EXPRESSION OF EXTRACELLULAR MATRIX METALLOPROTEINASE INDUCER (EMMPRIN) AS A BIOMARKER IN ORAL SQUAMOUS CELL CARCINOMA (OSCC)

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ABSTRACT

EMMPRIN is a widely distributed cell surface glycoprotein that belongs to immunoglobulin (Ig) super family. It is inducing the production of extracellular matrix metalloproteinase (MMP) enzymes and play important role in angiogenesis via stimulation of vascular endothelial growth factor (VEGF). The aim of the present work is to evaluate and asses the expression of extracellular matrix metalloproteinase inducer (EMMPRIN) in oral squamous cell carcinomas and to compare the expression of extracellular matrix metalloproteinase inducer (EMMPRIN) in different types of oral squamous cell carcinomas. Thirty diagnosed cases of Oral squamous cell carcinoma (OSCC) were selected and immunohistochemistry was performed for EMMPRIN. All cases showed positive EMMPRIN expression with different intensity. This study concluded that the elevated expression of EMMPRIN levels correlate with tumor proliferation, angiogenesis, metastasis and invasion.

KEY WORDS: Oral squamous cell carcinoma (OSCC), EMMPRIN.

INTRODUCTION

Oral squamous cell carcinoma (OSCC) is the sixth most common malignancy worldwide with an estimated 211,500 new cases per year (2.6% of all cancers) and over 120,000 deaths every year[(1)]. The etiology of oral cancer is multi-factorial, with multiple carcinogenic agents affecting the oral mucosa[(2)].

EMMPRIN is a widely distributed cell surface glycoprotein that belongs to immunoglobulin (Ig) super family[(3)]. It is expressed in numerous cells; including platelets, fibroblasts, T-lymphocytes and especially in cancer cells[(4-7)].

The major function of EMMPRIN has been implicated in many biological functions, such as; in the developing nervous system[(8)], embryo implantation, spermatogenesis[(9)] retinal development[(10)], and in immune cell activation[(11)]. Elevated EMMPRIN expression levels correlate with tumor proliferation, angiogenesis, metastasis and invasion[(12,13)]. It was found that over expression of EMMPRIN / CD147 in cancer tissues is associated with poor prognosis of patients with several types of solid tumors[(14)].

The number of studies focusing on EMMPRIN expression in oral squamous cell carcinoma (OSCC) is limited, so the aim of the present study is to detect the expression of EMMPRIN immunohistochemically in different oral squamous cell carcinoma (OSCC) and to compare between them**.**

MATERIALS AND METHODS

Biopsies:

Thirty patients clinically diagnosed with OSCC were selected from the Cranio-Maxillofacial and Plastic Surgery Department at the Faculty of Dentistry, Alexandria University. a written informed consent was taken from all the patients and 10 patients agreed to be photographed before surgery. Biopsies were taken from the tumor tissue and fixed in 10% neutral buffered formalin, processed and embedded in paraffin wax using the conventional procedures. Serial sections of 3-4 µm thick were placed on glass slides and stained using hematoxylin and eosin (H&E) for routine histopathological examination.

Immunohistochemical staining of EMMPRIN:

Immunohistochemical marker of primary rabbit polyclonal antibody EMMPRIN Glut 1 Cat. # E2260-03 (0.5 ml), (US Biological life science) was used. Strept-Avidin Biotin-peroxiadase complex method (LSAB) was used. Serial sections 4-5 µm thick were taken from the previously used for H&E blocks. The slide will be mounted on poly-L-lysine coated glass slides. Two sections will be obtained for the positive test slides and third one for the negative control by omitting the primary antibody. The tissue sections were deparaffinized in xylene, rehydrated in graded ethanol and incubated in 0.3% hydrogen peroxide solution to block the endogenous peroxidase. The specimens were washed with an appropriately characterized, diluted and were incubated with the primary antibody of EMMPRIN. Exposure to biotinylated link antibody and labeled streptavidin-biotin-peroxidase complex was done to bind the primary antibody. Staining was completed by incubation in substrate-chromogen solution and hematoxylin counter stain. Immuno-expression of EMMPRIN will be evaluated by using image analyzer to evaluate both mean area percent and mean optical density.

The results were recorded and statistically analyzed using (ANOVA) test.

RESULTS

In the present work, a total of 30 patients with OSCC were included. The patients' age ranged between 30 and 76 with a mean of (58.1years). Twenty patients (66.6%) were males and ten patients (33.3%) were females. As regards to location, the most common site of occurrence was the alveolar ridge, 15cases (50%), followed by the lateral side of the tongue 10 cases (33.3%) and the buccal mucosa 5 cases (16.6%).

Clinical data regarding the site of the lesion is presented in the **(table 1)**.

(Table 1) Clinical Data of Squamous Cell Carcinoma Cases According to the Location.

Diagnosis	Age (y)/sex	Location	Presence of Lymph Node Metastatic Deposits
Poorly differentiated squamous cell carcinoma	30/F	Alveolar ridge	x
Well differentiated squamous cell carcinoma	75/M	Alveolar ridge	√
Poorly differentiated squamous cell carcinoma	60/M	Alveolar ridge	x
Moderate differentiated squamous cell carcinoma	72/M	Alveolar ridge	x
Moderate differentiated squamous cell carcinoma	70/M	Alveolar ridge	x
Well differentiated squamous cell carcinoma	73/M	Alveolar ridge	x
Well differentiated squamous cell carcinoma	63/F	Tongue	x
Poorly differentiated squamous cell carcinoma	64//F	Tongue	x
Moderate differentiated squamous cell carcinoma	33/F	Tongue	x
Well differentiated squamous cell carcinoma	51/M	Tongue	x
Moderate differentiated squamous cell carcinoma	35/F	Tongue	x
Poorly differentiated squamous cell carcinoma	76/M	Buccal mucosa	x
Moderate differentiated Squamous cell carcinoma	50/F	Buccal mucosa	√
Moderate differentiated squamous cell carcinoma	59/M	Buccal mucosa	x
Well differentiated squamous cell carcinoma	61/F	Palatal mucosa	x

Immunohistochemical Results:

In the present immunohistochemical study, routinely formalin fixed, paraffin-embedded 30 patients with OSCC biopsies were used. This was done to detect the extracellular matrix metalloproteinase inducer (EMMPRIN) expression along with the normal control.

The intensity of immunostaining of EMMPRIN was calculated in terms of mean area percent and mean optical density by the computer image analyzer.

Pattern of EMMPRIN Immunostaining in Normal Control Sections:

All biopsies of normal oral mucosa (no=2) Showed positive immunosignals for EMMPRIN which is limited in the basal cell layer.

Pattern of EMMPRIN Immunostaining in Squamous Cell Carcinomas:

EMMPRIN expression was analyzed in 30 squamous cell carcinoma biopsies. They all showed positive expression.

Well differentiated SCC (n=10) showed diffuse positive cytoplasmic immunosignals of EMMPRIN in the malignant epithelial cells forming the keratin pearls, while the nuclei were free from any reaction (fig.a) *Moderately differentiated SCC* (n=8) showed positive cytoplasmic immunoreaction of EMMPRIN. The anaplastic cells formed epithelial nest, which demonstrated membranous, sometimes perinuclear immunosignals, the nuclei were free from any reaction (fig.b) *In the poorly differentiated SCC cases* (n=7) the intense cytoplasmic EMMPRIN immunopositivity was detected in the highly anaplastic malignant epithelial cells. It showed different abnormal mitotic figures (fig.c) *The metastatic lymph nodes* (n=5) of SCC revealed strong positive diffuse immunosignals of EMMPRIN within keratin pearls (fig.d).

Correlating EMMPRIN Immunoexpression in Different Grades of Squamous Cell Carcinoma:

Comparing different grades of squamous cell carcinomas (SCC) and metastatic lymph node of squamous cell carcinoma according to the area percent of EMMPRIN immunoexpression was done. The greatest mean value was in metastatic lymph node of squamous cell carcinoma (80.66±11.05) and the lowest value was in well differentiated SCC (61.85±1.678). ANOVA test revealed a statistically significant difference (p=0.003). Tukey's post hoc test revealed no significant difference between moderately or poorly differentiated squamous cell carcinoma and metastatic lymph node of squamous cell carcinoma, (table 2), (figure 1).

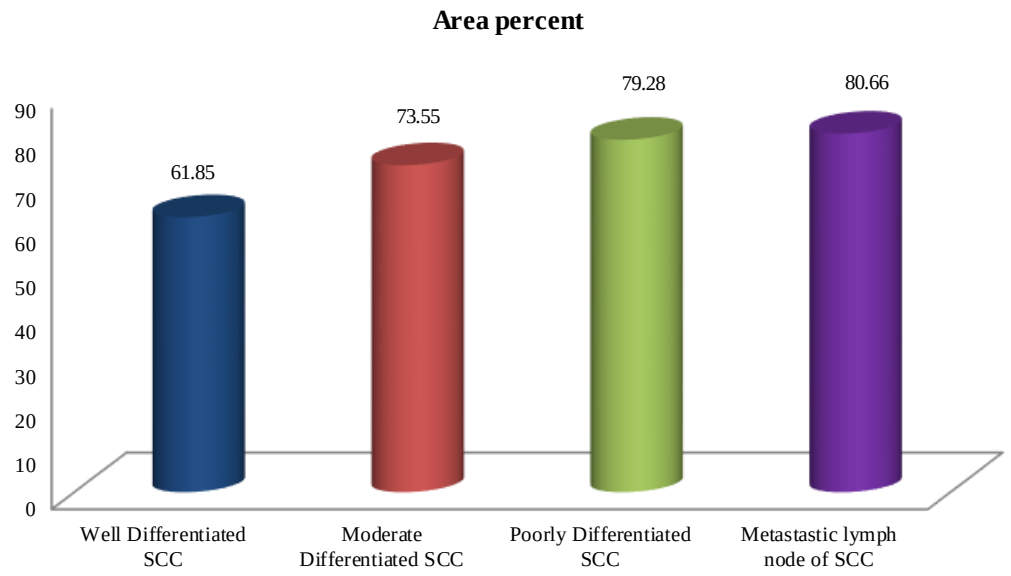
Comparing different grades of squamous cell carcinomas (SCC) and metastatic lymph node of squamous cell carcinoma according to the optical density of EMMPRIN immunoexpression was done. The greatest mean value was in metastatic lymph node of squamous cell carcinoma (83.49±7.32) and the lowest value was in well differentiated SCC (59.81±13.59). ANOVA test revealed a statistically significant difference (p=0.034). Tukey's post hoc test revealed no significant difference between moderately or poorly differentiated squamous cell carcinoma and metastatic lymph node of squamous cell carcinoma. (Table 3) and (figure 2).

(Table 2) Comparison between Different Histological Types of Squamous Cell Carcinomas (SCC) according to the Mean Area Percent of EMMPRIN immunoexpression using ANOVA Test.

	Well differentiated SCC (4 cases)	Moderate differentiated SCC (5 cases)	Poorly differentiated SCC (4 cases)	Metastatic lymph node of SCC (2 cases)
Mean ±SD	61.85a±1.68	73.55b±6.12	79.28b±3.66	80.66b±11.05
F value	8.85			
P value	0.003*			

**statistically significant*

Tukey's post hoc test: means with different superscript letters are significantly different

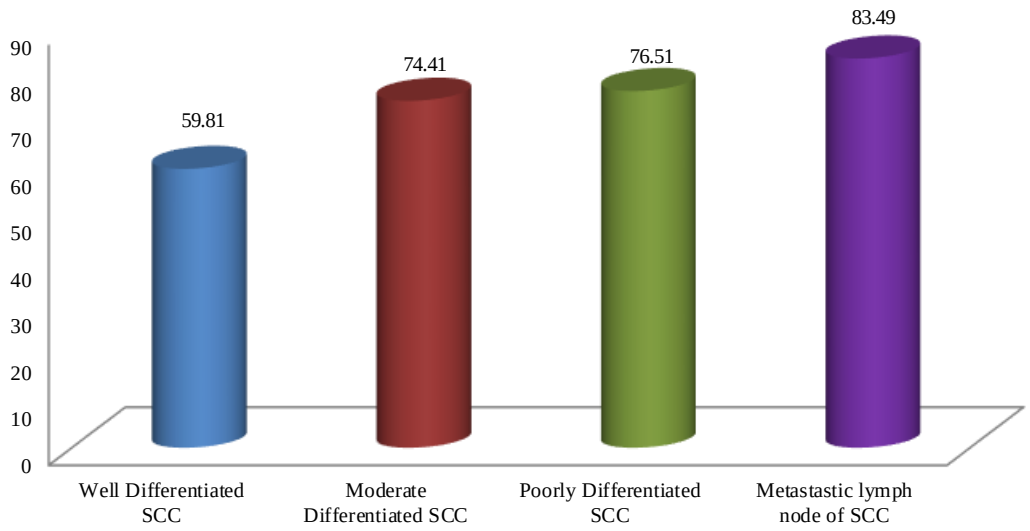


(Figure 1) Column Chart Showing the Mean Area Percent of EMMPRIN Immunoexpression in Different Histological Types of Squamous Cell Carcinomas.

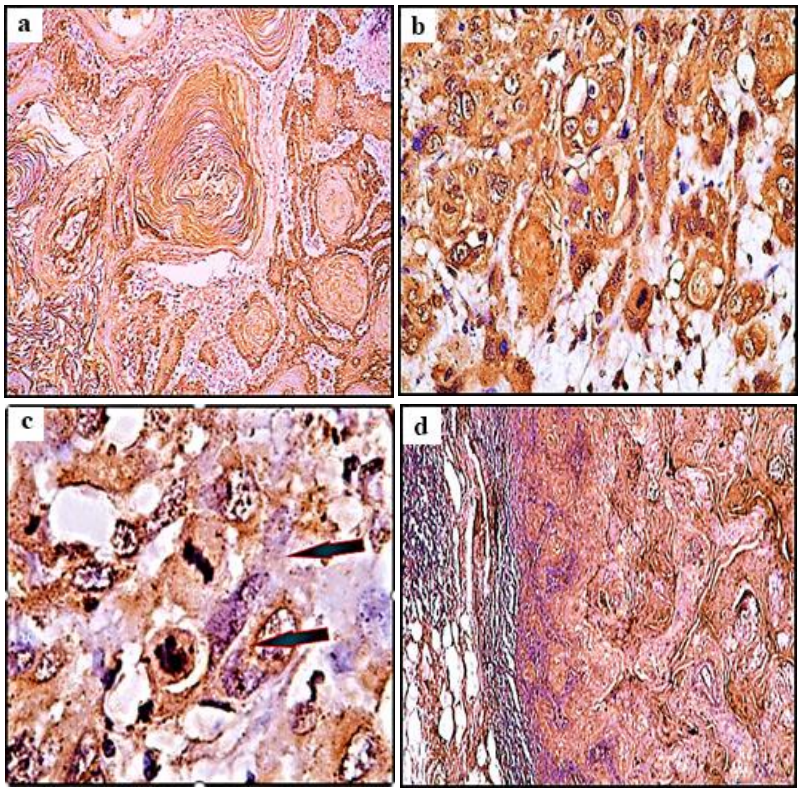
(Table 3) Comparison between Different Histological Types of Squamous Cell Carcinomas according to the Mean Optical Density of EMMPRIN Immunoexpression using ANOVA Test.

	Well differentiated SCC (4 cases)	**Moderate differentiated SCC (5 cases)**	**Poorly differentiated SCC (4 cases)**	**Metastatic lymph node of SCC (2 cases)**
Mean ±SD	59.81[a]±13.59	74.41[a, b]±5.07	76.51[a, b]±6.15	83.49[b]±7.32
F value	4.156			
P value	0.034*			

**statistically significant*
Tukey's post hoc test: means with different superscript letters are significantly different.



(Figure 2) Column Chart Showing the Mean Optical Density of EMMPRIN Immunoexpression in Different Histological Types of Squamous Cell Carcinomas.



(Figure 3)
(a) Well Differentiated Squamous Cell Carcinoma Showing Diffuse Positive Cytoplasmic Immunosignals of EMMPRIN in the Malignant Epithelial Cells Forming the Keratin Pearls and Epithelial Nests. (x100).
(b) Moderate Differentiated Squamous Cell Carcinoma Demonstrating Evident Positive Immunoreaction of EMMPRIN in All the Malignant Cell Nests. (x400).
(c) poorly Differentiated Squamous Cell Carcinoma Revealing Positive Intense Cytoplasmic Immunosignals in the Anaplastic Epithelial Cells. Note the Abnormal Mitotic Figures (Arrows). (x1000)
(d) Metastatic Lymph Node of Well Differentiated Squamous Cell Carcinoma Revealing Strong Positive Diffuse Immunosignals of EMMPRIN within Keratin Pearls (x32).

DISCUSSION

Oral cancer remains a major public health problem with almost 300,000 new cases worldwide[1,15]. New insights in cancer diagnosis and therapy have not changed significantly, during the last decades the survival rate for oral cancer is around 50%[15]. Oral tumorigenesis is a multistep process caused by accumulation of multiple genetic and epigenetic alterations. The comprehension of the molecular pathways involved in this process may originate special biological markers able to differentiate tumors with a more or less aggressive behavior. These markers may contribute to identify and stratify patients with greater precision to the most appropriate treatment plan[16].

The present study included 30 cases oral *squamous cell carcinoma*; the age range of these patients was between (30-76 years) with a mean age 58 years. It has been accepted for a long time that SCCs are associated with old age[17, 18].

In this research, the encountered squamous cell carcinoma cases showed that the alveolar ridge was the most prevalent site of occurrence, followed by the tongue and the buccal mucosa. This may be due to the poor oral hygiene of the presented cases. This is in accordance with *Effiom et al*[19].

An increasing body of evidence suggests that extracellular matrix metalloproteinase inducer (EMMPRIN), a transmembrane glycoprotein present on the surface of tumor cells modulates key steps of the metastatic cascade. Therefore, might play a crucial role in the progression of carcinomas[20,21]. It induces angiogenesis, tumor invasiveness and multidrug resistance depending on stimulation of VEGF and MMPs production overcome "natural" barriers, such as the basement membrane and to spread locally and subsequently also reach lymphatic and blood vessels and metastasize[22]. It also elevates urokinase-type plasminogen activator (uPA) that is important in tumor progression[23].

In the present research, *control sections* included normal mucosa and squamous cell papilloma. All the examined normal mucosa specimens showed only positive cytoplasmic EMMPRIN immunosignals in the basal cell layer. This is consistent with the findings reported by, *Riethdorf et al*[7], *Siu et al*[24] and *Ayva et al*[25], *Vigneswaran et al*[26].

In the present study, EMMPRIN immunoexpression was evaluated using the computer image analyzer. All the examined *squamous cell carcinoma* cases revealed high expression of extracellular matrix metalloprotein-

ase inducer. The expression was observed in cytoplasm and membrane of the malignant epithelial cells. This is in concordance with other studies[7,27,28]. Our examined squamous cell carcinoma cases (moderate and poorly differentiated type) as well as metastatic SCC to lymph nodes showed statistically significant overexpression of EMMPRIN more frequently than the well differentiated type. This is in accordance with *Riethdorf et al.*[7] This indicates that EMMPRIN might play an important role in SCC progression and invasion.

In a recent study, conducted by *Monteiro et al*[29]. In their work on squamous cell carcinomas, they found that a positive association of EMMPRIN expression with histological grade, where moderate and poorly differentiated tumors presented EMMPRIN expression more often than well differentiated ones. They also added that this glycoprotein overexpression occurs at an early step of oral carcinogenesis and contributes to oral tumorigenesis and that this marker may serve as a reliable biological marker to identify high risk subgroups[29]. These findings agree with the findings of the present work. It also suggests that increased EMMPRIN expression could be a negative prognostic factor in SCC.

Comparing different grades of squamous cell carcinomas (SCC) and metastatic lymph node of squamous cell carcinoma according to the area percent of EMMPRIN immunoexpression was done. The greatest mean value was in metastatic lymph node of squamous cell carcinoma and the lowest value was in well differentiated SCC. Comparing different grades of squamous cell carcinomas (SCC) and metastatic lymph node of squamous cell carcinoma according to the optical density of EMMPRIN immunoexpression was done. The greatest mean value was in metastatic lymph node of squamous cell carcinoma and the lowest value was in well differentiated SCC. Moreover, well, moderately and poorly differentiated SCC didn't significantly differ from each other. Well differentiated SCC revealed a significant difference from the metastatic lymph node of squamous cell carcinoma only. This in accordance with *Zucker et al*[21], *Nabeshima et al*[30] and *Huang et al*[13] studies, they reported that EMMPRIN expression is linked to a more aggressive type of cancer. They found that over expression of EMMPRIN is a frequent and important event in head and neck cancer invasion and metastasis. They also added that major function of such glycoprotein is to stimulate the synthesis of the extracellular matrix metalloproteinase family.

CONCLUSIONS

Based on the results of the present study, expression of EMMPRIN in moderate, poorly differentiated and metastatic SCC was higher than well differentiated ones. Therefore, it might play an important role in SCC progression and invasion. Further studies with larger sample size are required to clarify the correlation between EMMPRIN expression and squamous cell carcinomas (SCC).

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