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EXPRESSION OF OSTEOPONTIN IN ORAL SQUAMOUS CELL CARCINOMA – AN IMMUNOHISTOCHEMICAL STUDY

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ABSTRACT

Objectives: To analyze the expression of Osteopontin (OPN) in well differentiated, moderately differentiated, and poorly differentiated oral squamous cell carcinoma (OSCC).

Methodology: This study was conducted at the Department of Pathology, Peshawar Medical College, over a period of 12 months. A total of 50 formalin-fixed paraffin-embedded (FFPE) blocks (n=50) of confirmed cases with varying grades of OSCC were included in the study. Immunostaining was evaluated for intensity and percentage of positive cells and Immunoreactivity of OPN was assessed by the Immunoreactivity scoring system (IRS) method.

Results: The age of the patients was 58.44 ± 13.01 years ranging from 30 to 82 years on average. Maximum number of cases belonged to the age group 51 to 70 years having 27 (54%) participants. The most common category of OPN expression among well differentiated OSCC was moderate (n=16, 66.7%). For poorly differentiated OSCC the strong staining was found in 6 cases (50%) and was the most common category of OPN expression. In males the pattern of staining for OPN expression was not statistically significant among various grades of OSCC (P=0.455) while among females the results were highly significant (P=0.003). The mean IHC staining score by IRS method for OPN expression was highest among well differentiated (9.20 ± 4.31)

Conclusion: OPN can be used as a diagnostic marker for OSCC. It can also help in assessing the potential aggressive behavior of the tumor pointing at close follow up of such patients for recurrence.

Keywords: Oral squamous cell carcinoma; Osteopontin; Immunohistochemistry

INTRODUCTION

Globally the sixth most frequently occurring tumor is cancer of the head and neck accounting for 2.5% of all new cancer cases, with an annual mortality rate of 1.9%.¹ In the global ranking of oral cancer (OC) Pakistan ranks at number 10.¹ A Canadian study revealed that the 5-year survival rate for OC has stayed low, approximately 50%, for the past decades. According to annual registry report 2016 by Shaukat Khanum Memorial Cancer Hospital and Research Center, lip and oral cavity cancers rank 8th in malignancies among all age-groups and genders in Pakistan.² Lip and oral cavity tumor accounts for 8.6% of the entire new cases reported and 7.2% of all cancer deaths in Pakistan.^{3,4}

OSCC, is the commonest (90%) amongst oral neoplasms.⁵ The rising frequency of OSCC coupled with poor prognosis has urged researchers to discover novel biomarkers that can help to identify patients with poor prognosis and recurrence.⁶ Osteopontin (OPN) is a multifunctional phosphorylated glycoprotein which is secreted by a number of immune cells (macrophages,

leukocytes, and T lymphocytes). A variety of cancers show an over-expression of OPN including breast, lung and liver carcinoma.⁷ The function of OPN in malignant growth has been a significant focal point of exploration for the last decade.⁸

OPN may cause oral cancer via activating the PI3K/AKT/mTOR pathway, according to some investigations.⁹ A study in 2009 conducted on OSCC suggested OPN to be an important prognostic factor due to demonstration of high expression level of OPN in either the plasma or the tumor of the patients.¹⁰ Another study in 2019 demonstrated the expression of OPN in OSCC and correlated the expression of OPN with the histologic grades of OSCC. This study examined Osteopontin expression to evaluate oral squamous cell carcinoma (OSCC) in different grades (OPN).

METHODOLOGY

This comparative cross-sectional study was conducted in the Pathology department, Peshawar Medical College in a span of 12 months. The study consisted of 50 FFPE blocks of already diagnosed cases of various

grades of OSCC obtained from PIMS, Islamabad. The patient's age and gender along with clinical information like the site, size, and duration of the lesion were entered in a predesigned proforma from the biopsy reports. Immunohistochemistry was carried out using Primary Antibody (Rabbit Monoclonal Osteopontin Antibody, ab91655 -EPR3688, Abcam, UK, 1:250) and Secondary Antibody (ab80436, Abcam, UK).¹² Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) version 22.0. Mean and standard deviation was computed for continuous variables like age. Frequencies and percentages were calculated for all categorical variables like site of the lesion, level of OPN expression in normal mucosa and various histological grades of OSCC. Categorization of OPN expression by immunoreactive scoring (IRS) method was divided into strong, moderate, mild and negative

staining as described by Grizzle et al.¹³

One-way ANOVA was applied to assess immunoreactive score (IRS) of OPN in various grades of OSCC. Chi-square test was employed to compare strong, moderate, mild and negative staining score between various grades of OSCC. The analysis was stratified among gender, age groups and site of lesion to find effect modifiers. Post stratification Chi-square test was applied. P value ≤ 0.05 was considered as a statistically significant.

RESULTS

The study included 50 cases of OSCC. The mean age of the patients was 58.44 ± 13.01 years ranging from 30 to 82 years. Majority of the cases were of the age group 51 to 70 years having 27 (54%) participants followed by 30-50 years with 13 (26%)

cases and above 71 years with 10 (20%) patients. Male to female ratio was 1:1. The most prevalent site of OSCC was tongue (n=24, 48%) followed by buccal mucosa (n=16, 32%). Few participants had lesion at alveolar ridge (n=3, 6%). Involvement of floor of mouth and nasopharyngeal area was found in 2(4%) cases each. The occurrence of OSCC at lip was 1(2%) (n=1, 2%) case. In our study the most common grade of OSCC was 'well differentiated' (n=24, 28%) followed (by)'moderately differentiated' (n=14, 25%) and then 'poorly differentiated' (n=12, 24%).

The most common category of OPN expression among well differentiated OSCC was moderate (n=16,66.7%) followed by mild (n=4, 16.7%) and then strong (n=3, 12.5%). Among moderately differentiated OSCC the commonest category of OPN ex-

Table 1: Comparison of OPN expression in various grades of OSCC by IRS scoring method

OPN expression	Well-differentiated N (%)	Moderately differentiated N (%)	Poorly differentiated N (%)	p-Value
Strong	3 (12.5)	1 (7.1)	6 (50.0)	0.002
Moderate	16 (66.7)	5 (35.7)	2 (16.7)	
Mild	4 (16.7)	8 (57.1)	2 (16.7)	
Negative	1 (4.2)	Nil	2 (16.7)	

Table 2: OPN expression in various grades of OSCC stratified by gender

Variable		Well differentiated		Moderately differentiated		Poorly differentiated		p-Value*
		n	%	n	%	n	%	
Male	Negative	3	27.3	1	16.7	5	62.5	.455
	Strong staining	5	45.5	3	50.0	1	12.5	
	Moderate staining	2	18.2	2	33.3	1	12.5	
	Mild staining	1	9.1	0	0.0	1	12.5	
Female	Negative	0	0.0	0	0.0	1	25.0	0.003
	Strong staining	11	84.6	2	25.0	1	25.0	
	Moderate staining	2	15.4	6	75.0	1	25.0	
	Mild staining	0	0.0	0	0.0	1	25.0	

Table 3: Comparison of OPN score by IRS method among various grades of OSCC

OSCC Grade	OPN Score Mean ± SD	95% CI	p-value
Well-differentiated	9.20±4.31	7.38, 11.02	0.001
Moderately differentiated	7.42±3.435	5.44, 9.41	
Poorly differentiated	3.16±4.529	.28, 6.04	

pression was mild (n=8, 57.1%) followed by moderate (n=5, 35.75). For poorly differentiated OSCC the strong staining was found in 6 cases (50%) and was the most common category of OPN expression (Table 1).

In males, the pattern of staining for OPN expression was not statistically significant among various grades of OSCC (P=0.455) while among females the results were highly significant (P=0.003) (Table 2).

Among females for well-differentiated OSCC the common staining for OPN expression was strong (n=11, 84.6%) followed by moderate staining (n=2, 15.4%). For moderately differentiated OSCC among females the common staining for OPN expression was moderate (n=6, 75%). In all age groups the expression of OPN among various grades of OSCC was statistically different (P≤0.05).

The mean IHC staining score by IRS method for OPN expression was highest among well-differentiated (9.20±4.31) followed by moderately (7.42±3.435) and least in the poorly differentiated (3.16±4.529) (Table 03). These results were statistically significant (P=0.001).

■ DISCUSSION

In adults the most common occurring oral cancer is squamous cell carcinoma.¹⁴ The mean age of patients in our study was 58.44 ± 13.01 years with a range (30-82) years. In comparison to other studies there was a significant variation, that might be due to difference in the study design. A study conducted in Lahore Pakistan in 2016 on delayed diagnosis of OSCC on 246 patients reported a mean age of 46.7 ± 10.2 years ranging from 27-60 years. Interestingly the same study observed a male predominance of 3.7:1 in contrast to our 1:1.¹⁵ However most of the research conducted in Pakistan reveals that males are affected more by OSCC than females.¹⁶⁻¹⁸ Nevertheless one

study on 202 cases of OSCC conducted in Lahore, Pakistan reported female predominance.¹⁹ These variations can be explained by sample size, geographical locations along with use of snuff (naswar), pan chewing and betel nuts, etc., among both genders in Pakistan.

The current study results revealed that the most affected sites were tongue and buccal mucosa as previously reported by studies conducted in Lahore.^{20,21} This might be because tongue and buccal mucosa are exposed to carcinogens released from tar in cigarettes and naswar.

Our study showed that there was statistically significant association between OPN expression and histological grade of OSCC in females (table 2). Our findings are supported by a previous research findings that OPN expression was differently expressed among males and females.¹¹ However, the study by Avirović et al. concluded that expression of OPN among genders was statistically insignificant (P=0.19).²³

Our findings showed that in all age groups, the OPN expression was different in various grades of OSCC statistically (P=0.020). This shows genetic association of OSCC and OPN expression irrespective of age. Previous studies also found no association between age and expression of OPN conducted in India by Aravind found the expression of OPN to be decreasing with increasing grades of OSCC.²⁵ While Matsuzaki in Japan and Jeyasivanesan et al, Routray et al. in India noted that the intensity of OPN expression increases with loss of differentiation in tumor cells.^{8,11,24} Our results for OPN expression showed no harmony among various grades of OSCC.

A statistical association of histological grades and OPN expression has been previously reported by one of the studies.²⁵ However in other studies conducted on OPN

expression, statistically there was no difference between well-, moderate-, poorly-differentiated carcinomas.^{11,24,26} In our study a significant association was observed among histological grades of OSCC and OPN expression making our results similar to the study conducted by Aravind et al while in contrast to the study conducted by Jeyasivanesan et al., Devoll et al., and Matsuzaki et al.^{11,26,24} The variations in results could be due to sample size, OPN scoring methods along with genetic and ethnic factors.

■ CONCLUSION

Our study concluded that the histological grades of OSCC showed a significant relationship with OPN expression. Thus, high OPN expression may reflect the potential aggressive behavior of the tumor which points at close follow up of such patients for recurrence and subsequent management. Also there is a need for standardized IHC scoring system for OPN expression to curtail intra- and interobserver variability and create harmony between studies carried out in different parts of the world. A study with a large sample size including follow up may further elucidate our findings

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Author's Contribution

RA, MMK, and SS helped in the data collection and write-up of the manuscript. SN and AK contributed in data analysis and write up for the manuscript. SA conceived the idea and gave the final approval. Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Conflict of Interest

Authors declared no conflict of interest

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None

Data Sharing Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.