Evaluation of epidermal growth factor receptor (EGFR), proliferation (Ki-67) and apoptosis (P53) in salivary mucoepidermoid carcinoma in relation to tumor grade

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ABSTRACT

Background: Mucoepidermoid carcinoma (MEC) is the most common salivary gland malignancy. They display a variety of biological behaviours, and several systems have, therefore, been proposed to grade this neoplasm. Today, the most popular grading systems are Auclair et al, (1992) and Brandwein et al,(2001) grading systems. Assessment of cellular proliferation, apoptosis and growth molecules are important factors in tumor kinetic which may reflect tumor biological behaviour.

Materials and methods: Immunohistochemical analyses of seventeen cases of -fixed paraffin-embedded tissue blocks of MEC of salivary gland origin using (Ki-67, P53 and EGFR) monoclonal antibodies.

Results: The samples comprised of ten males and seven females to give male to female ratio (1.4:1). The mean age was (47.06±8.5) years. The submandibular salivary gland was the most predominant affected site (5 cases). 100% of cases were EGFR immunopositive. Only 47% of MEC cases showed Ki-67 immunopositivity, while P53 immunopositivity were shown in 94% of MEC cases. There was no statistically significant correlation regarding P53 or EGFR markers in relation with grading systems. There was a statistically significant correlation between the expression of Ki-67 marker and Auclair grading system. There were no significant statistical correlation among markers except between Ki-67 expression and P53.

Conclusions: Assessment of tumor biology in term of apoptosis (p53), proliferation (Ki-67) and EGFR are not reflected on tumor grade.


INTRODUCTION

Mucoepidermoid carcinoma (MEC) is one of the most common salivary gland malignancies. It demonstrates a wide age distribution with a mean of 45 years (1). Histologically MEC is composed of a mixture of mucous, intermediate, and epidermoid cells, with columnar, clear cell and oncocytoid features (2).

Mucoepidermoid carcinoma grading systems have a long history of controversy over the best grading system use and which facilitates inferences in the prognosis of this neoplasm (3). Different systems have been proposed with special characteristics for establishing tumor grade, which then require different types of treatment (4,5). In their original report, Stewart and colleagues in 1945 defined benign and malignant varieties of mucoepidermoid tumors. Nonetheless, subsequent metastases of a few of the previously benign tumors has led to all mucoepidermoid tumors being considered carcinoma (6).

A three-level system of dividing tumors into low, intermediate, and high grades has widely been used (2). Today, the most popular grading systems are Auclair et al. (4) and Brandwein et al. (5) grading systems. Both are point based, assigning point values to each adverse histologic parameters and with ascending point scores equating to a higher grade. However, the way in which each system correlates with outcome varies. The Auclair system appears to ‘down grade’ tumors while the Brandwein system appears to ‘upgrade’ tumors (7).

Epidermal growth factor receptor is the cell surface receptor for members of the epidermal growth factor family (8); it is a member of the ErbB family of receptors. It plays an important role in the differentiation and morphogenesis of many organs and proliferation and survival in mammalian cells (9). Mutations affecting EGFR expression or activity could lead to its constant activation which could result in uncontrolled cell division and cancer (10).

Antigen Ki-67 is a nuclear protein that is associated with and may be necessary for cellular proliferation (11). The cellular appearance and location of this protein throughout the cell cycle is not homogeneous, during interphase, the Ki-67 antigen can be exclusively detected within the cell nucleus, whereas in mitosis most of the protein is relocated to the surface of the chromosomes (12).
Apoptosis is a specific mode of cell death by which deletion of cells occurs (13). P53 tumor suppressor gene is a transcription factor which regulates cell proliferation and apoptosis to prevent division of potentially malignant cells (14). An alteration of the P53 gene is often observed in various human cancers (15).

MATERIALS AND METHOD
Seventeen formalin-fixed paraffin-embedded tissue blocks of mucoepidermoid carcinoma of salivary gland origin which were collected from laboratory archive of college of dentistry from the period between 1972 to 2011, in addition to cases from Al-Shaheed Ghazi Hospital/ Medical City/ Baghdad and private laboratories/ Baghdad included in this study.

Diagnosis was performed through examination of hematoxylin and eosin sections. Based on the criteria of the Auclair et al, and the criteria of Brandwein et al., All MEC tissue samples were scored and graded using a quantitative grading systems based on their histological features, and samples were separated into three grades; low, intermediate and high according with these grading systems.

Immunostaining
Five micrometer thick sections were cut and mounted on (Bio care, USA) positively charged slides, then deparaffinized and rehydrated. For p53 and EGFR (US biological); the sections were immersed in 0.3% hydrogen peroxide (H2O2) to block the endogenous peroxidase activity, washed in phosphate-buffered saline (PBS), and then incubated in 10% normal serum to block any non-specific binding of antibodies. The tissue sections were incubated with monoclonal mouse anti-human p53 (diluted 1:60) and EGFR (diluted 1:50) antibodies over night at 37 ºC. The bounded antibodies were detected by the streptavidin-biotin complex method, after an immunoreaction, the sections were counterstained with Hematoxylin. For Ki-67 monoclonal antibody (Abcam) the same steps were done in addition to epitope retrieving by citrate buffer solution pH 6.0 after blocking the endogenous peroxidase activity, the dilution of Ki-67 was 1:40.

Scoring system
The scoring of all markers was done by examining of at least 1000 cells per section in five different representative fields. The intensity of staining was not taken into consideration. For EGFR: (0) point for negative staining of the considered cells, (<10%, (+++)<10-50%, (++++) 51-80%, and (++++) ≥81% positive staining of the considered cells (16). For Ki-67 and p53: nuclear expression in ≤5% of tumor cells was scored as negative, 6-25% (+), 26-50% (++), and 51-100% (+++)(17).

Statistical analysis
The data were compiled into statistical software, statistical package of social sciences (SPSS) version 18. All variables were compared using Chi- square test. While Pearson correlation coefficient was applied to plot a correlation matrix among the different immunohistochemical markers expression values altogether. P values of less than 0.05 were considered significant.

RESULTS
The sample comprised of ten males and seven females to give males to females ratio (1.4:1). The mean age was (47.06±8.5) years (Table 1).

Table 1: Case distribution according to age groups

<table>
<thead>
<tr>
<th>Age groups years</th>
<th>No.</th>
<th>%</th>
</tr>
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<tbody>
<tr>
<td>30-39</td>
<td>3</td>
<td>17.65%</td>
</tr>
<tr>
<td>40-49</td>
<td>8</td>
<td>47.06%</td>
</tr>
<tr>
<td>50-59</td>
<td>4</td>
<td>23.53%</td>
</tr>
<tr>
<td>60-69</td>
<td>2</td>
<td>11.76%</td>
</tr>
</tbody>
</table>

According to site, the submandibular salivary gland was the most predominant affected site (5 cases) followed by parotid gland and buccal mucosa (4 cases for each), then palate (3 cases) and the lowest site was tongue (1 case).

According to Auclair grading system, 14 cases were found as low grade, non were intermediate and 3 cases were high grade. Brandwein grading system revealed 4 cases being as low grade, 7 intermediate and 6 cases were high grade. Concerning the site, sex and predominant cells, none of them had a significant statistical relationship with Auclair or Brandwein grading systems.

All cases (100%) were EGFR immunopositive in different scores (Figure 1). No statistical significant relationship was seen between this marker and both Auclair or Brandwein grading systems.

Only 47% of MEC cases showed Ki-67 immunopositivity (Figure 2). A significant statistical relationship with Auclair grading system in which all high grade cases were positive for this marker.
P53 immunopositivity was seen in 94% of MEC cases (Figure 3). 75% of these positive cases showed low expression, it had no significant statistical relationship with the tumor grading systems.

Table 2: Description of statistics concerning the results of immunohistochemical findings in relation with Auclair grading system

<table>
<thead>
<tr>
<th>Marker</th>
<th>Auclair grading system</th>
<th>Auclair grading system</th>
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<tbody>
<tr>
<td></td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>EGFR</td>
<td>++</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>+++</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>++++</td>
<td>2</td>
</tr>
<tr>
<td>Chi square test P value=0.815 NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ki-67</td>
<td>--</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>++</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>+++</td>
<td>1</td>
</tr>
<tr>
<td>Chi square test P value=0.045 *</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P53</td>
<td>--</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>++</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>+++</td>
<td>1</td>
</tr>
<tr>
<td>Chi square test P value=0.137 NS</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

There was no significant statistical correlation among markers except between Ki-67 expression and P53 expression.

Table 2 and 3 shows the relation between markers and Auclair and Brandwein grading systems respectively.

**DISCUSSION**

Mucoepidermoid carcinoma (MEC) is a malignant glandular epithelial neoplasm characterized by mucous, intermediate, and epidermoid cells. Grading of MEC is subjective with different criteria used in various series, and there is no universally accepted grading system (2).

Apoptosis is a pathway of cell death. P53 is a tumor suppressor and nuclear transcription factor (8). An alteration of the p53 tumor suppressor gene is often observed in various human cancers (15). In this study, 94% of cases were p53 positive and 75% of this positive cases were low expression, this finding is near the finding of Ehab et al, (18)
whom found that 80% of primary parotid MEC was p53 positive and 100% of recurrent cases were p53 positive. Jeanine et al. \(19\) study found p53 expressed in 75% of the MEC cases and had a weak expression, while Kiyoshima et al. \(15\) observed expression of p53 corresponding to 85% in MEC. The variation in the expression of p53 in this study and the aforementioned studies may be due to the use of different antibodies, different scoring systems, fixation times and concentrations of antibodies, and the sensitivity of the technique used. Only nuclear positive P53 immunoreactivity was considered in this study because p53 function depends on nuclear localization \(14\). Occasionally in some cases nuclear and cytoplasmic staining was observed at the same time. No statistical significance found between P53 expression and either Auclair or Brandwein grading systems. This sign that the parameters used for tumor grading don’t revealed the actual biological behavior of the MEC.

Ki-67 is a nuclear Ag, it is present throughout the complete cell cycle with the exception of early G1 phase \(20\). In this study; \(47\%\) of cases were Ki-67 positive. This finding is in agreement with the results of Brandwein et al. \(5\) who found no nuclear staining in 62% of the MECS evaluated, Alves et al. \(17\) study on 15 cases of MEC of the submandibular glands found that Ki-67 expression was 47% and was related to bad prognosis, Ki-67 positivity and negativity found in all grades. Because mucoepidermoid carcinomas are usually slow-growing tumors with proliferative rates lower than those observed in more aggressive carcinomas such as head and neck squamous-cell carcinomas. Therefore, it is not unexpected that low levels of Ki-67 may be observed. The relation of Ki-67 expression and Auclair grading system was statistically significant, this improves that Auclair grading system is down grading of MEC so only highly aggressive tumors are considered high grade.

The epidermal growth factor receptor is overexpressed in 80-100% of epithelial tumors of the head and neck \(8\). In this study; all cases were immunopositive for EGFR, this result is disagreed with the results of Jeanine et al. \(19\) whom found 75% of MEC cases showed score 2 & 3 positivity. This variation may be due to using of another scoring system, for instance Jeanine et al. \(19\) considered score 1 as a negative. Only membranous positive EGFR immunoreactivity was considered in this study because it indicates the receptor site. Occasionally membranous and cytoplasmic staining was observed at the same time but membranous staining was always stronger. Similarly Sarkis et al. \(9\) observed membranous and cytoplasmic EGFR staining in oral squamous cell carcinoma. The cytoplasmic staining may represent cytoplasmic synthesis or breakdown of the protein \(9\). There was no statistical significance found between EGFR expression and either Auclair or Brandwein grading systems. This sign that the parameters used for tumor grading don’t revealed the actual biological behavior of the MEC. The assessment of tumor growth using growth fraction and apoptosis as biological markers of tumorigenesis in MEC namely EGFR and Ki-67 proliferative index and P53 apoptotic marker, were irrelevant to tumor grade, as the results were insignificant except Auclair grading system which was significantly correlated with proliferation. This is in accordance to the conclusion drawn from a previous study of MEC done by Taher, 2011 \(21\) which assessed the metastatic behavior of the tumor, proven to be not significantly related to tumor grade.

**REFERENCES**


