Immunohistochemical Expression of EMMPRIN (CD147) in Salivary Gland Tumors

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Abstract

Extracellular matrix metalloproteinase inducer expression has been focus of research for variety of neoplasms owing to its potential role played in invasion, angiogenesis and metastasis through interactions with other molecules.

This study was designed to determine the immunohistochemical expression of EMMPRIN in benign and malignant salivary glands tumors in local population.

This descriptive study was conducted at the Department of Morbid Anatomy and Histopathology/ Oral Pathology, University of Health Sciences Lahore, Pakistan. Biopsies and detailed clinical data of 85 cases of salivary gland neoplasms (25 pleomorphic adenoma, 06 Warthin tumour, 25 adenoid cystic carcinoma, 25 mucoepidermoid carcinoma and 02 each basal cell adenocarcinoma and carcinoma ex pleomorphic adenoma) were obtained from different local tertiary care hospitals in Lahore from Jan. 2014 to Sep 2015. After confirming the histologic diagnosis on hematoxylin and eosin stained sections, immunohistochemical expression of EMMPRIN was determined. SPSS version 21.0 was used to determine the association between expression of EMMPRIN in benign, malignant and individual tumors. Chi-square and Fischer Exact tests were applied and p<0.05 was considered to be statistically significant.

Expression of EMMPRIN in malignant tumors was significantly higher than benign tumors (p< 0.0001). The staining pattern of cells was also significantly associated with type of tumour (p<0.0001). Significant EMMPRIN expression was noted with grades in AdCC (p <0.0001) & MEC (p=0.016).

The current study concludes that EMMPRIN expression is significantly higher in malignant salivary gland neoplasms and may help the pathologists for assessing tumor differentiation and malignant potential if added in a panel of other conventional markers.

ABBREVIATIONS

EMMPRIN: Extracellular Matrix Metalloproteinase Inducer; PA: Pleomorphic Adenoma; WT: Warthin Tumour; AdCC: Adenoid Cystic Carcinoma; MEC: Mucoepidermoid Carcinoma; BCA: Basal Cell Adenocarcinoma; CEPA: Carcinoma Ex Pleomorphic Adenoma; SPSS: Statistical Package for the Social Sciences; IgG1: Immunoglobulin G1; DAB: 3,3’-Diaminobenzidine; PBS: Phosphate Buffered Saline; DPX: Distyrene, Plasticizer, and Xylene ; USA: United States of America; F:M: Female: Male; PNI: Peri-Neural Invasion; VI: Vascular Invasion; MMP-2: Matrix Metalloproteinase-2; MMP-9:Matrix Metalloproteinase-9; mRNA: Messenger Ribonucleic Acid; OSCC: Oral Squamous Cell Carcinoma; FGFR: Fibroblast Growth Factor Receptor

INTRODUCTION

Extracellular matrix metalloproteinase inducer (EMMPRIN) or CD147 is a member of immunoglobulin super family [1]. It consists of three domains; cytoplasmic, transmembrane and intracellular [1]. Expressed on variety of cells [1], EMMPRIN plays critical roles in the invasion and metastasis by tumour cells through its complex interactions with various matrix metalloproteinases [2]. Over expression of EMMPRIN is noted in a number of human carcinomas including oral and head & neck squamous cell carcinomas [1]. The mechanism underlying the proliferation and metastasis in head & neck squamous cell carcinoma is via fibroblast growth factor (FGFR) while in oral squamous cell carcinoma (OSCC) invasion is mediated via epithelial-mesenchymal transition (EMT) by the activation of matrix metalloproteinases (MMPs) [1] (proteolytic enzymes which degrade the extracellular matrix) [3]. Other mechanisms include angiogenesis through the stimulation of vascular endothelial growth factor (VEGF) production [4].

Salivary gland tumors exhibit tremendous morphological variability in their histologic profile including features like hybrid tumors, anaplasia, lack of proper grading systems and
tendency for benign tumors to transform into malignant ones, so necessitating the use of specialized techniques for proper diagnosis and prediction of their biological behavior [5].

Owing to the diagnostic & prognostic roles played by EMMPRIN in various aspects of cancer progression [6], this study was designed to determine, for the first time in Pakistan, the expression of EMMPRIN in salivary gland tumors.

MATERIALS AND METHODS

This study was conducted at the Department of Morbid Anatomy and Histopathology/Oral Pathology, University of Health Sciences, Lahore. A total of 85 biopsies, 25 each of pleomorphic adenoma (PA), adenoid cystic carcinoma (AdCC) & mucoepidermoid carcinoma (MEC), 6 of Warthin tumour (WT) and 2 each of carcinoma ex pleomorphic adenoma (CEPA) and basal cell adenocarcinoma (BCA) of salivary glands reported at Histopathology Departments of University of Health Sciences, King Edward Medical College/Mayo hospital, Sheikh Zaid Hospital and Fatima Jinnah Medical College/Ganga Ram Hospital, Lahore from January, 2015 to September, 2015 were included in the study. Detailed clinical data was retrieved from the respective departmental records.

Hematoxylin and eosin staining

Paraffin embedded tissue sections were made from biopsy specimens. Tissue sections of 4µm were cut using rotary microtome and were stained with hematoxylin and eosin stain. Diagnosis was confirmed by 2 oral pathologists/histopathologists. Subtype determination of PA was done according to the criteria provided by Seifert [7].

Grading of AdCC was done according to the grading criteria provided by Spiero [8] where mostly tubular or cribriform (no stipulations or minor solid components) was given grade I, 50% solid pattern was grade II and mostly solid was named grade III.

Grading of MECs was done on the basis of less than 20% cystic component (+2), presence of neural invasion (+2), necrosis (+3), ≥ 4 mitoses per 10 high power fields (+3) and anaplasia (+4). Sum of the point values was used to determine low (0-4), intermediate (5-6) or high (7-14) grade MEC [9].

Immunohistochemistry

About 4 µm thick tissue sections were cut with the help of rotary microtome and taken on poly-L-lysine coated slides for immunohistochemical staining with anti-EMMPRIN (CD-147) antibody. Two sections were taken from each block, dried at 60°C for 50 minutes followed by de-waxing in xylene and rehydration in alcohol. Next, the slides were placed in Coplin jars containing citrate buffer (pH 6.0) solution and then in hot water bath (95°C) for 40 minutes in order to retrieve antigens (Heat Induced Epitope Retrieval). After removing the slides from hot water bath, they were allowed to cool at room temperature and hydrogen peroxide was added to block endogenous peroxidase activity followed by thorough washing with PBS (phosphate buffered saline). Sections were then incubated with 1-2 drops of protein blocker for 10 minutes to block endogenous enzymatic activity and then again washed with PBS. This was followed by incubation with primary antibody, mouse IgG1 kappa monoclonal CD147 antibody [HIM6] (Bio Legend; USA), diluted to concentration of 1:25 µg/ml (suggested dilution by the manufacturer) for 1 hour. Then, sections were incubated successively with Biotinylated Secondary Antibody for 10 minutes and Streptavidin Peroxidase Reagent for 10 minutes before application of DAB (di-aminobenzidine) (2 minutes) to avoid false positive staining. All incubation steps were separated by thorough washing with PBS. Counter staining with hematoxylin was done followed by dehydration and mounting of sections with coverslips using DPX. Positive (oral mucosa and skin) and negative (omission of primary antibody) controls were run with each batch of 20 histological sections of salivary gland tumors. EMMPRIN staining was evaluated on the basis of extent and intensity immunolabeling of tumor cells [10].

The intensity (qualitative variable) of staining was scored:
- 0 (absent), 1 (weak), 2 (moderate) and 3 (strong)

The extent/proportion (quantitative variable) of tumor cells staining was semi-quantitatively evaluated as:
- 0 (no or <10% positive tumor cells);
- 1 (10% - 24% positive tumor cells);
- 2 (25% to 49% positive tumor cells);
- 3 (50% to 74% positive tumor cells); and
- 4 (75% or more positive tumor cells).

Total/Final Score: The sum of the intensity and extent scores was the final score (0-7).

Negative: 0-1
Weak positive (1+): 2-3
Moderate positive (2+): 4-5
Strong positive (3+): 6-7

The clinical, histological and immunohistochemical data was analyzed statistically using SPSS 21.0. Chi-square and Fischer Exact tests were applied and p-value <0.05 was considered to be statistically significant.

RESULTS AND DISCUSSION

Results

The clinical parameters of the salivary gland tumors studied are summarized in Table 1.

The mean age for benign tumors was found to be 32.52 ± 16.326 years with an age range of 12-70 years. Most patients were seen in 2nd and 3rd (25.8% each) decades of life. Almost equal gender predisposition was noted (F:M, 1.06:1). Parotid gland (61.3%) was the most frequent site affected followed by minor salivary glands (22.6%) with palate being the commonest site (71.4%) (Table 1).

The mean age for the malignant cases was calculated to be 35.65 ± 14.130 years with an age range of 9-70 years. Most patients were seen in 5th decade (37%) of life. A slight male predilection of 1.125 was noted in malignant salivary gland tumors. Most of these tumors arose in minor salivary glands (48%) followed by...
Table 1: Frequencies, Percentages and P-Value Regarding Clinical Data of the Salivary Gland Tumours (n=85).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>PA</th>
<th>WT</th>
<th>AdCC</th>
<th>MEC</th>
<th>BCA</th>
<th>CEPA</th>
<th>Total</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>F %</td>
<td>F %</td>
<td>F %</td>
<td>F %</td>
<td>F %</td>
<td>F %</td>
<td>F %</td>
<td>F %</td>
</tr>
<tr>
<td>Mean Age</td>
<td>30.36 ± 4.838 41.50 ± 20.550 41.32 ± 11.022 31.44 ± 2.999 34.00 ± 19.799 19.00 ± 8.485 34.51 ± 14.949</td>
<td>31.44 ± 2.999 34.00 ± 19.799 19.00 ± 8.485 34.51 ± 14.949</td>
<td>0.028</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minimum (years)</td>
<td>12</td>
<td>18</td>
<td>22</td>
<td>9</td>
<td>20</td>
<td>13</td>
<td>09</td>
<td></td>
</tr>
<tr>
<td>Maximum (years)</td>
<td>70</td>
<td>70</td>
<td>70</td>
<td>70</td>
<td>48</td>
<td>25</td>
<td>70</td>
<td></td>
</tr>
<tr>
<td>Frequent decade</td>
<td>2nd and 3rd</td>
<td>6th</td>
<td>5th</td>
<td>3rd</td>
<td>-</td>
<td>-</td>
<td>5th</td>
<td>0.028</td>
</tr>
<tr>
<td>Gender</td>
<td>Female</td>
<td>14</td>
<td>56</td>
<td>2</td>
<td>33</td>
<td>9</td>
<td>36</td>
<td>12</td>
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<tr>
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<td>11</td>
<td>44</td>
<td>4</td>
<td>67</td>
<td>16</td>
<td>64</td>
<td>12</td>
<td>48</td>
</tr>
<tr>
<td>F:M</td>
<td>1.3 : 1</td>
<td>1:2</td>
<td>1:1.8</td>
<td>1:1</td>
<td>1:1</td>
<td>1:1</td>
<td>1:1</td>
<td>1:1</td>
</tr>
<tr>
<td>Gland</td>
<td>Parotid</td>
<td>15</td>
<td>60</td>
<td>04</td>
<td>66.7</td>
<td>04</td>
<td>16</td>
<td>17</td>
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<td>Submandibular</td>
<td>04</td>
<td>16</td>
<td>01</td>
<td>16.7</td>
<td>0</td>
<td>0</td>
<td>02</td>
<td>08</td>
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<tr>
<td>Sublingual</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>02</td>
<td>08</td>
</tr>
<tr>
<td>Minor</td>
<td>06</td>
<td>24</td>
<td>01</td>
<td>16.7</td>
<td>21</td>
<td>84</td>
<td>04</td>
<td>16</td>
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<tr>
<td>Laterality</td>
<td>Right</td>
<td>10</td>
<td>40</td>
<td>01</td>
<td>16.7</td>
<td>09</td>
<td>36</td>
<td>13</td>
</tr>
<tr>
<td>Left</td>
<td>10</td>
<td>20</td>
<td>05</td>
<td>83.3</td>
<td>09</td>
<td>36</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Not mentioned</td>
<td>05</td>
<td>20</td>
<td>0</td>
<td>0</td>
<td>07</td>
<td>28</td>
<td>11</td>
<td>44</td>
</tr>
<tr>
<td>Lymph node status</td>
<td>Positive</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>02</td>
<td>08</td>
<td>12</td>
</tr>
<tr>
<td>Negative</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>04</td>
<td>16</td>
<td>11</td>
<td>44</td>
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<tr>
<td>Not mentioned</td>
<td>-</td>
<td>19</td>
<td>76</td>
<td>02</td>
<td>8</td>
<td>02</td>
<td>100</td>
<td>0</td>
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</table>

Abbreviations: PA: Pleomorphic Adenoma; WT: Warthin Tumour; AdCC: Adenoid Cystic Carcinoma; MEC: Muco Epidermoid Carcinoma; BCA: Basal Cell Adenocarcinoma; CEPA: Carcinoma Ex Pleomorphic Adenoma; F: Frequency; n: Number of Cases.

parotid gland (42.6%). Of the minor salivary gland sites, palate (34.6%) was the most frequent site involved (Table 1).

Statistically significant association was noted among tumour type and age of patients (p=0.028), gland involved (p<0.001) and laterality (p=0.041) (Table 1).

Cell-rich or cellular subtype of PA (n=12; 48%) was the commonest subtype noted in PA, closely followed by classic (n=11; 44%). Only 2(8%) cases of stroma rich/hypo-cellular subtype were seen in the current study.

Regarding tumour morphology of malignant tumours, 17 (68%) cases of AdCC were of grade I and 8(32%) were grade III. Cribriform pattern (n=15; 60%) was the predominant pattern noted in AdCC followed by tubular (n=6; 24%) and solid (n=4; 16%). As for MEC, 9(36%) were grade I, 7(28%) were grade II and 9(36%) were grade III. Both cases of basal cell adenocarcinoma were of solid subtype characterized by solid nests of cells delineated by basement membrane like material.

Positive nodes were noted in 2(8%) of AdCC, 12(48%) cases of MEC and 2(100%) cases of carcinoma ex PA.

Perineural invasion (PNI) was noted in 9(36%) and 10(40%) cases of MEC and AdCC respectively. One (50%) case of basal cell adenocarcinoma showed PNI.

Vascular invasion (VI) was noted in 18(72%) cases in of AdCC and 1(50%) case of basal cell adenocarcinoma.

The staining reaction for anti-EMMPRIN (CD147) in the normal salivary gland tissue was strong membranous and cytoplasmic in the ductal structures while in acini the intensity was less profound than ducts and was limited to the cell membranes (Figure 1A).

The total scores for anti-EMMPRIN (CD147) in benign and malignant salivary gland tumours are summarized in Table 2. The staining pattern for anti-EMMPRIN (CD147) in individual tumours is shown in Table 3. The anti-EMMPRIN (CD147) positive scores were significantly higher in malignant neoplasms with a p-value <0.0001. Significant association was noted among the total score of EMMPRIN staining in the benign tumours (p=0.004), however, no significant association was noted within the malignant group (p=0.449). In contrast, the type of staining pattern was statistically significant (differed significantly) not only in benign and malignant tumours but also within the groups (p<0.0001).

The anti-EMMPRIN (CD147) positivity was moderate in 17(54.8%) of benign tumours followed by weak in 8(25.8%) and strong in 6(19.4%) (Table 2). The predominant staining pattern was cytoplasmic and membranous in 20(64.5%) cases while cytoplasmic in 10(32.3%). Only one case showed nuclear staining in addition to cytoplasmic and membranous. No significant association was noted between the staining pattern and type of tumour (p=0.172). Eighteen (72%) PA showed
Table 2: Anti-EMMPRIN (CD147) total score in benign and malignant salivary gland tumours (n=85).

<table>
<thead>
<tr>
<th>Tumour</th>
<th>Mean Score ± SD</th>
<th>Negative F %</th>
<th>Weak positive F %</th>
<th>Moderate positive F %</th>
<th>Strong positive F %</th>
<th>p-value &lt;0.0001</th>
</tr>
</thead>
<tbody>
<tr>
<td>PA</td>
<td>4.96 ± 0.020</td>
<td>0 0 0 0</td>
<td>13 52</td>
<td>4 67</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WT</td>
<td>5.67 ± 1.751</td>
<td>0 0 1 1.6</td>
<td>1 1.6</td>
<td>4 67</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AdCC</td>
<td>6.48 ± 0.823</td>
<td>0 0 0 0</td>
<td>03 12</td>
<td>22 88</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MEC</td>
<td>6.80 ± 0.406</td>
<td>0 0 0 0</td>
<td>0 0</td>
<td>25 100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BCA</td>
<td>6.50 ± 0.707</td>
<td>0 0 0 0</td>
<td>0 0</td>
<td>2 100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CEPA</td>
<td>7.00 ± 0.000</td>
<td>0 0 0 0</td>
<td>0 0</td>
<td>0 0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: EMMPRIN: Extracellular Matrix Metalloproteinase Inducer; PA: Pleomorphic Adenoma; WT: Warthin Tumour; AdCC: Adenoid Cystic Carcinoma; MEC: Mucoepidermoid Carcinoma; BCA: Basal Cell Adenocarcinoma; CEPA: Carcinoma Ex Pleomorphic Adenoma; F: Frequency; n: Number of Cases

Figure 1 Photomicrograph (A) showing normal salivary gland tissue showing strong cytoplasmic and membranous staining in ducts (arrows) and membranous only in acinar cells (EMMPRIN 100X), (B) moderate positive staining in epithelial component of PA (EMMPRIN 40X), (C) moderate staining in mesenchymal component of PA (EMMPRIN 40X) and (D) showing moderate positive staining cytoplasmic and membranous staining in WT (EMMPRIN 40X).

Figure 2 Photomicrograph (A) showing strong positive anti-EMMPRIN staining in tubular pattern of adenoid cystic carcinoma, (B) Cytoplasmic and/or membranous staining in most cells of tubular pattern of AdCC (EMMPRIN 40X), (C) Strong positive staining in cribriform pattern of AdCC (EMMPRIN 40X), (D) High power view of strong positive staining in cribriform pattern of AdCC (EMMPRIN 100X), (E) Moderate positive staining in solid pattern (EMMPRIN 40X) and (F) High power view of moderate positive staining in solid pattern of AdCC (EMMPRIN 100X).

All cases of AdCC showed cytoplasmic and membranous staining (Figure 2A-Figure 2F). On the other hand most MECs (n=17; 68%) showed membranous localization and only 8(32%) cases showed cytoplasmic reaction in addition to membranous staining (Figure 3A-Figure 3F). Basal cell adenocarcinoma showed membranous and cytoplasmic staining reaction in one case each showed cytoplasmic localization alone and 2(3.5%) showed nuclear reaction in addition to cytoplasmic and membranous staining (Table 2, Table 3) (Figure 2-Figure 4).

No significant association was noted between the subtypes of PA and anti-EMMPRIN (CD147) antibody staining reaction.

All the malignant tumours were strong positive for anti-EMMPRIN (CD147) antibody and the staining pattern was mostly cytoplasmic and membranous (n=33; 61.1%) followed by membranous alone in 18(33.3%) cases. Only 1 (1.9%) case showed cytoplasmic reaction in addition to cytoplasmic and membranous pattern (Figure 1B, Figure 1C) while most WT showed cytoplasmic reaction (n=4; 66.7%) with occasional cells showing both cytoplasmic/membranous pattern (Figure 1D). Stroma showed negative or weak staining in most cases (93.5%) and only 2 (9%) cases of PA showed moderate reaction in the stroma.

No significant association was noted between the subtypes of PA and anti-EMMPRIN (CD147) antibody staining reaction.

All the malignant tumours were strong positive for anti-EMMPRIN (CD147) antibody and the staining pattern was mostly cytoplasmic and membranous (n=33; 61.1%) followed by membranous alone in 18(33.3%) cases. Only 1 (1.9%) case
Figure 3 Photomicrograph (A) showing strong positive staining reaction in grade I MEC (EMMPRIN 40X), (B) Membranous staining in most cells (EMMPRIN 100X), (C) Strong positive staining in grade II MEC (EMMPRIN 40X), (D) Moderate membranous staining in grade II MEC (EMMPRIN 100X), (E) Strong membranous staining in grade III MEC (EMMPRIN 100X) and (F) Showing strong membranous staining in grade III MEC (EMMPRIN 100X).

Figure 4 Photomicrograph (A) showing strong positive cytoplasmic plus membranous staining reaction in BCA (EMMPRIN 100X), (B) Moderate membranous staining in most cells of BCA (EMMPRIN 100X), (C) Strong positive cytoplasmic plus membranous staining reaction in CEPA (EMMPRIN 40X) and (D) Showing high power view (EMMPRIN 100X). Note the strong positivity in the invaded nodal tissue.

(50%) (Figure 4A-Figure 4B) while both cases of CEPA showed membranous and cytoplasmic reaction (Figure 4C-Figure 4D).

Anti-EMMPRIN (CD147) staining score and staining pattern were significantly associated with the grades in AdCC and MEC (p<0.0001& 0.016 respectively). No significant association of anti-EMMPRIN (CD147) antibody staining was noted with lymph node involvement, peri-neural invasion or vascular invasion in both AdCC and MEC.

Stroma in AdCC and MEC was moderately reactive in 15(60%) and 10(40%) cases respectively.

Both cases of each BCA and CEPA were strong positive for EMMPRIN. Weak stromal reaction was noted in BCA while moderate to strong reaction was seen in CEPA.

Discussion

EMMPRIN (CD 147) is a potential regulator of cell-cell and cell-matrix interactions and its higher expression in various malignant neoplasms points towards the crucial role played by this molecular marker in tumour invasion, angiogenesis and metastasis [3].

Salivary gland tumors constitute 1-4% of all tumors occurring in human body [11]. Among these, pleomorphic adenoma is the most commonly occurring benign salivary gland tumour in any location mostly involving the parotid gland [12]. As regards the commonest malignancy of salivary glands, there is some debate. Some studies have reported adenoid cystic carcinoma to be the most commonly occurring malignant tumour of salivary glands [5,13] while others have named mucoepidermoid carcinoma as the commonest [14,15].

In the current study, we determined the expression of EMMPRIN (CD 147) in 85 cases (6 different types) of salivary gland tumors. Of these, 31 were benign [PA (n=25) and WT (n=06)] and 54 were malignant [AdCC (n=25, MEC (n=25), BCA (n=02) and CEPA (n=02)].

The mean age of the patients for benign (32.52 ± 16.326 years), malignant tumors (35.65 ± 14.130 years) and total (34.51 ± 14.949 years) salivary gland neoplasms studied in the current study is quite lower than other studies conducted worldwide [13,16]. Even when these tumors were considered individually (Table 1), they seem to affect younger age group in our population than reported in other studies [13,16,17]. These differences may be attributed to geographical, racial or ethnic dissimilarities among the various populations or to the variations in sample size of the study.

In line with the findings of current study, Kızıl [13] reported female predisposition for PA and MEC and male predilection for WT and AdCC. Other studies, however, have reported different findings [17].

Regarding site distribution of these salivary gland tumours, the present study is in accordance with other national and international studies with parotid being the commonest site for PA, WT, MEC, minor salivary glands (palate) for AdCC, both major and minor glands in basal cell adenocarcinoma and major glands for ca ex PA [13,16].
Byrd [17] reported lymph node involvement in 42.2% cases of MEC which is in accordance with the current study which reports nodal positivity in 48% cases. In contrast, Liu [18] reported only 13.8% positive nodes in MEC. Bianchi [19] reported 9% positive nodes in AdCC which is almost same as found in the current study.

McHugh [20] and Agarwal [21] reported peri-neural invasion in 28.7% and 32.4% cases of MEC and AdCC respectively which is close to the findings in the current study (MEC: 36%, AdCC: 40%).

Immunohistochemical expression of anti-EMMPRIN (CD147) antibody in the ducts of normal salivary gland tissue was both membranous and cytoplasmic which is similar to that reported by Yang [22]. However, in contrast to the current study, where moderately intense membranous expression in acini was noted, they reported negative expression in the acini of normal salivary glands [22] (Figure 1A).

Riethdorf [3] reported, only 2.3% positive expression in cell lines of PA which is highly different from the current study where moderate to strong positive reaction was noted.

In line with the current study, Yang [22] reported an increased expression of EMMPRIN in adenoid cystic carcinoma which was positively correlated with histopathological subtypes (grade). However, in contrast to the present study, they also reported positive correlation between EMMPRIN expression and perineural and vascular invasion. In addition, they reported that tumor size, clinical stage and metastasis were also significantly related to EMMPRIN expression [22].

In another study, Yang [23] further showed that silencing of EMMPRIN expression significantly reduces the proliferation of tumour cells via reduced secretion of MMP-2 and MMP-9. In addition, perineural invasion was also inhibited when EMMPRIN is silenced due to decreased adhesion of the tumour cells to the nerves [23].

The current study is in an agreement with the study conducted by Huang [24], who reported that expression of EMMPRIN in MEC and AdCC was significantly higher than that in normal salivary gland tissue and PA [24]. In addition to the immunohistochemical results, they reported significantly higher EMMPRIN mRNA expression in malignant salivary gland tumours as compared to the benign ones [24]. Also, they reported EMMPRIN to be a positive stimulator for angiogenesis in salivary gland neoplasms [24].

In contrast to the current study, Riethdorf [3] found statistically significant difference in EMMPRIN expression among the malignant salivary gland tumours (AdCC 7.5%, MEC 66.7%, Acinic cell CA, SCC, undifferentiated adenocarcinoma 100%) and very rare expression in benign tumours (PA).

As for EMMPRIN expression in basal cell adenocarcinoma and carcinoma ex pleomorphic adenoma of salivary glands, we did not find a study to compare. However, the high expression of EMMPRIN in undifferentiated adenocarcinoma of salivary glands [3] and colorectal adenocarcinoma a support the results of the current study.

As evident from the studies being conducted all over the world, EMMPRIN (CD147) is a potential diagnostic and prognostic marker in a large number of tumors [1]. Its higher expression in malignant salivary gland tumours may dictate its potential advantage as a biologic marker in characterizing these neoplasms [24]. In addition, it may be utilized as a promising target for treatment of malignant salivary gland tumours and may help to improve the overall patient survival which is the ultimate goal of all the research work being done internationally.

CONCLUSION
Salivary gland tumors, both benign and malignant, affect a younger age group in our population with a slight female predilection in benign tumors and male predilection in malignant tumours. Statistically significant difference was noted for anti-EMMPRIN (CD147) antibody expression and staining pattern between benign and malignant salivary gland tumors (<0.0001). It can be concluded that EMMPRIN (CD147) can be utilized as a marker to characterize benign and malignant salivary gland tumors if added in the panel of conventional markers.

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