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CORELATIONS BETWEEN THE CLINICAL AND MORFOLOGICAL ASPECTS AND THE MOLECULAR MARKERS IN THE MALIGNANT TRANSFORMATION OF THE ORAL LEUKOPLAKIAS

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ABSTRACT

The leukoplakia injuries represent the most frequent premalignant oral injuries. Their clinical and histopathological features are not always significant for the malignant transformation and, implicitly, for their diagnosis. The important progress achieved in the last three decades for the understanding of the cellular and molecular mechanisms of the leukoplakia lesions have not managed to significantly reduce the prevalence of the oral squamouscellular carcinomas. Therefore, it is necessary the identification and the characterization of the molecular markers which, together with the clinical and morphological aspects, can improve the management of these lesions.

Key words: leukoplakia, biomarkers, premalignant lesions

INTRODUCTION

Over the years, the suitable identification and treatment of the premalignant lesions constituted an important objective in the prevention of the cancer of the oral mucosa. Despite the progress achieved in the last decades, their incidence recorded a significant increase due to the incapacity of early diagnoses. This incidence varies a lot between the developed countries and the emerging ones. [1, 2, 3]

Described for the first time by the Hungarian dermatologist Erno Schwimmer, in 1877, as a lesion preceding the squamouscellular carcinoma of the tongue in patients with third degree syphilis, leukoplakia is a relatively frequent in the general population, varying between 1-5 %. This represents more than 80 % of the total premalignant lesions at the level of the oral mucosa, being followed by erythro-plasia/erithroleukoplakia, lichen planus, oral sub-mucus fibrosis, lupus erythematous, chronic keratosis cheilitis of the lower lip and the congenital oral fluoride dyskeratosic papillomatosis. [1, 4-8]

In the speciality literature there is yet no consensus with regard to the malignant transformation of the leukoplakia lesions. Although the majority of the studies indicate a rate smaller than 1 %, however, at the world level, this rate has been calculated at an average value of 1,36 %/year with variations between 3 % and 17 %, according to the morphological and ethiopathogenic features. [2, 4, 5, 9, 10]

The prognosis of the leukoplakia lesions is very diverse, specifying that 42,5 % of the untreated injuries are delayed after approximately 5 years of evolution. However, the persistent and aggressive lesions require attentive supervision and early treatment. [1, 9]

Given the fact that, presently, the risk of the oral leukoplakia lesions transforming in a squamouscellular carcinoma remains difficult to foresee, several studies tried to identify the

potential cellular and molecular markers which, together with certain clinical features, might indicate the potential to become malignant. [5, 10]

Clinical and ethiopathogenic characteristics

According to WHO, leukoplakia is "a white stain or plaque which cannot be clinically and histopathologically characterised as any other disorder". Thus, leukoplakia requires a complex differential diagnosis. [1, 2, 9]

Presently, the oral leukoplasia lesions, considered until recently specific to the old persons, know an increase of incidence and prevalence among the young population (31,5 %), with a bigger weight in men than women due to the larger exposure to various physical and chemical agents. [11]

Among the favoured etiological factors in favour for the development of the premalignant oral lesions we can remind: infections, chronical local mechanical irritations determined either by the radicular residues or by the inadequate dental prosthetics, the genetic predisposition, alcohol and tobacco consumption. The latter is responsible for the apparition of 70-90 % of the leukoplakia. [6, 11]

From the clinical point of view, the leukoplakia can be homogeneous, with the aspect of white, thin, uniform or inhomogeneous spots, manifesting as white lesions with a spotted and nodular appearance, such as the verrucous leukoplakia, determined by the infection with Human papilloma virus (HPV). It was noticed that the presence of the HPV-16 genotype is correlated with an increased rate of malignant transformation for this type of leukoplakia. [6, 9, 12, 13]

In the last ten years, there were registered significant increases of the infection with Treponema pallidum, especially in Eastern Europe. The risk of malignant transforming of the syphilitic leukoplakia was suggested by the increase of the prevalence of up to 60 % of the oral squamouscellular carcinomas developed on the dorsal side of the tongue in men with tertiary syphilis. [14]

Ever since 1965, Jepsen and Wither have associated the mycotic infection, caused by the serotypes of Candida albicans, with the increase of the risk of carcinomatosis transformation of the nodular leukoplakia in more than 9 %. [15]

Although these injuries can develop anywhere at the level of the oral cavity, however, it was noticed, ever since 1978, that the location at the floor level and the lateral parts of the tongue represent a predicting factor of the malignant transformation. Furthermore, leukoplakia is the most common form of the actinic cheilitis with undefined risk of malignant transformation. [4, 9]

Morphological characteristics

Within the malignant transformation process of the snot's epithelium, leukoplakia represents the first morphological change. [16]

Although it can be identified at the first inspection of the oral cavity, this being accessible to the early screening and diagnosis, however, it is considered that the bioptic exam, done with the scalpel and not with the punch, remains of reference. This hypothesis does not sustain the results of the recent studies, which have indicated the fact that there is no correspondence between the clinical shape of the leukoplakia and the histopathological exam. [1, 4, 9, 17-19]

The microscopic aspect of the leukoplakia lesions, together with the macroscopic features regarding the consistency, dimensions and shapes constitute a predicting factor of the malignant transformation. [1, 9, 20]

The photonic microscopy can emphasize the keratosis hyperplasia, hyperortho-keratosis, hyperparakeratosis, acanthosis or cellular atypia of different degrees which can indicate the presence of a slight, moderate or severe dysplasia. Among the morphological features of the dysplasia we remind the high cellular density, cells similar to the basal ones with hyperplasic and hyper-chromatic nuclei, increased nucleus-cytoplasmatic report and increased cellular proliferation with numerous anomalies in the upper layers. In this respect, the results of a study done by Rentrup et al.

showed that there is no correlation between the hyperkeratosis, acanthosis, dyskeratosis and carcinoma in situ, invasive squamocellular carcinoma and leukoplakia. [2, 17, 18]

The carcinomas transformation was identified in 17 % of the cases, in a 7 years interval from the diagnosis. [1, 2]

Until recently, the oral epithelial dysplasia represented the most important factor of prognosis of the malignant transformation in the oral leukoplakia, taking into consideration the fact that 15,6-39,2 % of these presented carcinomas changes. Presently, it is intensely debated the association between the dysplasia's degree of leukoplakia lesions and the risk of malignant transformation.(Fig.1) [4, 17, 21, 22]

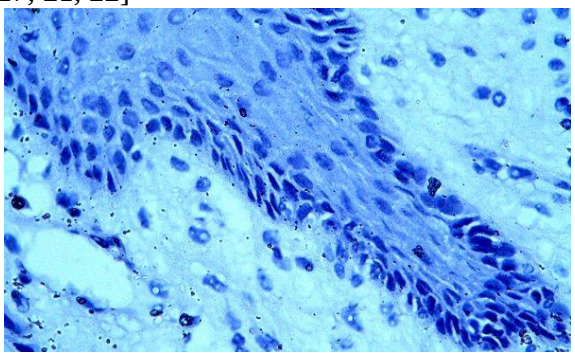

Figure 1. HE stains of leukoplakia tongue with intermediate grade keratinizing squamous lesion x 40X (Roy et al.,2014)

Although few, the electronic microscopic studies have objectified a series of ultra-structural features typical to the malignant transformation of the different types of leukoplakia lesions found at oral mucosa level. Thus, the transmission electronic microscopy (TEM) reveals certain details, superior to the histopathological exam, among which we remind the discontinuity of the basal membrane, the disorganization of the hemidesmosomes and the destruction of the inter-cellular junctions, to which it is added the alteration of the keratinization indicated by the reduction of tonofilaments, of the keratholian granules and of the Odland bodies. [17]

Since 1984, Dourov et al. mentioned that the scanning electronic microscopy (SEM) can identify, from the start, the aspects suggesting the malignant transformation through the examination of the epithelial surface of the oral mucosa. [23]

Molecular characteristics

A series of molecular markers were proposed, which, together with the clinical and histopathological aspects of these premalignant lesions, function as a valuable instrument of diagnosis and prognosis. [20]

The markers of the malignant transformation for the leukoplakia lesions can be divided in: 1. Genomic markers which include the alteration of the DNA content (ploidy), the chromosomal aberrations and the changes of the expression of the oncogenes and of the tumour-suppressing genes; 2. Markers of the proliferation such as PCNA (proliferating cell nuclear antigen) and the ki-67 non-histone nuclear protein; 3. Differentiation markers which include the keratins and the carbohydrates antigens. [20]

Although major progress was done in the study of the premalignant and malignant lesions at the snot's level, there are yet aspects not fully elucidated regarding the molecular mechanisms. It remains a challenge to establish the evolution of the injury through the identification and monitoring of a singular molecular market. [17]

The p53 protein

There is little data regarding the role of the p53 protein in the development and progression of the oral squamocellular carcinoma and of the premalignant oral lesions such as leukoplakia. [3]

It is thought that besides the identification of the epithelial dysplasia aspect, the p53 is a predictor of tumour differentiation, favouring the identification of the first stages of the malignant transformation. [3, 24]

Under normal circumstances, the p53 gene has a short halving time and it is capable of stopping the cellular cycle when DNA alterations are identified. The mutant p53 genes lose their capacity and, given the larger halving time, they tend to accumulate in the inside of the nucleus. The over-expression or the mutations of the p53 gene were identified

in 43-93 % of the oral squamocellular carcinomas, compared to the other types of human cancer. Their emergence not only in the oral dysplasia, but also in the normal microscopic mucosa, adjacent to the carcinomas, suggest the involvement in the early stages of the genesis of the carcinoma. The p53 expression is a sign of malignant transformation also on the oral pre-malignant lesions. In leucoplakia, the increase of the p53 expression, induced by the genotoxic stress, determined especially in smoking cases, is correlated with a high degree of malignant transformation. [10, 11, 20]

The immunohistochemical studies which compared the p53 expression in the premalignant injuries with that found in the malignant lesions emphasized that approximately 47 % of the premalignant lesions present an overexpression of the p53. The expression pattern, basal and supra-basal, is correlated with the progression of the squamocellular carcino-ma. Thus, at the level of the normal mucosa, p53 is expressed at the level of the basal cells, and the predominant expression in the upper layers indicates the progression towards leukoplakia, while the over expression in all the layers is a sign for the malignant transformation. [3, 11]

Cruzet et al. suggests that the p53 over expression in the supra-basal layers is an indicator of the carcinoma's development even in the absence of dysplasia. [20, 22]

Proliferation markers

Ever since 1998, Kaplan has emphasized at the oral hyperplasic epithelium level an increase of the immunopositivity of cells for p53 and PCNA. There is a close correlation between p53's positivity and the proliferation markers, such as, for example, ki-67, especially for the in situ cancers, where the markers of the normal oral epithelial differentiation, such as the cytokeratin K4, are absent. [20, 25, 26]

Ever since the beginning of this century, the results of the studies undertaken by De Paula et al. have indicated the Ki-67 and AgNORs cellular cycle modulators as the predicting markers of the malignant transformation. At the level of the cephalic extremity the increase of the local recurrence risk is associated, on one hand, with the reduction of the PCNA expression, while on the other hand, with the increase of the ki-67 (MBI) expression. The latter one is proposed as marker of the malignant transformation in case of leukoplakia. [2, 10, 20]

Markers of differentiation

Cytokeratins

The cytokeratins family is made of 20 cytokeratins grouped in heterodimers made of the type I (acids) and type II (basic) filaments, in a molarity proportion of 1:1 [21, 27]

The change in the cytokeratins' expression as factors of the cellular differentiation can constitute the main marker of the malignant lesions development. This hypothesis is supported by numerous molecular biology studies which emphasize their role in the development and progression of the oral squamocellular carcinoma. [16, 20]

The different expression of cytokeratins represents one of the parameters through which the stratified epithelial tissues differentiate themselves from the simple ones. Thus, if in the first case, normally, the 4, 5, 13 and 14 cytokeratins predominate, in the second case, it is observed the presence of the 7, 8, 18, 19, 20 cytokeratins. This distribution explains, on one hand, the different degree of differentiation and keratinization of each compartment of the oral cavity and, on the other hand, the important role during the genesis of the carcinoma. [16, 20, 21, 25]

The entire epithelium of the normal oral mucosa presents, in the basal layer, the K19 cytokeratin and the K5-K14 pair, whose expression is associated with the proliferative potential of these cells. The K4-K13 and K1-K10 cytokeratins are expressed in the supra-basal layers of the epithelial tissue and are associated with the cellular differentiation. [16, 21, 28]

Some studies support the idea that the epithelial dysplasia is characterised, on one hand, by the presence of the K5-K14 pair in

the para-basal layers in the thorny layer, while on the other hand, by the reduction of the K4-K13 and K1-K10 expression reduction. [28] However, recent research has emphasized that the K1-K10 expression represents a prognosis marker in the evaluation of the dysplasia lesions and that its reduction does not correlate with the presence and degree of dysplasia. Thus, the K1 cytokeratin's expression is much more reduced in the oral squamocellular carcinoma lesions compared to the oral, dysplasia and non-dysplasia leukoplakia. With regard to the K10 cytokeratin, this presents a much more stable expression in leukoplakia than in malignant lesions and it is considered that its presence is associated with a good prognosis. [16]

The identification of the K6-K16 pair, together with the cytokeratins' over expression of the K5 basal cells, K14 and K17, characterises the hyperproliferational condition, while the absence of K5 is encountered in the early stages of the carcinogenesis process associated with smoking. [18,29]

At the level of the epithelium of the oral mucosa, the presence of K8-K18 and the K19 expression in the supra-base layers is correlated with the malignant transformation. [16, 28]

The cytokeratins K18 and K18 normally expressed at the level of the epithelium of the tongue's mucosa until 27 weeks of gestation are frequently identified in the leukoplakia lesions compared to the normal mucosa, where it is emphasised an expression pattern of the embryonic type. It was noticed that their expression is much larger in the dysplasia leukoplakia compared to the non-dysplasia one, being correlated with a negative prognosis. Recent studies indicate their important role in the pathogenesis and invasion of the oral squamocellular carcinoma, reductions in the K8 expression being identified in the edges of the squamocellular carcinoma lesions.

(Fig. 2)[16, 20]

The cytokeratins K1, K8, K18 and K19 are markers of the tumor recurrence and progression. K18 was proposed as prognosis marker for the human oral cancer, while K19 was proposed as dysfunctional epithelial differentiation. [16, 21, 29]

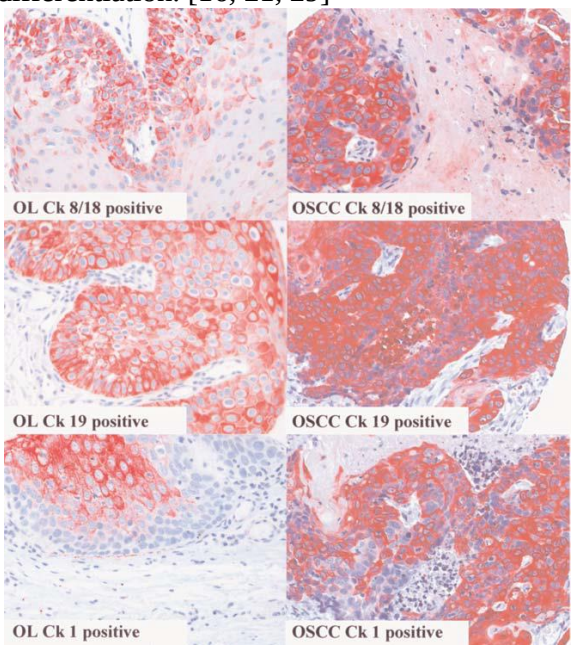

Figure 2. Immunohistochemical staining of oral leukoplakia and oral squamous cell carcinoma with Ck 8/18, Ck 19 and Ck 1,magnification x20.(Fillies et al.,2007)

CONCLUSIONS

The key to preventing the oral squamocellular carcinomas is represented by the early identification and adequate, individual and specific treatment of the leukoplakia, the most frequent oral premalignant lesions. Although major progress was done in the study of the pathology of the premalignant and malignant lesions at the oral mucosa's level, however, numerous molecular mechanisms remained incompletely elucidated. It remains a challenge the establishment for the evolution of a lesion the identification and monitoring of a singular molecular marker.

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