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Altered cytokeratin expression in actinic cheilitis

Background: Actinic cheilitis (AC) is a widely recognized precancerous lesion of the lip. Varying degrees of epithelial dysplasia may be present. However, no studies have correlated epithelial changes with cytokeratin expression that might reflect the disordered maturation that is probably occurring.
Methods: Thirty-four cases diagnosed as AC were classified according to dysplasia degree, and submitted to immunohistochemical staining for the detection of cytokeratins (CKs) 7, 8, 13, 14, 16 and 19. Normal mucosa adjacent to the lesions was also evaluated.
Results: The results obtained showed that CK10 immunostained only superficial keratinized epithelial layers in 11 cases, and also intermediate spinous layers in 18 cases. Cytokeratin 14 was expressed in all epithelial layers of 31 cases, in two cases its expression was in the basal and intermediate layers, and one case was negative. Cytokeratin 13 immunostained 26 cases and was negative in eight cases. In these eight cases, CK13 was apparently replaced by CK16. Cytokeratin 16, besides these eight cases, was also expressed in the spinous intermediate layers of a further eight cases. The remaining CKs tested were all negative. No relation between the degree of dysplasia and the CK expression was noted.
Conclusions: Cytokeratin expression in AC is different from that of normal oral mucosa, and is not related to the degree of dysplasia.

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Actinic cheilitis (AC) is a diffuse, premalignant condition of the lower lip vermilion resulting from long-term or excessive exposure to the ultraviolet component of solar radiation. This entity is analogous to actinic keratosis or solar keratosis and, as occurs with solar keratosis, it can undergo malignant transformation in approximately 10–20% of cases.[1,2] Actinic cheilitis usually affects White men in the 6th or 7th decades, and presents a variable clinical aspect, mainly with areas of erosion and hyperkeratosis.[3] Histopathologically, the lesion is characterized by an atrophic stratified squamous epithelium, often demonstrating marked keratin production. Varying degrees of epithelial dysplasia may be found. A mild chronic inflammatory cell infiltrate is usually present, subjacent to the dysplastic epithelium, in the underlying connective tissue, which also invariably demonstrates an amorphous, acellular, basophilic change known as solar elastosis; presumably a result of an ultraviolet light-induced alteration of collagen and elastic fibers.[3,4] It is well known that malignant transformation is often associated with changes in the expression of normal differentiation markers. In this way, many studies have demonstrated increased or even decreased expression of cytokeratins commonly expressed by the normal mucosa during its malignant transformation.[5] This study aimed at analyzing the expression of different types of cytokeratins (CKs) in actinic cheilitis exhibiting different degrees of dysplasia.

Materials and methods

A total of 34 cases of AC were studied. All samples were retrieved from the files of the Dermatology and Oral Pathology Departments at the University of São

Paulo. The specimens were fixed in 10% formalin and embedded in paraffin. Haematoxylin-eosin-stained sections from all specimens were reviewed. Clinical data obtained from patient records indicated that there were 23 men and 11 women, with ages ranging from 27 to 78 years. Grading of dysplasia was accomplished according to Bánóczy & Csiba's[6] parameters, based on epithelial changes determined by WHO:[7] loss of polarity of the basal cells, hyperplasia of basal cells, increased nuclear-cytoplasmic ratio, drop-shaped rete-ridges, acanthosis, atrophy, irregular epithelial stratification, cellular and nuclear pleomorphism, nuclear hyperchromatism, mitotic figures and multinucleated cells. Lesions were graded as follows: mild dysplasia when up to 2 epithelial changes were present, moderate dysplasia when 2–4 epithelial changes were seen, and severe dysplasia when 5+ epithelial alterations were present.

Three-μm thick sections from each specimen were obtained and mounted on silanated slides for immunohistochemical evaluation. Immunostaining was carried out using the streptavidin-biotin method on dewaxed tissue sections. The primary monoclonal antibodies used and their source, clone, concentration and time of incubation are listed in Table 1. All antibodies were diluted using a Tris-HCl buffer, pH 7.4, containing bovine serum albumin. Before incubating the anticytokeratin antibodies, the tissue sections were submitted to antigen retrieval using a water bath (95 °C), with the slides immersed in citric acid (10 mM), pH 6.0. For endogenous peroxidase inactivation, tissue sections were immersed in two baths of a methanol solution containing 0.3% H_20_2 for 5 min each. All steps were performed at room temperature.

Table 1. Monoclonal antibodies used

Antibody	Clone	Dilution	Incubation time (min)
CK7*	0 V-TL12/30	1 : 200	30
CK8*	C-51	1 : 50	60
CK10*	DE-K10	1 : 10	60
CK13*	AE8	1 : 80	120
CK14*	–	1 : 50	60
CK16*	LL025	1 : 600	60
CK18*	–	1 : 600	60
CK19*	RCK108	1 : 100	120

CK, cytokeratin.
*Biogenex, San Ramon, CA, USA.

After incubation with the primary antibody, sections were thoroughly rinsed using a Tris-HCl buffer, pH 7.4, exposed to secondary antibodies, and after further washing were exposed to a streptavidin-biotin complex. Diaminobenzidine was used as the chromogen and the slides were counterstained with Mayer's haematoxylin. Positive and negative appropriate controls were used. Normal labial mucosa, adjacent to the AC, was also studied for comparative purposes.

Results

Microscopic findings

Characteristically, the haematoxylin-eosin stained sections of the 34 cases of AC revealed a mucosal fragment lined with an epithelium, which disclosed some alterations such as loss of polarity of the basal cells, hyperplasia of basal cells, drop-shaped rete-ridges, acanthosis, and atrophy. Other features such as irregular epithelial stratification, nuclear hyperchromatism, mitotic figures and multinucleated cells were also observed. Regarding the thickness of the keratin covering, most of the cases showed thickening, varying from mild to marked, of ortho- or parakeratin. In some cases both parakeratin and orthokeratin were seen in the same section. All 34 cases showed some degree of basophilic change within the connective tissue. Mild inflammatory infiltrate was present in approximately half the cases, and in the other half it was moderate to intense (Fig. 1).

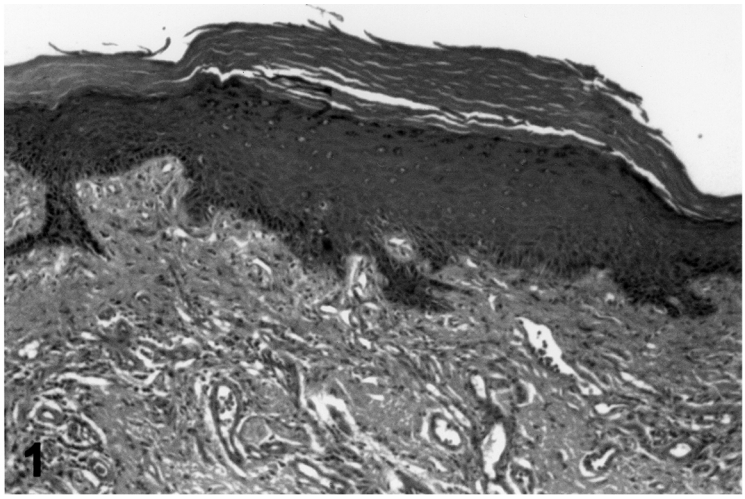
According to the Banoczy & Csiba[6] criteria for classification of intensity of dysplasia, eight cases were classified as mild dysplasias, 18 as moderate, and eight as severe.

Immunohistochemical findings

In the sections of normal mucosa obtained from lip, CK10 was expressed only in the parakeratin or orthokeratin layers. Cytokeratin 13 was expressed in all suprabasal layers, while CK14 stained only basal cells. The other CKs tested were negative in normal labial mucosa.

Immunostainings of the 34 cases of AC are presented in Table 2. It can be noticed that the expression of cytokeratins is not dependent on the degree of dysplasia. Cytokeratins 7, 8, 18 and 19 were negative in all cases studied.

Figs. 1–7. (1) General aspect of an actinic cheilitis showing epithelial dysplasia with marked thickening of keratin layer. HE ×100. (2) Expression of cytokeratin 10 in the superficial layers (keratin) of the epithelium. Basophilic degeneration within the connective tissue is present. Streptavidin-biotin ×100. (3) Cytokeratin 13 is expressed in the intermediate layers of the epithelium on the right-hand side. There is an abrupt change to negative epithelium in the area where hyperkeratosis is present. Streptavidin-biotin ×100. (4) Cytokeratin 13 is expressed in groups of cells of the intermediate spinous layer. Streptavidin-biotin ×100. (5) Expression of cytokeratin (CK) 16 by cells of the spinous layer below a marked parakeratosis. In this section CK13 was negative. Streptavidin-biotin ×100. (6) Expression of cytokeratin 14 in all spinous layers on a hyperkeratinized area. No inflammation is present. Streptavidin-biotin ×200. (7) High-power view showing the expression of cytokeratin 16 in suprabasal cells. Streptavidin-biotin ×640.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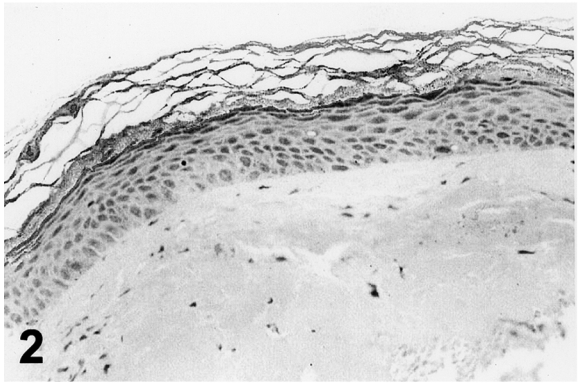
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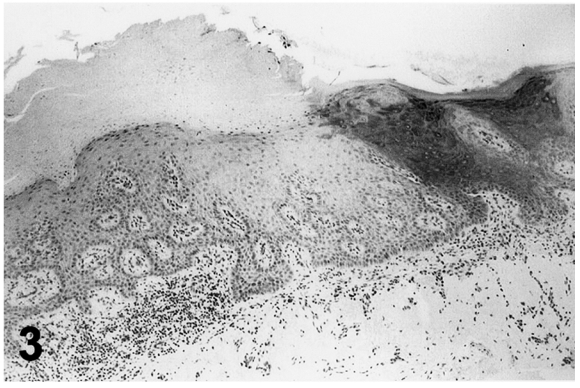
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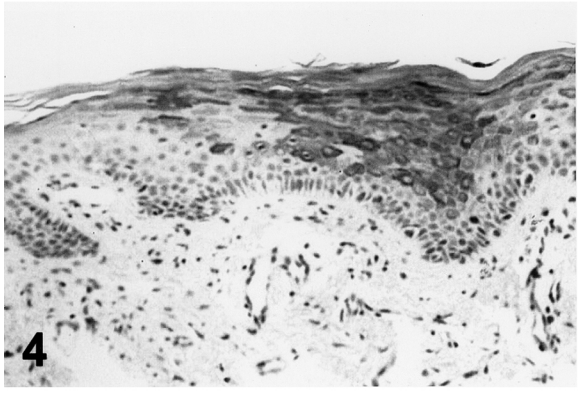
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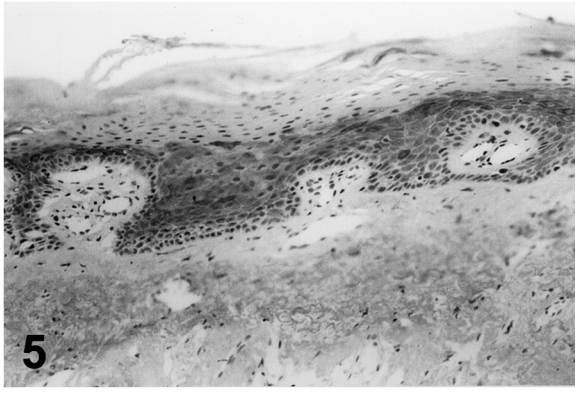
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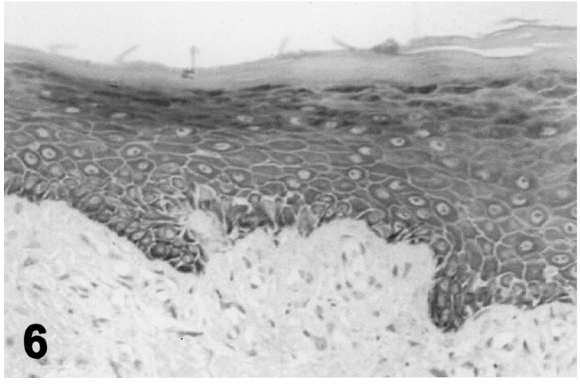
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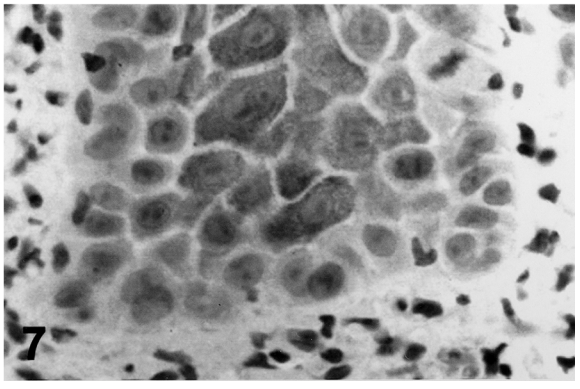
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Table 2. Expression of cytokeratins according to epithelial layer and degree of dysplasia

Case	Dysplasia	CK10	CK13	CK14	CK16
1	Mild	S	–	B,I	–
2	Mild	I,S	I,S	B,I,S	–
3	Mild	I,S	–	B,I,S	I
4	Mild	–	I,S	B,I,S	I,S
5	Mild	S	I,S	B,I,S	I,S
6	Mild	I,S	I,S	B,I,S	I,S
7	Mild	I,S	–	B,I,S	I,S
8	Mild	I,S	I,S	B,I,S	–
9	Moderate	I,S	I,S	B,I,S	I
10	Moderate	S	S	B,I,S	I
11	Moderate	I,S	I,S	B,I,S	I
12	Moderate	I,S	I,S	B,I,S	–
13	Moderate	S	S	B,I,S	–
14	Moderate	I,S	I,S	B,I,S	I,S
15	Moderate	I,S	I,S	B,I,S	I,S
16	Moderate	I,S	I,S	B,I,S	I,S
17	Moderate	I,S	–	B,I,S	–
18	Moderate	–	I,S	B,I,S	–
19	Moderate	S	I,S	B,I,S	I,S
20	Moderate	I,S	–	B,I,S	–
21	Moderate	–	I,S	B,I,S	–
22	Moderate	I,S	–	B,I	I,S
23	Moderate	–	I,S	B,I,S	–
24	Moderate	S	I,S	B,I,S	–
25	Moderate	S	I,S	B,I,S	–
26	Moderate	I,S	B,I,S	B,I,S	–
27	Severe	I,S	I	–	I
28	Severe	S	–	B,I,S	I
29	Severe	I,S	I,S	B,I,S	–
30	Severe	–	I,S	B,I,S	–
31	Severe	S	I,S	B,I,S	–
32	Severe	S	I,S	B,I,S	–
33	Severe	I,S	I,S	B,I,S	–
34	Severe	S	–	B,I,S	I,S

–, negative expression; B, expression in basal epithelial cells; I, expression in epithelial cell of the intermediate or spinous layer; S, expression in superficial epithelial layers.

Cytokeratin 10 was observed in 29 (85.3%) cases and negative in five (14.7%). In the positive cases CK10 was present in the intermediate to superficial layers, mainly in keratin laminas, in 18 (62%) cases. In the remaining 11 cases (38%), only the keratin layer was stained (Fig. 2).

Twenty-six cases (76.5%) were positive to CK13 (Fig. 3): 21 cases in all suprabasal cell layers, one case suprabasal plus the basal layer, and in four cases CK13 was seen in focal groups of cells in the spinous layer (Fig. 4). In six cases (17.6%) where there was intense hyperkeratosis, CK13 was negative, and in these cases CK16 was positive (Fig. 5). In two cases (5.9%), both CK13 and CK16 were negative.

Cytokeratin 14 was positive in 33 cases (97.1%) and negative in one case (2.9%). Positivity was present in all epithelial layers of 31 cases. Basal and a few suprabasal layers were stained in two cases (Fig. 6).

Sixteen cases expressed CK16 (47.1%): six cases in the intermediate layer, and 10 cases in both the intermediate and superficial epithelial layers (Figs. 5 and 7). Eighteen cases were negative (52.9%).

Discussion

Histopathological findings of the studied AC were within its histopathologic spectrum, as have been described in the literature. The lining epithelium showed either parakeratinization and/or orthokeratinization, with the thickness of the keratin layer accompanied by acanthosis, atrophy, and dysplasia. According to Kaugars et al.[4] the finding that acanthosis and thickening of the keratin are associated with increased epithelial change may mean that the damage to the basal cell layer occurred first, and that continued exposure to the sun or other etiologic factors then caused the subsequent thickening of the keratin and spinous cell layers. In the connective tissue, together with the amorphous basophilic degeneration of collagen and elastic fibers, a mild to intense chronic inflammatory infiltrate composed predominantly by lymphocytes, and rarely plasma cells was observed. The basophilic degeneration of collagen and elastic fibers renders the lamina propria with a characteristic 'amorphous' appearance and often extends over a large portion of the tissue. This collagen degeneration is the most consistent and prominent histopathologic finding in AC.[3]

Our results showed that AC expressed CKs 10, 13, 14 and 16, but staining varied depending on the epithelial cell layer, and was not related to the degree of dysplasia. Characteristics of dysplastic epithelium can be interpreted as manifestations of disordered differentiation;[8] therefore, it might be expected that disturbances in the expression of keratin might accompany dysplasias.

The expression of CK10 in the cases studied was different from that seen in the normal labial mucosa, as it was present in all suprabasal layers in some of the cases, and not only in the keratin layer. The presence of CK10 in the intermediate layers of the epithelium supports the thought that an altered keratinization process is taking place.

Cytokeratin 14 was expressed in all epithelial layers in 31 of the studied cases, mainly in areas where inflammatory infiltrate was present. This finding has been demonstrated in the epithelium next to inflamed tissue from other pathologies as periodontopaties.[9] Thus, inflammation induces marked changes in the keratin expression and these changes might indicate a role of some components of the inflammatory exudate in epithelial modification.[9] Cytokines produced by lymphocytes might alter protein expression. Moreover, inflammatory infiltrate is not the only cause of altered epithelial maturation, as the presence of malignant neoplasms in the connective tissue also leads to the expression of CK14 in all epithelial layers of adjacent epithelium. Then, dysplastic phenotype may play a role in the suprabasal expression of CK14, as has been suggested by other authors.[10]

Cytokeratins typical of simple epithelia[7,8,18,19] were not positive in AC, although their increased expression in carcinomas has been shown.[11,12] Studies have correlated CK19 with premalignancy;[8] however, it was not detected in any epithelial layer of the studied AC. The non-detection of CK19 in AC could be explained by keratinocytic maturation, as occurs in SK.[15]

Suprabasal expression of CK13 in AC was observed, as it is in normal labial mucosa. Focal cellular distribution was also observed. Although this last finding may indicate that in dysplastic conditions the process of differentiation of the epithelium is more deeply disturbed,[14] in our study CK13 expression was independent of the degree of dysplastic change.

Among the results obtained with the CKs, the most interesting was the replacement of CK13 by CK16, mainly in hyperparakeratinized lesions. Expression of CK16 has been reported in other diseases, such as squamous cell carcinomas[15] as well as hypertrophic areas of solar keratosis.[16] However, in our study, CK16 was expressed mainly in atrophic mucosa. Other authors[16] have observed that keratin distribution in atrophic areas is similar to that of epidermis in solar keratosis. Cytokeratin 16 has been referred as a marker of hyperproliferative or activated keratinocytes.[15,17] Probably, keratinocytes in the epithelium of the AC are altered as a result of the damage caused by ultraviolet radiation, as CK16, among others, is expressed as a consequence of an increase in the cell cycle rate.[16] The down-regulation of CK13 seems to be associated with a loss of differentiation in squamous cell carcinomas of the larynx.[11] It has been suggested that extracellular growth factors might be important in the regulatory process of these cytokeratins. There seems to be an absence of CK13 in tumors giving rise to recurrences and distant metastasis.[12,18]

In conclusion, the pattern of the CK antigen expression does not correlate with morphologic changes occurring in epithelial dysplasia. Our study demonstrates that deep changes in the phenotype of AC epithelium can occur before malignant transformation and tumor progression. These changes are related to epithelial differentiation status, indicating that the immunoprofile of CK may be useful in the study of AC.

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