

Histological analysis of the actinic cheilitis: an interobserver approach

Análise histológica da queilite actínica: uma abordagem interexaminadores

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INTRODUCTION

Actinic Cheilitis (AC) was first described in 1923 as a chronic inflammatory disorder of the lips apparently due to actinic or solar rays¹.

Manganaro et al.², defined AC as a premalignant condition that can be focal or

ABSTRACT

Actinic Cheilitis (AC) is a clinical entity caused by excessive solar radiation exposure. This disease affects almost exclusively fair-skinned people with outdoor occupations or those who have activities under prolonged exposure to sunlight. Due to its slow progression, the patient attributes his condition to the ageing process, ignoring its evolutive and malignant nature. The scope of this study is to establish a criteria standardization for being used as a reference in the histological evaluation of the epithelial changes, levels of atypia and connective tissue behavior that compromise the lip epithelium with AC. 48 slides were analyzed by two independent observers with different levels of experience and academic backgrounds. In the connective tissue, solar elastosis was found in 100% of the patients with varied degrees of inflammatory infiltrate and vessel dilatation. The presence of epithelial dysplasia was also seen in the whole sample, but one. The inter-examiners agreement degree by Kappa varied from substantial to perfect. We concluded that the inter-examiners previous calibration as well as the criteria standardization helped in the inter-examiner agreement degree, and that the academic background as well as the slide preparation influenced the study.

Key words: Actinic; cheilitis; inter-examiners; epithelial; dysplasia; lips.

RESUMO

A queilite actínica (QA) é uma entidade clínica causada pela excessiva exposição à radiação solar. Esta doença afeta quase exclusivamente pessoas de pele clara que possuem ocupações externas ou aquelas que fazem atividades expostas por longos períodos à luz solar. Devido à sua lenta progressão, o paciente atribui à sua condição ao processo de envelhecimento, ignorando sua natureza evolutiva e de malignidade. O objetivo deste estudo foi estabelecer uma padronização de critérios para serem usados como referência na avaliação histológica das mudanças epiteliais, níveis de atipia e comportamento do tecido conjuntivo que comprometem o epitélio labial com QA. 48 lâminas foram analisadas por dois examinadores independentes com diferentes níveis de experiência e formação acadêmica. No tecido conjuntivo foi encontrada elastose solar e 100% dos pacientes com graus variados de infiltrado inflamatório e dilatação vascular. A presença de displasia epitelial foi também vista em toda a amostra. O grau concordância inter-examinadores determinado pelo valor de Kappa variou de substancial a perfeito. Concluiu-se que a calibração prévia dos examinadores, assim como a padronização dos critérios auxiliou no grau de concordância inter-examinadores, e que tanto a formação acadêmica quanto a preparação da lâmina influenciaram o estudo.

Palavras-chave: Queilite actínica; inter-examinadores; epitelial; displasia; lábios.

diffuse, shows epithelial atrophy or/and erythema and occasionally can present ulcers and vesicles. It affects almost exclusively fair-skinned men with outdoor occupations or those who have activities under prolonged exposure to sunlight^{3,4}, being more frequent on the protrusive and unprotected lower lip⁵. It is rarely recognized in its early stage and due to its

slow progression; the patient attributes his condition to the ageing process that is why it is neglected until it reaches more advanced stages enabling the occurrence of neoplasia⁶.

Histological, the epithelium of the lip vermilion must be acanthotic, hyperplastic and/or atrophic with several degrees of keratinization and shows disorder in the maturation evidenced by cellular atypias and alteration of mitotic activity⁷. There is also the proposal for the unification of the "pre-malignant lesions" classification, taking in consideration the histological pattern and the clinical correlation adopted to the gynecological model histological characterized as intraepithelial neoplásica⁸.

The medical literature presents countless works that show a constant search in the evaluation agreement or/and interpretation of the epithelial dysplasia degrees, but there is no ideal standard in the study of the epithelial dysplasia degree agreement⁹⁻¹³. The Kappa index is one of the methods used to evaluate inter and intra observers agreement degree¹¹.

AC, for being a premalignant disease strongly involved in the etiology of the lip cancer, with multifocal clinical and histopathologic findings, demands better defined criteria to evaluate the alterations of the epidermis and the subjacent dermis. In an attempt to search for criteria to be used as reference in the histological evaluation of the epithelial alteration, we have carried out an inter-observer preliminary study of the levels of atypia that compromise the lip epithelium with AC.

MATERIALS AND METHODS

48 "Research Subjects" with AC were studied. They were informed that the condition was a premalignant lesion and that the biopsy was indicated to the verification of the tissue histomorphological behavior and, in order to do that, their permission was needed to carry out the procedure of the histopathologic diagnosis, afterward helping in the prevention and following up AC. The place of the biopsy was chosen mainly a white area and an erythematous atrophic area, it means, with mottled aspect, although in most cases the white lesion was predominant.

All the histological cuts were read by two independent observers. We will identify observer 1 as E1 and observer 2 as E2. The gradation of atypia and dysplasia was

standardized using the interpretation criteria of the cellular atypia gradation established by the WHO described by Pindborg et al.⁷ and of the epithelial dysplasia preconized by Banócz, Csiba¹⁴.

A record card was used to the register of the histomorphological readings and the inter-observer agreement was studied among the variables through the Kappa test.

The following characteristics of the epithelium were analyzed:

a) keratinization: Absent; Parakeratin: keratin layer in which the epithelial nuclei are retained; Hyperparakeratin: increase of the parakeratin layer thickness; Orthokeratin: keratin layer in which there is loss of epithelial nuclei; Hyperorthokeratin: increase of the orthokeratin layer thickness.

b) covering epithelium changes related to the cellular proliferation. Atrophy: decrease of cell layers and consequently of the epithelium thickness, usually at the cost of the prickle layer; Hyperplasia: noticeable, deep, irregular epithelial projections in the subjacent propria lamina (Fig. 1), increased number of epithelium layers, this change can extend and cover various epithelium layers; Acanthosis: thickening of the prickle layer due to the increased number of cells; Normal: epithelium with normal thickness.

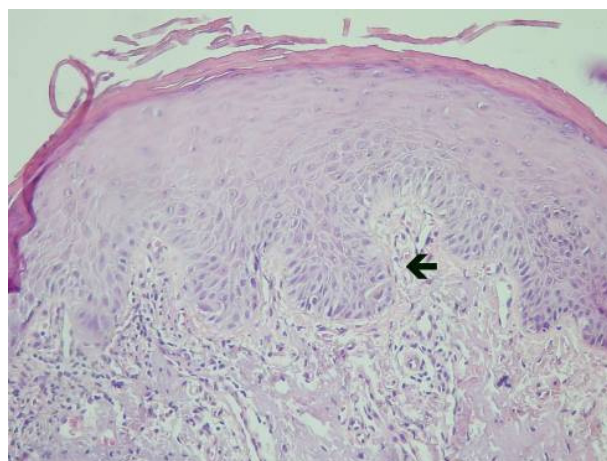


Figure 1. Irregular epithelial projections in the subjacent propria lamina

c) histological criteria of epithelial atypia – the presence of cellular changes related to changes in the proliferation and differentiation of the epithelial cells: Loss of basal cell polarity; Duplication of the basal layer; Drop-shaped rete pegs; Increased nucleus/cytoplasm ratio; Nuclear hyperchromatism; Enlarged nucleoli (Fig. 2); Increased number of mitotic figures; Atypical mitosis; Mitotic figures in the middle portion of the epithelium; Cellular

and nuclear pleomorphism (Fig. 3); Loss of epithelial stratification; Loss of cellular cohesion; Individual keratinization or keratinization in the group of cells in the prickle layer or in deeper layers.

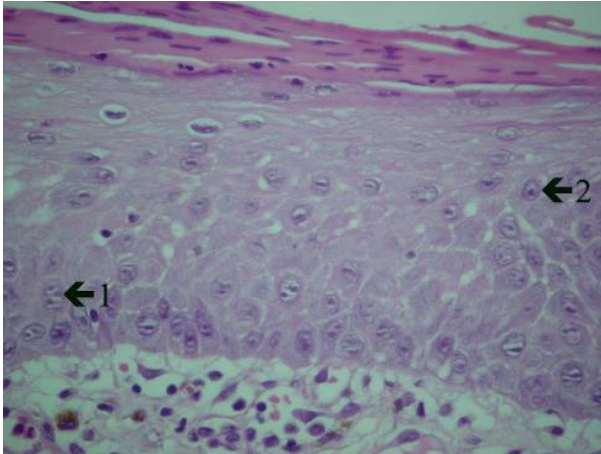


Figure 2. Enlarged nucleoli

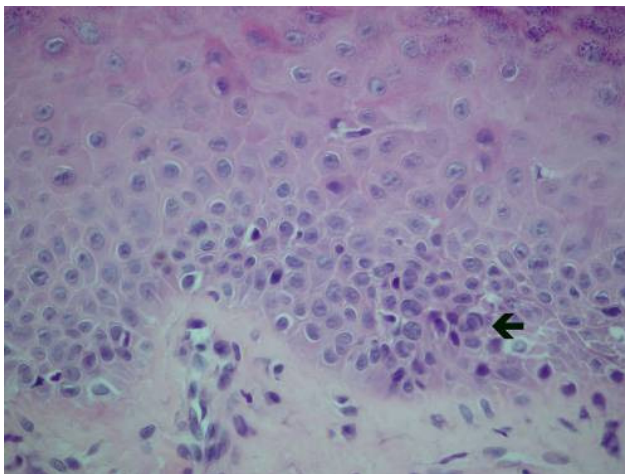


Figure 3. Cellular and nuclear pleomorphism.

d) connective tissue: presence of solar elastosis: characterized by the basophilic degeneration of collagen fibers.

e) inflammatory infiltrate: it is considered as a result of the immunologic reaction against the lesion: characterized by mild, moderate and severe infiltrate.

RESULTS

The results of the microscopic readings carried out by observers E1 and E2 are shown on Table 01.

Table 1. Levels of atypia in the 48 "Research Subjects"

	Observer 1		Observer 2	
	Yes	No	Yes	No
Loss of Basal Cell Polarity	46	2	43	5
More than one Basal Cell Layer	4	44	13	35
Drop-Shaped Rete Pegs	8	40	11	37
Increased Nuclear-Cytoplasmic Ratio	8	40	11	37
Cellular Hyperchromatism	8	40	11	37
Enlarged Nucleoli	21	27	17	31
Increased Number of Mitotic Figures	0	48	1	47
Atypical Mitosis	0	48	0	48
Mitotic Figures in the Middle Portion of the Epithelium	0	48	0	48
Nuclear and Cellular Pleomorphism	43	5	45	3
Irregular Epithelium Stratification	30	18	22	26
Loss of Cohesion among Cells	15	33	11	37
Individual Cell Keratinization	3	45	0	48
Mild Dysplasia (1 to 2 Atypia Findings)	6	42	6	42
Moderate Dysplasia (3 to 4 Atypia Findings)	21	27	13	35
Severe Dysplasia (5 to 13 Atypia Findings)	20	28	29	19

DISCUSSION

The medical literature has shown in the various works involving histopathologic interpretation of the epithelial dysplasia that there is subjectivity in the precognition of the correct diagnosis. In the AC case, because of its multifocal aspect, if we don't improve the calibration of the clinical and histological criteria it will be impracticable to have an appropriate orientation of the patient. The interpretation in the meaning of epithelial dysplasia is consequence of the lack of objectivity in the established criteria evaluation; arbitrary division of the graduations; lack of criteria calibration and graduation and lack of enough knowledge about which criteria is important to the measurement of the malignant potential¹⁵.

Individual criteria to the measurement of the malignant transformation in studies of adjacent epithelial dysplasia and oral carcinomas are based on the presumption of the dysplastic findings in this region are truly premalignant¹⁶, and the presence of mild to moderate epithelial dysplasia on the borders of the excisional oral squamous carcinoma carries a significant risk of local reoccurrence¹⁷. If we consider that in the last years we have had as reference of premalignant lesion the presence of dysplasia, these statements confirm a thesis still accepted in the medical literature. However, conditions like reactive hyperplasia (hyperplasia induced by

prosthesis), chronic inflammatory lesion (lichen planus) and benignant neoplasia (papilloma of squamous cells) can show mild degrees of dysplastic findings¹⁸ and the presence of inflammation can modify the reliability in the diagnosis of oral lesions because the inflammatory process seems to induct reactive atypia and still be associated with dysplastic alterations and/or reduce the ability of the pathologist in these observations¹².

In inter-observer studies, the histopathologic diagnosis of the epithelial dysplasia is variable because some works that evaluate the agreement among pathologists have shown that they themselves don't agree with previous opinions¹⁹. In many studies, the Kappa test has been used to evaluate the reliability intra and inter observer when you are studying quantitative and categorical variables²⁰. We have chosen the Kappa test to measure the agreement among the observers because the subjective answers can many times coincide by chance, taking in consideration the premise that the observers have already a preconceived opinion in reason that previously there is a probability to agree or disagree²¹.

For highly misbalanced situations, the Kappa test will have low results independently of the observers. It occurs because when the marginal totals are asymmetrically disbalanced²², similar result in our research (Table 2) referring to the loss of basal cells polarity in which the inter-observer agreement was of 89,58% and the Kappa index was 0,24, considering the median Kappa index agreement, almost insignificant.

Karabulut et al.¹³ in 1995, investigated the gradation agreement of the epithelial dysplasia among pathologists with same academic background, and pathologists with different academic backgrounds. They examined one hundred slides of oral leukoplakia, varying from dysplastic absence to Ca in situ. The inter-observer agreement varied from 49% to 69%, and the measured Kappa value increased from 27% to 45%, showing that the agreement level among the pathologists varied from poor to moderate. The authors concluded that the divergences occurred were due to the differences in academic background although there are divergences in the epithelial dysplasia diagnosis even among experienced professionals with the same academic background. Because of that, the

previous calibration of the histological aspects in our research was of great importance, specially because the observers involved had different academic backgrounds and work experience. Similar data was observed by Brothwell et al.¹¹.

Table 2. Inter-observer evaluation, Hits ratio, Kappa index and Kappa Index Agreement

Levels of atypia	E1/E2 Yes	E1/E2 No	Hits	Kappa	Kappa Index Agreement
Loss of Basal Cell Polarity	42	1	89.58%	0,24	Median
More than One Basal Cell Layer	4	35	81.25%	0,39	Median
Drop-Shaped Rete Pegs	7	36	89.58%	0,67	Substantial
Increased Nuclear-Cytoplasmic Ratio	7	36	89,58%	0,12	Insignificant
Cellular Hyperchromatism	7	36	89.58%	0,35	Median
Enlarged Nucleoli	1	23	50.00%	0,48	Moderate
Increased Number of Mitotic Figures	0	47	97.92%	1,00	Almost Perfect
Atypical Mitosis	0	48	100%	1,00	Almost Perfect
Mitotic Figures in the Middle Portion of the Epithelium	0	48	100%	1,00	Almost Perfect
Nuclear and Cellular Pleomorphism	41	1	87,05%	0,19	Insignificant
Irregular Epithelium Stratification	20	16	75.00%	0,51	Moderate
Loss of Cohesion among Cells	10	32	87.05%	0.69	Substantial
Individual Cell Keratinization	0	45	93.75%	1,00	Almost Perfect
LEVEL OF DYSPLASIA					
Mild Dysplasia (1 to 2 Atypia Findings)	3	39	87.50%	0.43	Moderate
Moderate Dysplasia (3 to 4 Atypia Findings)	9	23	66.67%	0.29	Median
Severe Dysplasia (5 to 13 Atypia Findings)	17	16	68,75%	0.40	Median

Odell and Morgan²³, declared that several studies of calibration revealed lack of reproducibility in the definition of oral epithelial dysplasia among pathologists in different occasions. In our study, the inter-observer agreement varied from substantial to perfect depending on the histological aspect evaluated. In our study, the inter-observer agreement varied from one end to the other, it means, it varied from insignificant to almost perfect depending of the level of atypia evaluated. It led us to accept the considerations of Warnakulasutriya¹⁵ when he says that the interpretation problem in the meaning of the epithelial dysplasia is consequence of the lack of objectivity in the evaluation of the established criteria; arbitrary division of the graduations; lack of criteria calibration and graduation and lack of enough knowledge about which criteria is important to the measurement of the malignant potential.

Epstein et al.²⁴, declared that the histological interpretation is subjective and varies among the pathologists, so this

variation can also induct to the improper diagnosis and treatment.

Pinto²⁵, still emphasized the importance of the experience that the observer must have in the identification of the parameters to be studied, once that histotechnical factors can interfere in their evaluation.

Based on the results obtained and based on the medical literature we conclude that the inter-observer previous calibration as well as the criteria standardization help in the inter-observer agreement degree, diminishing the possible lack of reproducibility in the histological definitions among pathologists and also that the same inter-observer academic background as well as the slide preparation can influence the studies involving agreement analysis.

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