**Immunohistochemical Expression of Caspase 7 and Annexin V as Apoptosis Markers in Oral Squamous Cell Carcinoma (A clinicopathological study)**

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**ABSTRACT**

**Background:** Oral squamous cell carcinoma (HNSCC) is an aggressive and lethal malignancy. It is an epithelial malignancy with morphologic features of squamous cell differentiation without additional features suggestive of other differentiated tissues. Caspase 7 is a caspase involved in the execution phase of apoptosis. The genetic alteration of caspase 7 might be involved in the development of human cancer. Annexin V belongs to a family of phospholipid binding proteins. It binds in the presence of Ca+2 ions with high affinity to negatively charged phospholipids like phosphatidylserine (PS) exposed on cell surface during apoptosis.

**Materials and Methods:** The study is a prospective one in which twenty cases of oral squamous cell carcinoma and eleven retrospective of paraffin embedded formalin fixed of oral squamous cell carcinoma blocks. H&E stain was done for each block for reassessment of histological examination. An immunohistochemical staining done by using monoclonal antibodies for caspase-7 and annexin V. The aim of the study: The aim of the study is to evaluate the immunohistochemical expression of caspase 7 and Annexin V as apoptosis markers in correlation with various clinicopathological parameters in oral squamous cell carcinoma.

**Results:** The results of 31 oral squamous cell carcinoma cases included in this Study. Age group of studying samples was more than 50 years. Immunohistochemical expressions were as follows in all cases of oral squamous cell carcinoma for caspase 7 and Annexin V tumor markers: The expression of caspase-7 was weak positive in 12 cases (38%), strong positive in 11 cases (35%) and 8 (25%) cases were negative. The expression of Annexin V was weak positive 16 cases (51%), strong positive 10 cases (32%) and 5 cases (16%) were negative.

**Statistical result for caspase 7 and Annexin V** with clinicopathological parameters was no significant correlation between them.

**Conclusions:** Immunohistochemical expression were observed in studying samples of oral squamous cell carcinoma of both annexin V and caspase 7, however, statistically non significant correlation with all clinicopathological findings were found and between both markers.

**Key words:** oral squamous cell carcinoma, apoptosis, caspase 7, annexin V. (J Bagh Coll Dentistry 2011;23(74-77).

**INTRODUCTION**

Oral squamous cell carcinoma (OSCC) is the most common malignant tumor occurring in the oral cavity and one of the 10th most common causes of death in the world. It arises from dysplastic oral squamous epithelium. In the majority of cases, it is diagnosed at an advanced stage and consequently present with a poor prognosis. In the last few years relevant advances in the diagnosis and treatment of oral cancer has been observed. Nevertheless, despite this recent improvement, there are still many difficulties in evaluating the prognosis of OSCC. Thus, various studies attempting to establish the role of protooncogenes, antioncogenes and apoptosis-regulating genes in the tumoral progression have recently been performed in order to find more significant information to evaluate and predict the biological behavior of this neoplasm. At certain stages in tumor development, the balance between proliferation and apoptosis is interrupted, resulting in deregulated cell proliferation.

**MATERIALS AND METHODS**

This study was performed on thirty-one formalin- fixed paraffin-embedded blocks histologically diagnosed as OSCC. Twenty cases of them were prospectively collected during 2009 from the histopathological laboratory in surgical specialties Hospital (SSH). Eleven cases were retrospectively collected randomly from 2000 to 2006 from the archives of Oral Pathology laboratory of, College of Dentistry, Baghdad University during eight months period of collection for all cases. The clinicopathological information regarding age, gender, tumor sites, clinical presentation, tumor size, lymph node involvement (if present), in addition to any other information were obtained from the case sheets presented with the tumor specimens. 4µm thickness sections were mounted on clean glass slides for routine Haematoxylin and Eosin.
staining (H&E), from each block of the studied sample and the control group for histopathological re-examination. Other 2 sections of 4µm thickness were mounted on positively charged microscopic slides (ESCO, SuperFrost plus/USA) to obtain a greater tissue adherence for immunohistochemistry. Two types of monoclonal antibodies were used in the present study; the information and specification of each antibody obtained from the data sheets. Monoclonal mouse anti human caspase 7 was used from (Abcam Company) United Kingdom. Monoclonal mouse anti-human Annexin V was used from (Abcam Company) United Kingdom. Immunohistochemical scoring of Caspase-7 and Annexin V: The scoring was done under light microscope and because the staining intensity was not uniform among different lesion, we scored the antibodies with the criteria combined intensity with the rate of positive cell. The intensity was graded as follows: 0: negative 1: weak 2: moderate 3: strong. The rate of positive cells was graded as follows: 0: < 5%: 1: 5-25%: 2: 26-50%: 3:51-75%: 4: > 75% A final score was achieved by multiplication of the two scores above: Scores of 0-4 were defined as negative expression. (-) Scores of 5-8 were defined as weakly positive expression. (+) Scores of 9-12 were defined as strongly positive expression. (++) (7).

RESULTS

The immunohistochemical staining of caspase-7 was positive in 23 examined OSCC and control samples.

Immunostaining of caspase-7 was detected as brown (granular) in the cytoplasm especially of the tumor cells.

Caspase-7 expressions for 31 OSCC cases were summarized in Table -1.

For all 31 cases 26%(8 cases) show negative expression for caspase-7, 39%(12 cases) showed weak positive expression while 35%(11 cases) showed strong positive expression. (Figure 1,2, and 3). The immunohistochemical staining of annexin-v was positive in 26 examined OSCC and control samples Immunostaining of annexin-v was detected as brown (granular) staining in the cytoplasm especially of the tumor cells. Annexin-v expressions for 31 OSCC cases were summarized in Table -2. For all 31 cases 16%(5 cases) show negative expression for annexin-v, 52%(16 cases) show weak positive expression, while 32%(10 cases) show strong positive expression (Figures 4-6).

DISCUSSION

It is still unclear whether all procaspases are expressed in all types of tumor cells and, if so, to what extent their presence relates to the susceptibility of cancer cells to Apoptosis. Manipulation of the apoptotic pathway is becoming a basis for elaboration of new anticancer therapies. (8). However, previous Iraqi study showed over expression of procaspase-3 in OSCC with significant positive correlation with tumor grade which predict poor prognosis since most caspase-3 remains uncleaved and inactivated (9). Other Study showed that resistance to apoptosis in renal cancer cell lines correlates with an almost complete loss of caspase 3 and variable down regulation of caspase 7,8 and 10 (10). These findings confirm the result of present study which showed caspase 7 expression in (23)74.1% of OSCC cases with majority of them (38.7%) having weak expression score. These data support the view that disruption of the caspase pathway may play a role in tumorigenesis or possibly confers resistance to tumor therapy. This work showed no correlation between caspase-7 immunohistochemical staining and traditional clinicopathological parameters. This finding was in line with many authors (11-15). The expression of caspase -7 in different normal tissue was strongly positive (16, 17). From the studying findings it is apparent that caspase-7 has no significant correlation with clinicopathological parameters, may be due to small samples and randomly collected, in addition to exclude tumor size and metastasis according to data sheets information.

Annexin v is a member of annexin family, which can bind (annex) to cellular membranes in a calcium-dependent manner. Studies had been showed that annexin might be a cell signaling protein and be implicated in the signaling process of inflammation, differentiation, apoptosis, coagulation, immune response and proliferation (18). The role of annexin in tumor biology is attracting growing interests. This is partly because characteristic distribution and expression of annexin have been found in different normal tissues, and dysregulation of annexin expression has been described in a Varity of cancerous and precancerous lesions (19,20).

The result of the present study showed positive annexin-v expression in (83.8%) of OSCC cases. Our results revealed that (51.6%) of positive cases showed weak expression score. This finding confirm previous study on esophageal squamous cell carcinomas that demonstrated annexin v down regulation in all
studied cases compared with patient-matched normal epithelial and suggested that annexin might be an essential component for maintenance of normal esophageal epithelial phenotype and its loss might be correlated with tumorigenesis. (21) Annexins are commonly dysregulated in cancers (22) and their frequent down-regulation has suggested a possible homeostatic or tumor suppressor role (23). We prioritized annexin V for follow-up analysis on the basis that this protein is normally wellexpressed in a wide range of organs and tissues, is specifically implicated in epithelial differentiation and growth regulation (24). Regarding the correlation of annexin v with clinicopathological parameters in present study there was no correlation between them while other studies showed there was a significant correlation between annexin v and tumor staging ,with presence of metastasis in esophageal carcinoma this finding was in line with (21,25). From the results of annexin v with clinicopathological parameters as mentioned above indicate that no correlation due to exclude tumor size and metastasis according to data sheets information.

Concerning the correlations between the two tumor markers ,the present study showed no correlations between the markers while other studies showed different correlations between them (26,27). Bouker et al was found that down regulation of caspases specially caspase 3,7 in breast cancer that determined by flurecent molecules annexin v. Economopoulou et al suggested that positive correlation between caspases and the level of apoptosis that was determined by annexin v in bone marrow of Myelodysplastic syndrome.

Table 1: Expression of caspase 7 in OSCC case

<table>
<thead>
<tr>
<th>Annexin-v expression</th>
<th>NO.</th>
<th>%</th>
</tr>
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<tbody>
<tr>
<td>Negative(0~4)</td>
<td>5</td>
<td>16.1</td>
</tr>
<tr>
<td>Weak positive(5~8)</td>
<td>16</td>
<td>51.6</td>
</tr>
<tr>
<td>Strong positive (9~12)</td>
<td>10</td>
<td>32.2</td>
</tr>
<tr>
<td>Total</td>
<td>31</td>
<td>(100%)</td>
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Table 2: Expression of Annexin-v in OSCC cases

<table>
<thead>
<tr>
<th>Caspase-7 expression</th>
<th>NO.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative(0~4)</td>
<td>8</td>
<td>25.8</td>
</tr>
<tr>
<td>Weak positive(5~8)</td>
<td>12</td>
<td>38.7</td>
</tr>
<tr>
<td>Strong positive (9~12)</td>
<td>11</td>
<td>35.48</td>
</tr>
<tr>
<td>Total</td>
<td>31</td>
<td>(100%)</td>
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REFERENCES

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