epithelium are probably responsible for the hyperplasia and intensive hyperkeratinization.

Two other morphological aspects that indicate aggression to the epithelium may be the intense vacuolization observed in epithelial cells on the superficial layers and the large number of cells with pyknotic nuclei. The cytoplasmic vacuolization has been regarded as a degenerative response of the epithelia to high alkalinity of the SLT products [31]. Alkalinity is used to enhance nicotine delivery, but it also maximizes the toxic effects of SLT products [32]. Pyknosis, in turn, occurs in an unspecific manner in the presence of cell aggression and represents chromatin condensation [33].

Despite the frequent and long period of use among SLT users, as characterized in the present sample, there was probably an insufficient amount of time for the SLT byproducts to act on the oral mucosa and promote significant alterations in the pattern of cell proliferation, which could morphologically result in epithelial dysplasia. Another possible explanation may be the very young age range of the users, which would imply insufficient time for the occurrence of failures in antigenic recognition or in the mechanism of cell control to allow for the disorganized proliferation of altered cells, which would

thus be destructed by apoptosis or by the extracellular control mechanisms, such as the action of natural killer (NK) cells [33].

The leukoplakia lesions in chronic SLT users demonstrated a significant increase in the number and concentration of Langerhans cells and absence of epithelial dysplasia. Therefore, it may be suggested that there is an adaptive response of the peripheral immune system to the functional demand increased by the greater penetration of antigens through the oral mucosa. The increased epithelial desquamation with a consequent increase in the rate of cell proliferation, possibly induced by nicotine and its byproducts, explain the increased number of cells immunolabeled by Ki-67 and p63 observed in SLT-induced leukoplakia lesions. Similarly, considering the results of this study and the results of the scientific literature reviewed, it is clear that there is a direct relationship between the number of Langerhans cells present and the current stage of the injury. Thus, it is also possible to suggest that the increase in the number of these cells represents the initial stage of leukoplakia. These findings emphasize the necessity to understand these relationships in order to promote the development of prevention strategies.

Acknowledgements

The authors would like to sincerely thank Mrs. Kim Kubitza for correcting the English language in this article. This work was supported by a grant of the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), Brazil.

Conflict of interest statement

The authors do not declare any conflict of interest or financial support in this study.

References

1. Christen AG, Swanson BZ, Glover ED, Henderson AH. Smokeless tobacco: the folklore and social history of snuffing, sneezing, dipping, and chewing. J Am Dent Assoc 1982;105:821-829.
2. Hoffmann D, Djordjevic MV. Chemical composition and carcinogenicity of smokeless tobacco. Adv Dent Res 1997;11:322-329.
3. Changrani J, Gany F. Paan and Gutka in the United States: an emerging threat. J Immigr Health 2005;7:103-108.
4. Gregory RL, Kindle JC, Hobbs LC, Malmstrom HS. Effect of smokeless tobacco use in humans on mucosal immune factors. Arch Oral Biol 1991;36:25-31.
5. Zatterstrom UK, Svensson M, Sand L, Nordgren H, Hirsch JM. Oral cancer after using Swedish snus (smokeless tobacco) for 70 years - a case report. Oral Dis 2004;10:50-53.
6. Boffetta P, Hecht S, Gray N, Gupta P, Straif K. Smokeless tobacco and cancer. Lancet Oncol 2008;9:667-675.
7. Sinha DN, Gupta PC. Tobacco use among teachers [corrected] in Uttar Pradesh & Uttaranchal, India. Indian J Public Health 2004;48:132-137.
8. Lyon H, Poulsen HE, Pindborg JJ. Studies in oral leukoplakias. Deposits of amyloid in the oral submucosa induced by prolonged use of snuff. Acta Pathol Microbiol Scand 1964;60:305-310.
9. Pindborg JJ, Poulsen HE. Studies in oral leukoplakias. I. The influence of snuff upon the connective tissue of the oral mucosa. Preliminary report. Acta Pathol Microbiol Scand 1962;55:412-414.
10. Shklar G, Niukian K, Hassan M, Herbosa EG. Effects of smokeless tobacco and snuff on oral mucosa of experimental animals. J Oral Maxillofac Surg 1985;43:80-86.
11. Daniels TE, Chou L, Greenspan JS, Grady DG, Hauck WW, Greene JC, et al. Reduction of Langerhans cells in smokeless tobacco-associated oral mucosal lesions. J Oral Pathol Med 1992;21:100-104.
12. Wright-Browne V, McClain KL, Talpaz M, Ordonez N, Estrov Z. Physiology and pathophysiology of dendritic cells. Hum Pathol 1997;28:563-579.
13. Schmitt D. [The Langerhans cell: from in vitro production to use in cellular immunotherapy]. [Article in French]. J Soc Biol 2001;195:69-74.

14. Pindborg JJ, Reichart PA, Smith CJ, van der Waal I. Histological Typing of Cancer and Precancer of the Oral Mucosa. In Collaboration with Sobin LH and Pathologists in 9 Countries. World Health Organization International Histological Classification of Tumors, Springer-Verlag Berlin Heidelberg, Germany, 1997.
15. Donato R. Functional roles of S100 proteins, calcium-binding proteins of the EF-hand type. Biochim Biophys Acta 1999;1450:191-231.
16. Nakajima T, Kameya T, Watanabe S. S-100 protein distribution in normal and neoplastic tissues. In: Sternberg S, DeLellis R (eds.). Advances in Immunohistochemistry, Masson Publishing, New York, 1984;141–158.
17. Tomar SL. Trends and patterns of tobacco use in the United States. Am J Med Sci 2003;326:248-254.
18. el-Hakim IE, Langdon JD. Arachidonic acid cascade and oral squamous cell carcinoma. Clin Otolaryngol Allied Sci 1991;16:563-573.
19. Arenberger P, Kemény L, Rupec R, Bieber T, Ruzicka T. Langerhans cells of the human skin possess high-affinity 12(S)-hydroxyeicosa tetraenoic acid receptors. Eur J Immunol 1992;22:2469-2472.
20. Metzger K, Angres G, Maier H, Lehmann WD. Lipoxygenase products in human saliva: patients with oral cancer compared to controls. Free Radic Biol Med 1995;18:185-194.
21. Ali A, Rautemaa R, Hietanen J, Beklen A, Konttinen Y. A possible CD1a Langerhans cell-mast cell interaction in chronic hyperplastic candidosis. J Oral Pathol Med 2007;36:329-336.
22. Wenghoefer M, Pantelis A, Najafi T, Deschner J, Allam JP, Novak N, et al. Gene expression of oncogenes, antimicrobial peptides, and cytokines in the development of oral leukoplakia. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2010;110:351-356.
23. Benharroch D, Guterman G, Levy I, Shaco-Levy R. High content of Langerhans cells in malignant lymphoma--incidence and significance. Virchows Arch 2010;457:63-67.
24. Sokolova IN, Pavlovskaia AI, Smirnov AV, Bukaeva IA. [Sarcoma arising from Langerhans cellsī]. [Article in Russian]. Vopr Onkol 1999;45:641-644.
25. Scholzen T, Gerdes J. The Ki-67 protein: from the known and the unknown. J Cell Physiol 2000;182:311-322.
26. Choi HR, Batsakis JG, Zhan F, Sturgis E, Luna MA, El-Naggar AK. Differential expression of p53 gene family members p63 and p73 in head and neck squamous tumorigenesis. Hum Pathol 2002;33:158-164.
27. Chen YK, Huse SS, Lin LM. Differential expression of p53, p63 and p73 proteins in human buccal squamous-cell carcinomas. Clin Otolaryngol Allied Sci 2003;28:451-455.
28. Nylander K, Coates PJ, Hall PA. Characterization of the expression pattern of p63 alpha and delta Np63 alpha in benign and malignant oral epithelial lesions. Int J Cancer 2000;87:368-372.
29. Arredondo J, Nguyen VT, Chernyavsky AI, Jolkovsky DL, Pinkerton KE, Grando SA. A receptor-mediated mechanism of nicotine toxicity in oral keratinocytes. Lab Invest 2001;81:1653-1668.
30. Merne M, Heinaro I, Lähteenoja H, Syrjänen S. Proliferation and differentiation markers in snuff-induced oral mucosal lesions. J Oral Pathol Med 2002;31:259-266.
31. Axéll T, Mörnstad H, Sundström B. The relation of the clinical picture to the histopathology of snuff dipper"s lesions in a Swedish population. J Oral Pathol 1976;5:229-236.
32. Tomar SL, Henningfield JE. Review of the evidence that pH is a determinant of nicotine dosage from oral use of smokeless tobacco. Tob Control 1997;6:219-225.
33. Kumar V, Abbas AK, Fausto N. Acute and chronic inflammation. In: Kumar V, Abbas AK, Fausto N (eds). Robbins and Cotran Pathologic Basis of Disease, Elsevier Saunders, Philadelphia, 2005; 47–86.

DOI: 10.5455/aces.20120229052919