ORIGINAL RESEARCH

Comparative Study of Immunohistochemical Expression of Moesin and FLOT 1 in OSCC and Their Correlation with Histopathological Prognostic Factors

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Abstract

Aim: To study immunohistochemical (IHC) expression patterns of Moesin and FLOT 1 in oral squamous cell carcinoma (OSCC) and to correlate it with histopathological prognostic factors.

Materials and methods: A cross-sectional study design was conducted on histopathologically diagnosed cases of OSCC. The inclusion criteria were carcinoma of buccal mucosa, tongue, alveolar mucosa, palate, gingiva, the floor of the mouth, retromolar area, and soft palate. The exclusion criteria included cases of squamous cell carcinoma from sites other than the oral cavity, potentially malignant disorders (PMDs), and any pseudomalignancies of the head and neck. Tissue sections were subjected to IHC staining for Moesin and FLOT 1 and the results were subjected to statistical analysis.

Results: Moesin showed strong positivity and was significantly associated with the histopathological variables such as lymph nodes and the worst pattern of invasion, whereas FLOT 1 was not associated with any clinical, histopathological, or demographical variable in this study.

Conclusion: Cytoplasmic detection of Moesin (35.19%) was higher than FLOT 1 (15.74%). There was no statistically significant relationship between the grade of the lesion and Moesin and FLOT 1.

Clinical significance: New emerging prognostic biomarkers can aid to assess the rate of malignant transformation (epigenetic and molecular changes), thereby resulting in early prophylactic conciliation of the disease progression in OSCC. There is an urgent need for introducing these as an interventional therapy for effectively addressing OSCC at an early stage, thus preventing it from further proceeding to the advanced severe stage.

Keywords: Biomarkers, FLOT 1, Moesin, Oral squamous cell carcinoma, Prognostic.

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Introduction

One of the most challenging diseases of the oral cavity that delineates high morbidity and high mortality rates in the Indian population is Oral Squamous Cell Carcinoma (OSCC).[1] Being the 6th most common malignancy affecting the head and neck region, it is now the most studied and researched malignancy due to its complexity and biological heterogeneity of the tumor behavior.[2]

OSCC includes a group of epithelial neoplasms of the squamous cells of the oral cavity, pharyngeal regions, and salivary glands. It constitutes about 4.7–20.3% of all head and neck malignancies, 40% involving the lateral border of the tongue, followed by 30% in the floor of the mouth and 20% in the lower lip.[3–6] Recent analysis of SEER in 2019 (Surveillance, Epidemiology and End result) database has inferred that OSCC of the tongue has the highest mortality rate when compared to other sites.[7] Both exogenous and endogenous traditional risk factors and predisposing factors such as chronic use of Tobacco (Smoke/Smokeless forms), Alcohol (synergistic effect), Human *Papilloma Virus* (HPV), *Epstein–Barr Virus* (EBV), Hepatitis C, Nutritional deficiencies like Vitamin A, Vitamin C, *Candida* infections, *Genetic* mutations, Immune deficiencies play a major role in the etiopathogenesis of OSCC, thereby making it multifactorial in origin.[8–19] The overall 5-year survival rate for OSCC is as low as 40–50%. The high rate of recurrence, poor prognosis, aggressive nature, metastasis to locoregional lymph nodes, the concept of Field cancerization proposed by Slaughter et al.[5] in 1953, the emergence of secondary primary tumors (SPTs) and high failure rates of surgical treatment have strongly recommended the urge to improve the diagnostic capabilities of this malignancy.[20]

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Over the yester years, a lot of interest has also been evoked in studying the molecular changes and biomarkers in OSCC. Biomarkers, being the products of malignant cells, can be used to assess the rate of malignant transformation, thereby aiding in early prophylactic conciliation of the disease progression. The epigenetic and molecular changes that can occur within a cell during the carcinogenetic process can be detected by an in-detail study of cell moiety using new emerging biomarkers. Hence, there is a need for introducing new emerging prognostic biomarkers as an interventional therapy for effectively addressing OSCC at an early stage, thus preventing it to further proceed to the advanced severe stage.[21]

MOESIN, a 577amino acid polypeptide, belongs to a group of Ezrin/Radixin/MOESIN (ERM) proteins whereas FLOT 1 is a ubiquitous protein associated with specific caveolin-independent cholesterol- and glycosphingolipid-enriched membrane microdomains. Both play a major role in various signaling pathways, such as EGFR signaling, cadherin signaling, cell adhesion, membrane trafficking, and anon regeneration. Recent findings have revealed that the expression of both these biomarkers was upregulated in various types of human cancers (breast cancers, prostate cancers, pancreatic cancers, lung cancers, melanoma, and OSCC) and are associated with poor patient survival prognosis and high risk of metastasis formation.[21,22] Based on this platitude, the present study was conducted with the aim to compare and correlate the expression patterns of Moesin and FLOT 1 with histopathological prognostic factors of OSCC. The primary objectives were to study the immunohistochemical expression pattern of Moesin and FLOT 1 in OSCC and to correlate it with histopathological prognostic factors. The secondary objectives was to compare the expression patterns of Moesin and FLOT 1 in OSCC and to correlate it with histopathological prognostic factors.

Materials and Methods

This is a cross-sectional study design, bearing the protocol number 0578/2018-2019, was conducted, during the period of December 2019 – July 2021, on a total of 115 biopsy tissue/surgical excision/resection tissue of OSCC patients admitted to the Department of Oral Pathology and Microbiology, SDS, KIMSDU, Karad, Maharashtra, India and Department of Pathology, Krishna Institute of Medical Sciences, Karad, Maharashtra, India. Cases of histopathologically diagnosed OSCC of buccal mucosa, tongue, alveolar mucosa, palate, gingiva, floor of the mouth, retromolar area, and soft palate were included in the study. All cases of SCC from sites other than the oral cavity, potentially malignant disorders (PMD's) and any pseudo malignancies of head and neck region (ameloblastoma, angiolymphoid hyperplasia with eosinophilia, calcifying epithelial odontogenic tumor, inflammatory myofibroblastic tumor, juxtaoral organ of Chievitz, necrotizing sialometaplasia, nodular fasciitis, pseudoepitheliomatous hyperplasia, radiotherapy, respiratory epithelial adenomatoid hamartoma, spindle cell/pleomorphic lipoma, squamous odontogenic tumor) were excluded from the study.

A detailed anamnesis, information on the anatomic site, nature of the specimen, a surgical procedure performed, grossing details, and histopathology report were collected from the biopsy requisition form of the Department of Oral Pathology and Microbiology, SDS, KIMSDU, Karad, Maharashtra, India and Department of Pathology, Krishna Institute of Medical Sciences, Karad, Maharashtra, India. Stained H and E slides of histopathologically graded (well differentiated, moderately differentiated and poorly differentiated) as mentioned in the biopsy requisition from histopathology report for OSCC of buccal mucosa, tongue, alveolar mucosa, palate, and gingiva, the floor of mouth, retromolar area and soft palate were collected. Archival blocks of the same cases and tissue sections (2-micron thickness) were prepared and mounted on slides (Thermo Fisher Superfrost microscopic slides) and then subjected to IHC staining (K802321-2 EnVisiona, FLEX Mini Kit) for Moesin (Anti Moesin antibody ab52490 100 UL) and FLOT1 (Anti – Flotillins antibody ab41927 100 UL) applying the principle of IHC by two step Polymerase Chain Indirect Technique. The appropriate double-blinding method was followed wherein the coding for all slides (H and E stained; IHC stained) as well as the requisition forms, was done and comparison of both immunohistochemical markers with the traditional H and E staining for different above-mentioned grades was randomly sequenced for observation to avoid any selection or performance bias. The coded slides were analyzed by two intraobservers for reporting the staining intensity for different grades of OSCC, i.e., well-differentiated OSCC, moderately differentiated OSCC, and poorly differentiated OSCC. Additionally, the intensity variations of staining within the malignant cell were noted (cytoplasmic, mixed, or membranous type). This variation in type was correlated with the different grades of OSCC.

The Allred scoring system was done for positively stained cells as none (0 cells/HPF) as score 0; weak (> 1 cells/HPF) as score 1; intermediate (1–10 cell/HPF) as score 2; strong (11–33 cells/HPF) as score 3; (34–66 cells/HPF) as score 4 and ≥ 67 cells/HPF as score 5 with

FINAL SCORE RANGE (A + B) : 0–8

The data was collected in Microsoft Excel and subjected to statistical analysis.

Results

Amongst the total samples, males (78.70%) outnumbered females (21.02%) in the study. The age ranged from 13 to 86 years with mean age of 53 ± 13.71 years. The nature of the specimen sample collected in most cases (57.41%) was hemimandibulectomy. The most commonly occurring grade of OSCC cases were moderately differentiated (47.22%), with free lymph nodes (76.85%), the worst pattern of invasion was type III (34.8%), and free margin (88.89%). Barely 3.70% showed angiolymphatic invasion but this result can be uncertain due to almost 93.5% missing data. Cytoplasmic detection of Moesin (35.19%) was higher than FLOT 1 (15.74%) respectively (Table 1). There was no statistically significant relationship ($p < 0.10$) between the grade of the lesion and Moesin ($p < 0.10$) and FLOT 1 ($p < 0.75$) respectively (Tables 2 and 3). Although *grade and gender* were the only variables of significance in this sample. Furthermore, Moesin was significantly associated with the variables: Lymph nodes and worst pattern of invasion (Table 4) FLOT 1 was not associated with any clinical, histopathological, or demographical variable in this sample (Table 5, Fig. 1). Immunostaining for Moesin and FLOT 1 are illustrated in Figures 1 to 3.

Inferences of the Present Study

- Moesin was significantly associated with the variables: Lymph nodes and the worst pattern of invasion.
- Cytoplasmic detection of Moesin was higher than FLOT 1 among the OSCC cases.
- There was no statistically significant relationship between the histopathological grade of the lesion and Moesin and FLOT 1 respectively.

Discussion

The present study revealed a statistically non-significant cytoplasmic expression of Moesin to be higher among moderately differentiated ($n = 24$), and low in well-differentiated ($n = 29$) cases. This can be attributed to the datum that shows a higher number of moderately (47.22%) and well-differentiated cases (36.11%) in our sample. This finding is consistent with a study[23] where Moesin expression was statistically significant among well and moderately

Table 1: Association between the histopathological pattern of the grade of the lesion and the clinicopathological characteristics of patients with oral squamous cell carcinoma

	Moesin			
Grade	*High*	*Intermediate*	*Low*	*p-value (Chi-square test)*
Dental follicle	0	0	6	NS (Kruskal-wallis Rank Test)
Moderately differentiated	24	9	18	
Poorly differentiated	9	2	1	
Well differentiated	5	5	29	
	Gender			$p < 0.04$ (Chi-square test)
	Males	*Females*		
Dental follicle	3	3		
Moderately differentiated	9	42		
Poorly differentiated	5	7		
Well differentiated	5	34		
	Flot 1			
Dental follicle	0	0	6	NS (Kruskal-wallis rank test)
Moderately differentiated	8	13	30	
Poorly differentiated	3	4	5	
Well differentiated	6	9	24	
	Lymph node			
	Missing data	*Free*	*Involved*	*Not involved*
Dental follicle	6	0	0	0	NS (Chi-square test)
Moderately differentiated	0	43	7	1	
Poorly differentiated	0	6	6	0	
Well differentiated	0	34	5	0	
	Angiolymphatic invasion			
	Absent	*Free*	*Involved*	*Present*	
Dental follicle	6	0	0	0	NS (Chi-square test)
Moderately differentiated	47	0	2	2	
Poorly differentiated	10	0	0	2	
Well differentiated	38	1	0	0	
	Worst pattern of invasion							
	Missing Data	*Type I*	*Type II*	*Type III*	*Type IV*	*Type V*	*Type VI*	*Type VII*	
Dental follicle	6	0	0	0	0	0	0	0	NS (Chi-square test)
Moderately differentiated	0	4	9	13	19	4	1	1	
Poorly differentiated	0	2	3	4	3	0	0	0	
Well differentiated	0	3	14	17	3	1	1	0	

(p^* < 0.05 by Chi-square test; p^* < 0.05 by Kruskal-Wallis rank test). NS, non-significant

differentiated grades of OSCC cases. On the other hand, FLOT 1 showed higher expression among moderately differentiated (30) and well-differentiated (24) cases (NS). But overall, our sample although statistically non-significant, had a higher cytoplasmic expression of Moesin as compared to FLOT 1.

The sample in our study was dominated by the male gender like all other similar OSCC cases where the male population in India and worldwide finds a higher consumption of alcohol, tobacco, and areca nut[9,24–32] The average age in our sample (53 ± 13 years) was unswerving with other studies as oral cancer is known to occur from the fifth and sixth decade of life.[4,31] Both males and females in our sample were in their fifth decade of life and females had a statistically significant higher propensity for being moderately ($n = 42$ cases) and well-differentiated ($n = 34$ cases) as compared to the males. This finding can be attributed to the circumstance that women in India have a habit of betel nut chewing and application of misri (burnt tobacco powder) also termed as smokeless tobacco (SLT) as a part of their oral hygiene routine.[24] Almost 73% percent of women in India are known to indulge in the habit of consumption of smokeless tobacco.[24] The nature of our specimen had a greater tendency for being: Moderately differentiated; type III worst pattern of invasion; free lymph nodes (76.85%); free margins (88.89%); and 57% who had undergone hemimandibulectomy and were in their fifth decade of life.

The most distinguishing feature of cancer is its ability to invade the surrounding tissues starting with the epithelia in OSCC and proceeding to the connective tissue. The degree of invasion affects the prognosis as any depth of invasion to the tune of 2–4 mm spells an increased rate of lymph node metastasis. Therefore, in most head and neck cancers, elective neck dissection is suggested even if there is no clinical spread to the lymph nodes.[33] In our sample, even though there was a non-significant association

Table 2: Association between histopathological grade of the OSCC lesion and expression of Moesin among OSCC cases

	Moesin			
Grade	*High*	*Intermediate*	*Low*	
Dental follicle	0	0	6	Kruskal–Wallis rank test *p*-value = 0.10 (NS)
Moderately differentiated	24	9	6	
Poorly differentiated	9	2	18	
Well differentiated	5	5	29	

NS, non-significant

Table 3: Association between histopathological grade of the OSCC lesion and expression of FLOT 1 among OSCC cases

	FLOT 1			
Grade	*High*	*Intermediate*	*Low*	
Dental follicle	0	0	6	Kruskal-Wallis rank test *p*-value = 0.75 (NS)
Moderately differentiated	8	13	30	
Poorly differentiated	3	4	5	
Well differentiated	6	9	24	

NS, non-significant

between the grade of the lesion and all other variables except gender (<0.04). The worst pattern of invasion, free lymph nodes, and absence of angiolymphatic invasion were highest among moderately differentiated. Most of the samples were obtained from hemimandibulectomy cases (57.41%).

In our study, Moesin expression configuration was statistically associated with lymph nodes, the worst pattern of invasion, and not with margins, grade, and nature of invasion or gender. This finding is partially similar to a study on Moesin where they found Moesin correlated to cervical lymph nodes metastasis, mode of invasion, tumor size, and lymphocytic infiltration.[34] The association between lymph node metastasis and Moesin expression is a sign of tumor aggressiveness and poor prognosis in OSCC cases. FLOT 1 did not show any association with any of the variables of our study.

In highly invasive OSCC cases, Moesin is known to translocate from the plasma membrane to the cytoplasm and diminishes the capability of cell-to-cell adhesions and interfere with the remodeling of the cytoskeleton and enhance the tumor invasion process, especially in the higher stages of cancer.[35] This outcome is commensurate with our study where cytoplasmic expression of Moesin was predominant in the moderately differentiated grade of carcinoma. This result was similar to other studies but differed from which did not find any association with demographic, clinical, or microscopic variables of the study.[36,37] A systematic review of FLOT 1 states that flotillins

Table 4: Association between the expression patterns of Moesin and clinicopathological characteristics of patients with oral squamous cell carcinoma

	Lymph nodes				
Moesin	*Missing data*	*Free*	*Involved*	*Not involved*	*p-value (Chi-square test)*
High	0	29	9	0	*p*-value = 0.030*
Intermediate	0	12	3	1	
Low	6	42	6	0	

	Gender		
	Males	*Females*	
High	28	10	*p*-value = 0.355 (NS)
Intermediate	12	4	
Low	46	8	

	Margins			
	Missing data	*Free*	*Involved*	
High	0	35	3	*p*-value = 0.18 (NS)
Intermediate	1	15	0	
Low	6	46	2	

	Worst pattern of invasion								
	Missing data	*Type I*	*Type II*	*Type III*	*Type IV*	*Type V*	*Type VI*	*Type VII*	
High	0	4	7	8	14	4	0	1	*p*-value = 0.005**
Intermediate	0	2	2	10	1	1	0	0	
Low	6	3	17	16	10	0	2	0	

	Nature of specimen				
	Excisional	*Hemiglossectomy*	*Hemimandibulectomy*	*Incisional*	
High	0	1	25	12	*p*-value = 0.29 (NS)
Intermediate	0	0	1	6	
Low	2	2	4	31	

p-value, probability of observed results

Table 5: Association between the expression patterns of FLOT 1 and clinicopathological characteristics of patients with oral squamous cell carcinoma

	Lymph nodes				
FLOT 1	*Missing data*	*Free*	*Involved*	*Not Involved*	*p-value (Chi-square test)*
High	0	12	4	1	*p*-value = 0.11 (NS)
Intermediate	0	22	4	0	
Low	6	49	10	0	
	Gender				
	Males	*Females*			
High	14	3			*p*-value = 0.68 (NS)
Intermediate	22	4			
Low	50	15			
	Margins				
	Missing data	*Free*	*Involved*		
High	0	17	0		*p*-value = 0.06 (NS)
Intermediate	0	23	3		
Low	7	56	2		
	Worst pattern of invasion				
	Missing data	*Type I*	*Type II*	*Type III*	*Type IV*	*Type V*	*Type VI*	*Type VII*	
High	0	2	3	8	3	1	0	0	*p*-value = 0.65 (NS)
Intermediate	0	2	7	6	9	2	0	0	
Low	6	5	16	20	13	2	2	1	
	Nature of specimen				
	Excisional	Hemiglossectomy	Hemimandibulectomy	Incisional	
High	0	2	10	5	*p*-value = 0.66 (NS)
Intermediate	0	2	17	7	
Low	2	2	35	24	

p-value, probability of observed results

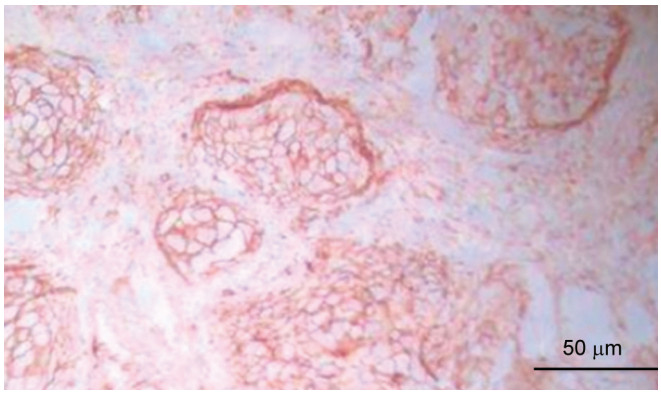

Fig. 1: Moesin positivity (membranous pattern) in moderately differentiated OSCC (100 μm)

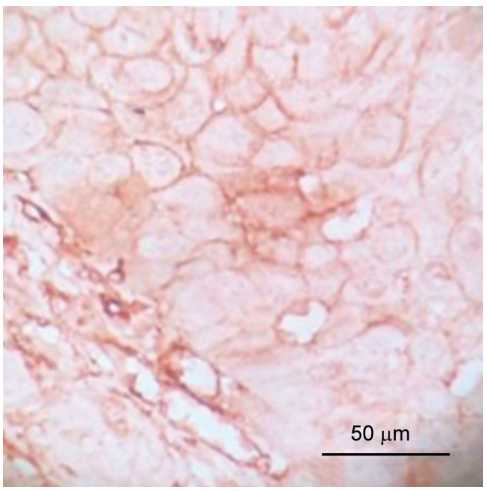

Fig. 2: FLOT 1 positivity in keratin pearls in well-differentiated OSCC (100 μm)

are associated with cancer metastasis, cell infiltrations, multiple tumors, and poor prognosis.[33] The overall survival rate was pegged at (HR = 2.17 CI-1.87–2.52).[35] But in this study FLOT 1 did not find any association between any of the clinical or demographical or microscopic variables. FLOT 1 was not correlated to the prognostic value of OSCC cases. FLOT 1 did not find any association between any of the clinical or demographical or microscopic variables. FLOT 1 was not correlated to the prognostic value of OSCC cases.

Limitations of the Study

Smaller sample sizes and unequal distribution of samples of well-differentiated OSCC, moderately differentiated OSCC, and poorly differentiated OSCC were the major limitations of this study.

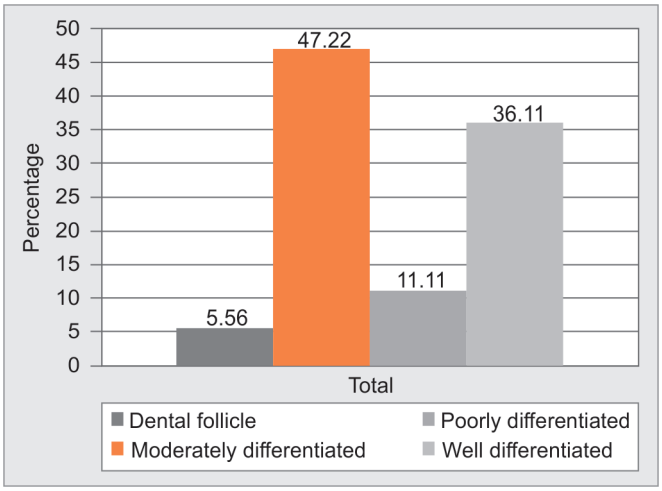

Fig. 3: Graphical representation of histopathological pattern of the grade of the lesion among the OSCC cases

Future Directions

The overall 5-year survival rate for OSCC is as low as 40–50%. The high rate of recurrence, poor prognosis, aggressive nature, and metastasis to loco-regional lymph nodes have strongly recommended the urge to improve the diagnostic capabilities of this malignancy.

Introducing new emerging prognostic biomarkers can aid as an interventional therapy for effectively addressing OSCC at an early stage, thus preventing it to further proceed to advance severe stage and combat the issue of mortality and deal effectively with the concept of field cancerization and the emergence of secondary primary tumors (SPT's) and high failure rates of surgical treatment.

Conclusion

Cytoplasmic detection of Moesin (35.19%) was higher than FLOT 1 (15.74%). There was no statistically significant relationship between the grade of the lesion and Moesin and FLOT 1. More research studies should be conducted for further confirmation and application in diagnostic pathology.

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