A Comparative Study of Immunohistochemical Expression of Moesin, Cytokeratin 14 andMMP7 in Oral Squamous Cell Carcinoma and Oral Verrucous Carcinoma

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ABSTRACT

Background: Squamous cell carcinoma (SCC) is the most prevalent malignant neoplasm of the oral cavity that exhibits certain histological variations. Verrucous carcinoma (VC) is an uncommon exophytic low-grade well-differentiated variant of SCC. Cellular differentiation and morphology play important roles in cell functions and maintenance of structural integrity .As the cancer is a malignant process in which disorder of the cell growth and behavior occurs, such changes may differ in different tumor types and within different grades of the same tumor.

Materials and Methods:Forty two formalin – fixed, paraffin – embedded tissue blocks were included in this study (30 blocks were diagnosed as OSCC and 12 blocks were diagnosed as OVC). An immunohistochemical staining was performed using anti moesin, anti CK14 and anti MMP7 monoclonal antibodies.

Results: Moesinimmunoreactivity was recognized in all the studied groups with predominant cytoplasmic expression in OSCC & membranous expression in OVC. No difference was noticed between 2 studied groups &between different grades of OSCC. Cytokeratin 14 positivity was noticed in all studied groups with significant difference between OSCC&OVC (p=0.012) & there was a significant difference between the different grade of OSCC (p=0.047). MMP7 expression was observed in the all studied groups with predominant cytoplasmic pattern in OSCC & nuclear pattern in OVC. No difference was found between the 2studied groups &between the different grades of SCC. A Strong positive linear correlation between MMP7&CK14 was noticed.

Conclusion: Verrucous carcinoma has a specific pattern for moesin and MMP7 that differs from OSCC, however; the difference is not significant. CK14 immunoreactivity indicated a significant difference in the degree of cellular differentiation between OSCC &OVC.

Keywords: Oral squamous cell carcinoma,oral verrucous carcinoma, moesin,CK14,MMP7. (J Bagh Coll Dentistry 2016; 28(2):52-57).

INTRODUCTION

The most common oral cancer (OC) of epithelial origin is oral squamous cell carcinoma (OSCC) which is the most frequent malignant neoplasm of the oral cavity, corresponding to almost 95% of all lesions and to about 38% of malignant tumors of the head and neck [(1)]. Oral verrucous carcinoma (OVC) is an uncommon exophytic low-grade well-differentiated variant of squamous cell carcinoma. It is well known for its locally aggressiveness and for its clinically slow-growing behavior with minimal metastatic potential and represent 0.3% to 10% of all oral cavity SCC [(1)].

OSCC is different from OVC in its clinical and histopathological aspects, as well as in its prognosis [(2)]. Several physiologic functions including cell shape, adhesion and motility require a connection between cell membrane proteins and the cortical cytoskeleton [(3)].

A subgroup of superfamily, ezrin-radixin-moesin (ERM), is known to function as a link between the cell membrane and actin cytoskeleton [(4)]. Altered expression of particular ERM proteins is believed to contribute to carcinogenesis and metastasis [(5,6)].

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A well controlled balance of cellular proliferation and differentiation is necessary for the development and maintenance of normal epithelium throughout the body. Keratins are cytoplasmic intermediate filament proteins [(7)], that regulate/modulate different signaling pathways associated with various cellular processes such as protein synthesis, cell growth, and cell differentiation [(8,9)].

Several cellular alterations occur in the cytoskeleton during oncogenic development that can be assessed through the expression of these proteins [(10)]. Degradation of the basement membrane and invasion of the underlying connective tissue by neoplastic cells are recognized as fundamental steps in the progression of many epithelial cancers. MMP-7 (matrilysin) is among the smallest members of the MMP family, has the capacity to start an activation cascade of MMPs and is able to degrade a variety of extracellular matrix (ECM) substrates [(11)].

Tumor growth and progression depends critically on the ability of tumor cells to proliferate, cell motility, receptors mediated cell adhesions, production of proteolytic enzymes, and certain extracellular matrix proteins. Moesin, cytokeratin 14 and MMP7 will be used in this study to show the degree of cellular changes,

assessing tumor behavior and invasiveness; comparing these biological parameters in OSCC and OVC.

This study aimed to compare the immunohistochemical expression of CK14, moesin and MMP7 in oral squamous cell carcinoma and verrucous carcinoma and to correlate their expression with the histopathological grading.

MATERIALS AND METHODS

Forty two formalin – fixed, paraffin – embedded tissue blocks (30 OSCC and 12 OVC) were collected from labrotaries archives and included in this study.After histopathological reassessment of haematoxylin& eosin stained sections for each block.

Four micrometer thick sections were cut and mounted on positively charged slides and stained immunohistochemically with monoclonal antibodies using anti moesin, anti CK14 and anti MMP7 monoclonal antibodies(Abcam UK). Abcam expose mouse and rabbit HRP/DAB immunohistochemical detection kit (Catalog No. ab80436, Cambridge, UK) was used.

RESULTS

The age of the studied samples ranged between 24-99 years old. The mean age was 59+/- 15.3 years in SCC while it was 61.3 +/- 14.4 years in OVC. Male predominance was found with 60% of SCC group and 75% of VC group. No statistically significant differences in age and sex distribution were observed between the 2 studied groups (Table 1).

The most affected site in SCC was the tongue 26.7% (8 cases) while buccal mucosa and alveolar ridge 33.3% (4 cases for each) were the common sites in OVC. Histopathological examination showed that 14 cases (46.7%) were well differentiated SCC, 12 cases (40%) moderately differentiated and 4 cases (13.3%) were poorly differentiated (Table 2).

Moesinimmunoreactivity was recognized in all the studied groups with predominant cytoplasmic expression in SCC and membranous expression in OVC(Table 3and figure1).

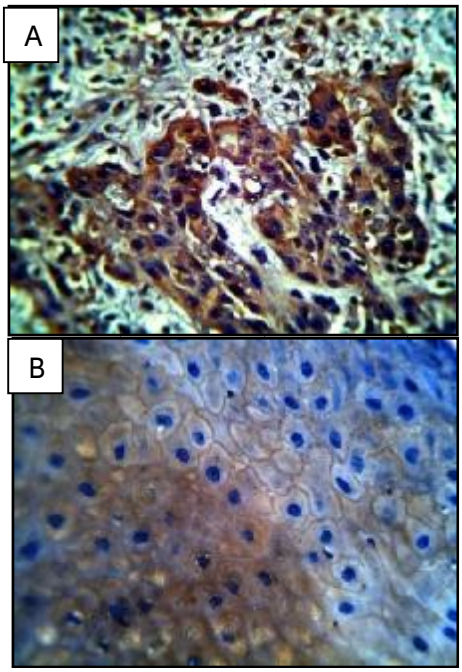

Figure1:Moesinimmunostainig,A: cytoplasmic expression in SCC, B: membranous &cytoplasmic in VC

No difference was noticed between 2 studied groups and between different grades of SCC. Cytokeratin 14 positivity was noticed in all studied groups with significant difference between SCC and OVC p=0.012 and there was a significant difference between the different grade of OSCC (p=0.047) (figure2).

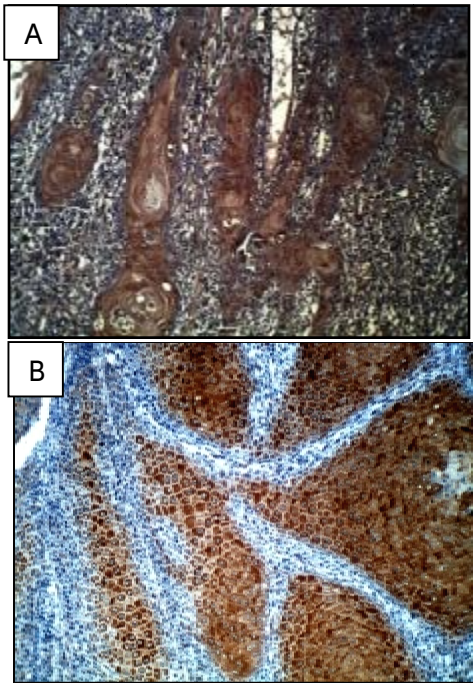

Figure2: CK14 immunostaining, A: cytoplasmic expression in SCC,B: cytoplasmic expression inVC

Collectively, MMP7 expression was observed in the all studied groups with predominant cytoplasmic pattern in SCC and nuclear pattern in OVC(figure3).

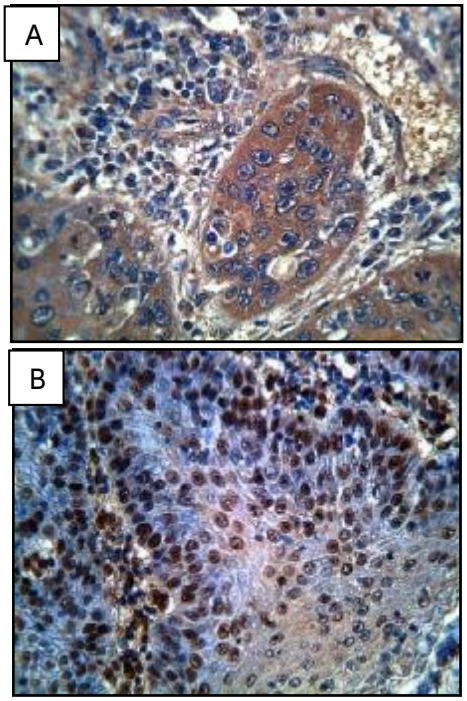

Figure 3:MMP7 immunostaining, A:cytolasmic expression in SCC,B: nuclear expression in VC

No difference was found between the 2studied groups and between the different grades of SCC. A Strong positive linear correlation between MMP7and CK14 was noticed (Tables 4 and 5)

DISCUSSION

Assessment of moesinimmunohistochemical expression:

All the studied cases showed positive expression for moesin, interestingly, moesin cytoplasmic expression was detected in 86.7% of SCC group which is higher than that of VC group (66.7%), while VC showed membranous expression(16.7%) compared to SCC group (3.3%). This would corroborate the finding of [12], however in this studied series the difference in this protein expression failed to reach the level of statistical significance which may be attributed to the small sample size.

There are several explanations for the shift in localization: Firstly, conformational and functional changes of moesin results in redistribution of this molecule in tumor cells [13]. Secondly, CD44(a cell surface receptor) has been cleaved by MMP-1 in carcinoma cells at a membrane proximal domain, thereby suggesting that functional moesin migrates with CD44 degraded from the cell surface to the cytoplasm [14]. Thirdly, according to carcinogenesis, it is possible that increased membranous degredation in more aggressive neoplasms and mutation of moesin gene cannot cross-link between plasma membrane and actin filament [15].

The last reason may explain the present work results regarding the grades in which proportional decrease in the moesin score with increasing histopathologic grade was observed, however no statistical significance in median moesin score was noticed between the different grades of SCC.

Assessment of cytokeratin 14 immunohistochemical expression:

The results showed that SCC cases showed CK14 immunostaining in all the neoplastic cells, irrespective of the grade. These findings are in contrast to those ofSu et al.[16]. WhileChu et al. [17]found that over 90% of cases of SCC of various origins were CK14 positive. In previous studies performed by Morgan and Lane[18], CK 14 expression was detected regardless of the differentiation compartment. In the current study, all oral epithelial layers in VC exhibited strong immunostaining for CK 14 and that in accordance withOliveira et al.[10].

The difference was significant regarding the CK 14 profile between OSCC and OVC, p=0.012,which emphasized the biological behavior of the studied lesions, especially pattern of OVC as the CKs profile was similar to the CKs profile in well differentiated oral squamous cell carcinoma. On the other hand there was significance among the different grades of OSCC(p=0.047). Moreover, it confirms the use of this protein as a marker of cellular differentiation.

Assessment of MMP7 immunohistochemical expression:

In the present work, all the studied cases showed positive reaction to MMP7. In VC, nuclear immunostaining was the main staining pattern of the cells, which also reported in other studies [19]. The explanation of nuclear expression is based on findings of a recent study carried out by [20] which focused on epithelial cell adhesion molecule (EpCAM, a single-transmembrane protein), MMP7 is a target of EpCAM, which is induced at the transcriptional level upon nuclear translocation of EpICD.

Expression patterns and levels of EpCAM and MMP7 correlated closely in vivo in tumors suggesting that there is biological relevance of this association during malignancy [20].While the absence of MMP-7 from epithelial cells of OVC was found in a study conducted byImpola et al.[21], who consideredthat matrilysin expression

correlates with the aggressive phenotype of many cancers. While the cytoplasmic stain was the predominant one in the SCC; this is accordance withWeber et al.[22], who found that cytoplasmic MMP-7 is mainly produced by tumor cells themselves and is associated with short survival times in head and neck cancer[23], also demonstrated that MMP-7cytoplasmic expression is significantly correlated with lymph node metastasis in oral cavity cancers. As OSCC &OVC are carcinoma,both of them have the ability to induce angiogenesis and local invasion of the basement membrane so,both of them expressed MMP7 positive immunoreactions, no difference was observed between both tumors and among the 3 grades of SCC concerning the degree of expression.

As conclusion; in this project moesin and CK14 are present normally in squamous cells. They changed their cellular localization and expression pattern in neoplastic epithelia. As VC is a very well differentiated variant of SCC, it has specific pattern for moesin, CK14 and MMP7 that differed from SCC, however, the current work not reach the level of significance in moesin and MMP7. CK14 immunoreactivity indicated a significant difference in the degree of cellular differentiation between OSCC &OVC.

Table 1: The difference in age and sex between (SCC) group and VC group

Variables	SCC		Verrucous		P
	N	%	N	%	
Age group (years)					**0.67 [NS]**
<50	9	30.0	2	16.7	
50-69	13	43.3	6	50.0	
70+	8	26.7	4	33.3	
Total	30	100.0	12	100.0	
Range	(24-99)		(32-83)		
Mean	59		61.3		
SD	15.3		14.4		
Sex					**0.36 [NS]**
Female	12	40.0	3	25.0	
Male	18	60.0	9	75.0	
Total	30	100.0	12	100.0	

Table 2: Frequency distribution of SCC group by grading

Grading for SCC	N	%
Well differentiated	14	46.7
Moderately differentiated	12	40.0
Poorly differentiated	4	13.3
Total	30	100

Table 3: The difference in type of moesin tissue stain expression between SCC and VC groups

Moesin tissue stain expression	Verrucous Ca (Vs Squamous cell Ca)				P
	SCC		Verrucous		
	N	%	N	%	
Mainly cytoplasmic	26	86.7	8	66.7	0.24 NS
Mainly membranous	1	3.3	2	16.7	
Mainly mixed	3	10.0	2	16.7	
Total	30	100.0	12	100.0	

Table 4: The difference in median score of the 3 markers staining between SCC and VC groups.

	Verrucous Ca (Vs Squamous cell Ca)				
	SCC		Verrucous		
	N	%	N	%	P
Moesin score					**0.18[NS]**
Score-I	0	0.0	0	0.0	
Score-II	9	30.0	7	58.3	
Score-III	15	50.0	3	25.0	
Score-IV	6	20.0	2	16.7	

Total	30	100.0	12	100.0	
Median	Score-III		Score-II		
Mean rank	23		17.8		
MMP7-score					**0.09[NS]**
Score-I	1	3.3	0	0.0	
Score-II	15	50.0	3	25.0	
Score-III	14	46.7	9	75.0	
Score-IV	0	0.0	0	0.0	
Total	30	100.0	12	100.0	
Median	Score-II		Score-III		
Mean rank	19.8		25.9		
Cytokeratin score					**0.012**
Score-I	1	3.3	0	0.0	
Score-II	17	56.7	2	16.7	
Score-III	12	40.0	10	83.3	
Score-IV	0	0.0	0	0.0	
Total	30	100.0	12	100.0	
Median	Score-II		Score-III		
Mean rank	18.9		28.1		

Table 5: The difference in average score of 3 types of markers staining between the three grades of SCC

	Grading						
	N	**%**	**N**	**%**	**N**	**%**	**P**
Moesin score							**0.12[NS]**
Score-I	0	0.0	0	0.0	0	0.0	
Score-II	3	21.4	3	25.0	3	75.0	
Score-III	7	50.0	7	58.3	1	25.0	
Score-IV	4	28.6	2	16.7	0	0.0	
Total	14	100.0	12	100.0	4	100.0	
Median	Score-III		Score-III		Score-II		
Mean rank	17.4		15.8		8		
r=-0.314 P=0.09[NS]							
MMP7-score							**0.18[NS]**
Score-I	0	0.0	0	0.0	1	25.0	
Score-II	10	71.4	4	33.3	1	25.0	
Score-III	4	28.6	8	66.7	2	50.0	
Score-IV	0	0.0	0	0.0	0	0.0	
Total	14	100.0	12	100.0	4	100.0	
Median	Score-II		Score-III		Score-III		
Mean rank	13.1		18.7		14.3		
r=0.216 P=0.25[NS]							
Cytokeratin score							**0.047**
Score-I	0	0.0	0	0.0	1	25.0	
Score-II	4	28.5	8	66.7	2	50.0	
Score-III	10	71.5	4	33.3	1	25.0	
Score-IV	0	0.0	0	0.0	0	0.0	
Total	14	100.0	12	100.0	4	100.0	
Median	Score-III		Score-II		Score-II		
Mean rank	19.7		13.1		11.4		
r=0.17 P=0.37[NS]							

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