Immunohistochemical expression of Cyclooxygenase 2 and Caspase 7 in oral lichen planus

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ABSTRACT

Background: Oral lichen planus (OLP) is one of the most common dermatological diseases presenting in the oral cavity. Although relatively frequent, OLP is the target of much controversy, especially in relation to its potential for malignancy. This study was conducted to find biological changes in the expression of caspase 7 and cyclooxygenase 2 (COX2) in OLP by immunohistochemistry and to explore the correlation between them.

Materials and Methods: Fifteen cases of randomly chosen paraffin embedded tissue blocks of OLP with 5 normal oral mucosa cases were included in this study. Immunohistochemistry was performed to evaluate COX2 and caspase 7 proteins expression.

Results: The expression of COX2 was positive in all studied cases of OLP with negative expression in normal oral mucosa. Caspase 7 expression was positive in (73%) of the cases of which (36.5%) showed strong positive expression score. Non-significant positive correlation was found between the two markers.

Conclusion: This study provided further evidence that epithelial cells in OLP undergo apoptotic death, on the other hand they develop high rate of inflammation which may create a good environment for malignant transformation.

Key words: oral lichen planus, cox2, caspase 7, immunohistochemistry.

INTRODUCTION

Oral lichen planus is a chronic inflammatory disease of oral mucosa. The world health organization has defined it as potentially precancerous disorder, representing a generalized state associated with a significantly increased risk of cancer (1, 2).

Cyclooxygenase-2 which is an inducible enzyme in most cell types including keratinocytes, fibroblast and T cell, catalyzes the synthesis of prostaglandins (3). Several processes in cancer may be influenced by COX2 including cell proliferation, apoptosis, and angiogenesis. Cyclooxygenase 2 may inhibit apoptosis via different pathways like down-regulation of arachidonic, up regulation of proto-oncogene Bcl2 and down-regulation of Bax, thus contributing to increased survival (4).

Caspase 7 is a member of the caspase family and has been shown to be an executioner protein of apoptosis. The precursor of this caspase is cleaved by caspase-3 and caspase-9 and 10. It is activated upon cell death stimuli and induces apoptosis (5). It is a 303-amino acid protein with high Similarity to caspase-3. Caspase -3 and caspase-7 are functionally similar substrate specificities (6). Caspase -7 is important to caspase -3 in apoptosis execution, especially in the cells with deficient or under expressed caspase-3 (7).

MATERIALS AND METHODS

Fifteen retrospective tissue samples of paraffin embedded blocks histologically verified as oral lichen planus were randomly chosen from the archives of oral pathology department, College of Dentistry, Baghdad University.

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Immunohistochemistry staining procedure

Immunohistochemical staining technique was performed to examine COX2 and caspase 7 protein expressions. Four um thickness sections of each case were cut and mounted on positively charged slides for immunohistochemical staining with monoclonal antibodies COX2 and caspase 7 (Abcam).

Positive and negative tissue controls were obtained according to antibodies manufacturer data sheets and included in each run. Normal oral mucosa (5 cases) from voluntary healthy individuals was also included in the study.

Assessment of immunohistochemical results:

The immunoreactivity of COX2 was evaluated according to (8). Immunostained regions at 400 magnification were scored as follows: positive expression was evaluated by taking three fields per case and staining intensity was scored as 0 (negative), 1 (weak), 2 (weak), and 3 (strong). Staining extent was scored as 0 (<5%), 1 (5-25%), 2 (26-50%), 3 (51-75%), and 4 (76-100%) according to the percentage of positively stained cells. The sum of intensity and extent scores was used as the final staining score. All cases were divided into four groups as follows: 0 (negative), 1-3 (low), 4-5 (moderate), and 6-7 (high). If the scores were moderate or high, cases were classified as COX2 over expressed.

Regarding Caspase 7 immunostaining evaluation, the staining extent was scored as follow: 0 (<5%), 1 (5-25%), 2 (26-50%), 3 (51-75%), and 4 (>75%) and the Staining intensity was graded as follows: 0 (negative), 1 (weak), 2 (moderate), and 3 (strong). The final score was achieved by multiplication of the two scores above and scores of 0-4 were defined as negative expression (-).
scores of 5-8 as weakly positive expression (+) and scores of 9-12 were defined as strongly positive expression (+++) (9).

Statistical analysis: Numerical values were used to describe variables which include, No, Mean, SD for age, cox2 and caspase 7. Pearson correlation coefficient of correlation (r) was used to find the relation between the two markers. The statistical analysis achieved by using SPSS (statistical package for social sciences).

RESULTS

The mean age of the study sample was (44 years ±stDv (13.8), ranged from (22-68) years, eight of them were males and seven were females. The most predominant site was the buccal mucosa (66.7%) followed by the tongue (20%).

Cox-2 expression was indicated as brown granular cytoplasmic and membranous staining in both basal and parabasal epithelial cells, Fig. (1). The results of this study showed positive expression of cox2 enzyme in all OLP cases with strong positive expression in 5 (33%) cases, moderate positive expression in 4 (27%) cases and weak positive in the remaining 6 (40%) cases (Table 1).

Table 1: Cox2 expression in 15 cases of OLP

<table>
<thead>
<tr>
<th>Cox2 expression</th>
<th>No.</th>
<th>%</th>
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<tbody>
<tr>
<td>Low</td>
<td>6</td>
<td>40</td>
</tr>
<tr>
<td>Moderate</td>
<td>4</td>
<td>27</td>
</tr>
<tr>
<td>High</td>
<td>5</td>
<td>33</td>
</tr>
</tbody>
</table>

Caspase 7 expression was detected as brown granular mostly cytoplasmic immunohistaining of the basal and parabasal cells, Fig. (2). Positive expression was found in 11 (73%) of the cases. Of them, 4 (36%) cases showed strong positive expression and 7 (64%) with weak positive expression as clarified in table (2).

Table 2: Caspase 7 expression in 15 cases of OLP

<table>
<thead>
<tr>
<th>Caspase 7 expression</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative (0-4)</td>
<td>4</td>
<td>27</td>
</tr>
<tr>
<td>Weak positive (5-8)</td>
<td>7</td>
<td>64</td>
</tr>
<tr>
<td>Strong positive (9-12)</td>
<td>4</td>
<td>27</td>
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DISCUSSION

In order to investigate potential biological objective predictive marker, the expression of cox2 and caspase 7 were evaluated on biopsies of oral lichen planus by immunohistochemistry.

The results of cox2 immunohistochemistry revealed positive expression in all investigated olp cases in basal and parabasal cell layers with negative expression in normal oral mucosa. This finding is in agreement with a previous study (10).

Similarly, a varying degree of cox2 expression was observed in sub epithelial infiltrate of olp. In previous studies on oral sequamous cell carcinoma, results revealed positive expression of cox2 in all studied cases with negative expression in normal oral mucosa (12). These findings supported the suggested link between chronic inflammation and the development of oral squamous cell carcinoma in olp.

Regarding caspase 7 expressions, results showed increased expression in olp cases in comparison to normal oral mucosa with varying degree of expression among different OLP lesions. Up to our knowledge there are no earlier reports on caspase 7 expressions in OLP, however caspase cascade pathway had been investigated in olp (13). Similarly caspase 3 expression in OLP was studied that revealed high expression in olp lesions compared with normal oral mucosa with co-localization in basal and supra basal epithelial layers suggesting that proliferating epithelial cells may be targeted for destruction in OLP. (14). There are several studies on Bcl-2 expression in OLP lesions, which have all shown only weak Bcl-2 expression in OLP keratinocytes, supporting the role of apoptosis in OLP (14-16). These findings supported the present finding since caspase 3 and caspase 7 are functionally similar in substrate specificities and caspase 7 is important for caspase 3 in apoptosis. Similarly, previous study on caspase 7 expression in OSCC showed positive expression in 74% of cases. (17). This study confirmed the view that apoptosis may play a role in olp tumor genesis.

Regarding the correlation between cox2 and caspase 7 expressions, the present finding showed a non significant positive correlation, however further studies with larger samples are needed to find out the relation between these two markers.

As a conclusion, this study provided further evidence that epithelial cells in olp die by apoptosis, on other hand they develop high rate of inflammation which may create a good environment for malignant transformation.

REFERENCES

4. Fernandes AT, Armstrong RC, Krebs J, Srinivasula


