Immunohistochemical study of BCL-2, PCNA and VIMENTIN markers in oral and laryngeal squamous cell carcinoma (Comparative study)

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ABSTRACT

Background: Squamous cell carcinoma (SCC) may arise in any stratified squamous epithelium or muosa that has undergone squamous metaplasia. The aims of the study were immunohistochemical evaluation and comparison of PCNA, BCL-2 and VIMENTIN expressions in oral and laryngeal squamous cell carcinoma and correlating such expressions with the clinicopathological behavior in both sites.

Materials and Methods: This study was performed on thirty formalin-fixed, paraffin-embedded pathologically diagnosed oral and laryngeal SCC blocks for the period of June 2006 till July 2010. Age, sex, site and histologic grades were recognized. The samples were immunohistochemically stained with monoclonal antibodies to BCL-2, PCNA and VIMENTIN.

Results: The age of the patients with squamous cell carcinoma of the oral cavity was between 40 and 86 and between 32 and 66 years in larynx. The male/female ratio was 10/5 and 8/7 for oral and Larynx respectively. The majority of the cases of Oral squamous cell carcinoma were located on the buccal mucosa, whereas Laryngeal squamous cell carcinoma cases were distributed on glottic mainly. No significant statistical difference in the age and gender between the two groups. Histological grading was recognized for each case of oral and laryngeal squamous cell carcinoma. Two thirds of the oral cases were well differentiated. While 8 of the fifteen cases were moderately differentiated SCC. Regarding histological grading there was no statistically significant difference between the two groups. PCNA expressed in 14 of the fifteen oral cases whereas in laryngeal, 12 cases were positive. Collectively, 9 of the 30 cases were positive for vimentin antibody with different score values, Bcl-2 was expressed in one case only from fifteen immunostaining laryngeal cases and all the oral squamous cell carcinoma cases were negative. No significant relationship in immunoexpression of the above markers between the two groups.

Conclusions: The results of this study proved that the biological behavior namely the PCNA, VIMENTIN and BCL-2 activities was comparable between SCC of the oral cavity and larynx with a spectrum of clinical behavior, due to the differences in location.

Keywords: Squamous cell carcinoma of the oral cavity and larynx, PCNA, BCL-2, VIMENTIN. (J Bagh Coll Dentistry 2011; 23(sp. issue):82-86).

INTRODUCTION

Squamous cell carcinoma of the head and neck is the sixth most common human malignancy, although it only accounts for 2% of the cancer in Western population. The incidence of head and neck cancer in particular tumors of the oral cavity and larynx, are increasing in developed countries, with the increase of risk being seen in young people (1).

The incidence of oral cancer is variable from region to region and the highest rates are seen in India, Sri Lanka, Hong Kong and Taiwan (2).

Carcinogenesis is multistep process at both phenotypic and genetic levels, resulting from multiple mutations. Non lethal genetic damage lies at the heart of carcinogenesis, this genetic damage can be defined in 4 classes:

1. Damage in normal regulatory genes-growth-promoting proto oncogen
2. Damage in gene regulates programmed cell death (apoptosis)
3. Damage in genes involved in DNA repair mechanism

The first step of cancer invasion is to break down the collagen component of the basement membrane (mainly collagen IV) by brotiolytic enzymes and then degrade the intermediate protein filament of the connective tissues. Vimentin is the main intermediate filament in the C.T (4). Although most intermediate filament are stable structures in fibroblast cells, Vimentin exists as dynamic structure serves as a biochemical carrier for low density lipoprotein (LDL) during cholesrol estrification and maintaining the flexibility of the cells (5,6). Tissue growth depends on both the rate of cell proliferation and cell death. The increased ability of cells to proliferate is one of the main features of malignancy. In many tumors cell kinetics has been demonstrated to correlate with malignant behavior, they are of importance in planning certain type of therapy. PCNA, a cofactor of DNA polymerase δ is generally
detected in cell nucleus between G1 and M phases of cell cycle mainly in the S phase. It is a useful immunohistochemical marker of cell proliferation because its expression and distribution correlate with cellular proliferation rate and DNA synthesis. Expression of PCNA has been investigated in many cancers including oral cancer. 

Recently, basic cancer research has produced remarkable advances in our understanding of cancer biology and cancer genetics. Among the most important of these advances is the realization that apoptosis and the gene that control it have profound effect on the malignant phenotype and it is now well documented that most cytotoxic anticancer agents induce apoptosis. Raising the intriguing possibility that defect in apoptotic programs contribute to treatment failure.

Bcl-2 (B-cell lymphoma 2) is the founding member of the bcl-2 family of apoptosis regulates proteins encoded by the bcl-2 gene. Bcl-2-derived its name from the B-cell lymphoma 2.

MATERIALS AND METHODS

Fifteen formalin fixed paraffin embedded tissue blocks of Oral squamous cell carcinoma, and another fifteen of Laryngeal squamous cell carcinoma were retrospectively collected from laboratories’ archives and included in the study. Diagnostic confirmation was performed through examination of hematoxylin and eosin (H&E) sections. Five micrometer thick sections were cut and mounted on positively charged slides and stained immunohistochemically with monoclonal antibodies to PCNA to assess the proliferative capacity, BCL-2 to assess the apoptotic potential and Vimentin to assess the invasion. Comparisons regarding the aforementioned markers’ expressions were carried out between the two sites involved in the study.

Assessment of immunohistochemical results of PCNA

PCNA expression was evaluated semi-quantitatively. It was obtained by counting the number of positive nuclear staining, regardless to the intensity, in 1000 tumor cells from five different randomly selected representative fields in each sample using 40X objective. Immunoreactivity was classified as: (-) negative <5%, (+) low 6-25%, (++) moderate 26-50% and (+++) high 51-100%.

Assessment of immunohistochemical results of BCL-2

Only the number of cells showing cytoplasmic expression of bcl-2 was quantified by counting at least 1000 cells in five representative fields at 40X objective in each case, The extent of immunoreactivity was graded 1+ (+) if less than 25% of tumor cells were positive, 2+ (++) if 25 to 50% of cells were positive, and 3+ (+++) if more than 50% of cells were positive.

Assessment of immunohistochemical results of VIMENTIN

Each histological section was examined at 40 to identify areas of maximum tumor staining. At X400, 200 cells were analyzed (in the areas of maximum tumor staining), and the percentage of positive cells was recorded. This procedure was repeated, and the average of the two percentages was recorded. These averaged values were originally stratified into four scoring groups: (a) no immune positive cells identified; (b) >10% positive tumor cells; (c) 10–50% positive tumor cells; and (d) >50% positive tumor cells.

RESULTS

The age range of the patients with squamous cell carcinoma of the oral cavity was between 40 and 86 years with a mean of (62.13±12.39). For squamous cell carcinoma of the larynx, the age ranged between 32 and 66 years with a mean of (54.73±8.14). No significant statistical difference in the age distribution between the two groups was found (p=0.063). The male/female ratio for oral squamous cell carcinoma was 10/5 (2:1), and it was 8/7 (1.4:1) for Laryngeal squamous cell carcinoma. No statistically significant difference was found regarding gender distribution between the groups. (Table 1) Regarding the locations, the majority of the cases of oral squamous cell carcinoma were located on the buccal mucosa 7 cases (46.6%), followed by 4 (26.6%) on the maxillary bone; The other 4 sites were distributed between alveolar ridge and the tongue. Whereas Laryngeal squamous cell carcinoma cases were distributed among glottic (10 cases) 66.7%, supraglottic(4 cases) 26.7% and subglottic(1 case) 6.7%.

Histological grading was recognized for each case of oral and laryngeal squamous cell carcinoma. Two thirds of the oral cases (11 of 15) were well differentiated, another four were moderate and poorly differentiated squamous cell carcinoma. While 8 of the fifteen cases were moderately differentiated, and the remaining six were well with one case of poorly differentiated SCC. Regarding histological grading there was no statistically significant difference between the two groups (oral and Laryngeal squamous cell carcinoma). (Table 2)

PCNA immunoreactivity was recognized in 14 of the fifteen oral cases whereas in laryngeal, 12 cases were positive for PCNA and the other three were negative (figure 1, 2). No statistically
significant difference in the PCNA immunooexpression between oral and laryngeal squamous cell carcinoma was found.

Collectively, 9 of the 30 cases (30%) were positive for vimentin antibody with different score values. There was no statistically significant difference in the vimentin immunooexpression between oral and laryngeal squamous cell carcinoma (figure 3, 4).

Bcl-2 was expressed in one case only from fifteen immunostaining laryngeal cases (figure 5, 6) and all the oral squamous cell carcinoma cases were negative.

Figure 1: Photomicrography showing Well differentiated SCC of the oral cavity with positive PCNA immunostaining of the malignant nuclei (X40)

Figure 2: Photomicrography showing Well differentiated SCC of the larynx with positive PCNA immunostaining of the malignant nuclei (X40)

Figure 3: Photomicrography showing Moderately differentiated SCC of the oral cavity with positive Vimentin immunostaining of the malignant cytoplasm (X40)

Figure 4: Photomicrography showing Moderately differentiated SCC of the larynx with positive Vimentin immunostaining of the malignant cytoplasm (X40)

Figure 5: Photomicrography showing Moderately differentiated SCC of the larynx with positive BCL-2 immunostaining of the malignant cytoplasm (X10)

Figure 6: Photomicrography showing SCC of Oral cavity with 87 positive BCL-2 immunostaining of the lymphoid tissue (internal control) (X40)

Figure 7: Photomicrography showing SCC of the larynx with positive BCL-2 immunostaining of the lymphoid tissue (internal control) (X40)
Table 1: Case distribution according to age and sex

<table>
<thead>
<tr>
<th>Tumors</th>
<th>Sex</th>
<th>Age Range</th>
<th>Mean±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral</td>
<td>Male</td>
<td>Female</td>
<td>40-86</td>
</tr>
<tr>
<td></td>
<td>N=10</td>
<td>N=5</td>
<td></td>
</tr>
<tr>
<td>Laryngeal</td>
<td>Male</td>
<td>Female</td>
<td>32-66</td>
</tr>
<tr>
<td></td>
<td>N=8</td>
<td>N=7</td>
<td></td>
</tr>
</tbody>
</table>

Tests
- Pearson Chi square test= 0.456
- t-test= 0.063

Total N=18 Male 60% Female 40% 32-86 40.562±13.649

Table 2: Case distribution according to histological grading

<table>
<thead>
<tr>
<th>Tumors</th>
<th>Well</th>
<th>Moderate</th>
<th>Poor</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral</td>
<td>11</td>
<td>2</td>
<td>2</td>
<td>15</td>
</tr>
<tr>
<td>Laryngeal</td>
<td>6</td>
<td>8</td>
<td>1</td>
<td>15</td>
</tr>
</tbody>
</table>

Pearson’s Chi-square = 0.135

NS Non-significant difference (p>0.05)

DISCUSSION

Head and neck squamous cell carcinoma (HNSCC) is one of the most common cancers. Although potentially curable by local radiotherapy and surgical resection, the overall 5–years survival rate is only 50%, largely because of the propensity of some HNSCC tumors to disseminate via the lymphatic (14).

Therefore, a factor that consistently identifies patients at risk for recurrent disease would help to improve disease free by allowing physician to select high–risk patients for more aggressive treatment. In addition, identifying a biologic marker of aggressiveness would help to provide new avenues for rational drug disintegrated specific molecular defect (15).

This study attempted to investigate a possible correlation between the biological behavior of oral and laryngeal SCC, in relation to proliferation, apoptosis and invasion and their histological appearance.

PCNA immunohistochemical expression

The current result showed positive PCNA nuclear staining in 14 out of 15(93.3%) of oral SCC cases and 12 out of 15(80%) of laryngeal SCC cases, which reflect the high proliferative activity of cancerous tissue. The increased PCNA expression in malignant tissue is thought to occur as a repair response, PCNA was found to play a role in DNA damage repair by combining with hMsH3 (the sub unite of LMuts alpha and LMuts beta that act as cofactors in DNA mismatch repair system) . Since malignant tissue is characterized by high frequencies of DNA mismatch, breakage and mutations, thus, leading to increased proliferative rate reflected by PCNA over expression (16).

Assessment of the Vimentin expression

Vimentin, a mesenchymal cell marker, associates with components of the cytoskeleton and membrane adhesions. Studies of human epithelial carcinomas have shown that vimentin expression can be correlated with tumour invasion and a poor prognosis, (17,18)

The epithelial to mesenchymal transition theory is a potential mechanism to explain the dissemination and metastasis of carcinoma and may explain the aberrant expression of vimentin in epithelial tumors (19). To disseminate and metastasize, cohesive epithelial cells have to lose their stable, polarized, non migratory properties and transdifferentiate into migratory cells acquiring mesenchymal characteristics (20).

Elevated and aberrant expression of vimentin have been found to correlate well with these features. In the current study vimentin expression was 33.3% and 23% from 15 cases of LSCC and 15 OSCC respectively; This may correlate with a better prognosis than tumors with high vimentin expression.

Assessment of BCL-2 expression

By means of immunohistochemistry we estimated the level of expression of bcl-2 proteins in a series of the 30 formalin fixed, paraffin-embedded samples of oral and laryngeal squamous cell carcinoma and the results was 1(6%) case in laryngeal and none of the oral cases was positive ,while other studies found, none of the cases were positive in SCC of the skin, 26.2% from149 LSCC, 17%from 90 OSCC and 25%from 154 LSCC(21,22,23,24)respectively. The discrepancies might be attributed to numbers of reasons one of them is small sample size, the other one is lost of bcl-2 from the basal layer that may be result from Transcriptional suppression of BCL-2 which may also result from modulation of the negative response element located at the 5’ untranslated region (25).

BCL-2 is known to have antiproliferative effects, by delaying progression to S phase from quiescence (26). In addition, the antiproliferative effect of BCL-2 has been shown to inhibit tumour progression in animal tumours. (27,28). Therefore, loss of the suppressive effects of BCL-2 may be advantageous for potentially malignant and malignant oral keratinocytes. A suppressive effect of BCL-2 on proliferation is consistent with the
study of (29) who reported increased proliferative activity (as assessed by Ki-67 labelling) in basal cells of OSCC, which had lost BCL-2. Therefore, loss of BCL-2 in oral keratinocytes may be involved in dysplastic and malignant progression of oral epithelium by making oral keratinocytes more responsive to mitotic stimuli.

In conclusion, no significant relations were found among the immunohistochemical findings obtained by PCNA, BCL-2 and VIMENTIN considering the proliferation, apoptosis and invasion potential when correlated with the clinical finding and the histological grading systems obtained by the traditional H&E staining in both tumor sites.

REFERENCES


26. Huang DC, Reilly LA, Strasser A. The anti-apoptosis function of Bcl-2 can be genetically separated from its inhibitory effect on cell cycle entry. EMBO J 1997; 16: 4628-38.

