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Research Article

Fibrosis Squamous Cell Carcinoma

Study of Oral Squamous Cell Carcinoma with and without Oral Submucous Fibrosis.

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Abstract: Oral submucous fibrosis (OSMF) is a precancerous condition associated with areca nut chewing. Arecoline which is a component of areca nut is considered a carcinogen. The OSMF in patients with oral squamous cell carcinoma (OSCC) indicates areca nut-induced carcinogenesis. The molecular pathway for oral carcinogenesis caused by areca nut is discrete and considered a different clinicopathological entity. Thus the present study aims to study and compare the demographics, risk factors, and clinical characteristics of OSCC patients with and without OSMF. It was a retrospective study done by medical record review. About 40 OSCC cases were selected, of which 20 were with OSMF, and 20 were without OSMF. Demographic data, risk factors including history of habits such as areca nut chewing, tobacco, etc., and clinical characteristics such as anatomical location, extent, tumor size, and lymph node metastasis were noted, and data were analyzed. The mean age of OSCC cases with OSMF was younger than those without OSMF. The study groups did not observe a significant difference in habit type and duration. Most OSCC patients with OSMF were younger than those OSCC without OSMF. The buccal mucosa was the most common anatomical location involved with ulcers- proliferative growth in both study groups. The incidence of multiple sites and lymph node enlargement involvement is higher in OSCC without OSMF compared to those with OSMF. There was no significant difference concerning histopathological changes between OSCC with and without the OSMF group.

Keywords: Oral squamous cell carcinoma, oral submucous fibrosis, areca nut, malignant transformation, arecoline.

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1. INTRODUCTION

Oral submucous fibrosis(OSMF) is a chronic disease characterized by fibrosis of the submucosa lining the oral cavity.[1] It has the potential for malignant transformation, and WHO considers it an oral potentially malignant disorder.[2] Previously, it was predominantly seen in Southeast Asian countries, but now it has been observed in many countries.[3] Oral cancer is one of the most common cancers in India and accounts for approximately 30% of all cancers.[4] The incidence of oral squamous cell carcinoma arising with the background of OSMF was reported as 25.77%.[5] The incidence of oral cancer is highest in India, mainly due to the high prevalence of chewing areca nut and smokeless tobacco. [6] The cause of OSMF was believed to be unknown; there were multiple factors contributing to the cause of the OSMF. All consumption of areca nut in any form is considered the major causative agent. Areca nut chewing is the fourth most common addictive habit in the world. [7,8] Add about etiopathology of OSMF is a chronic debilitating disease of the oral cavity that presents with a burning sensation, blanching of the oral mucosa, submucosal fibrosis, restricted mouth opening, etc., OSMF is caused by multiple aetiological factors such as areca nut chewing, capsaicin in chilies, nutritional deficiencies of iron, zinc, and essential vitamins.[9] It is most commonly associated with the habit of areca nut chewing. OSMF is a disease that has a high risk of malignant transformation. [10] OSMF causes atrophy in the epithelium that can increase the risk of penetration of carcinogens.[11]Arecoline is a component of areca nut. This desiccating agent causes the cells to shrink enough, so the cells permit carcinogens through the epithelium to reach the basal layer. , The basal cell layer is where the neoplastic cellular transformation may occur.[12] Areca nut contains high copper content that stimulates fibrosis by up-regulating the activity of Lysyl Oxidase (LOX). [13] Based on a recent study from India, the incidence of oral squamous cell carcinoma (OSCC) with OSMF was reported as 25.77% which is very high. The malignant transformation potential of OSMF is underestimated. [5] Malignant transformations of OSMF were substantiated by Pindborg et al., using 5 main criteria. As per his observations, the frequency rate of OSMF was high in patients with OSCC, the prevalence of OSCC was more in OSMF patients, the histopathological findings of OSCC were seen in patients without any obvious tumors in OSMF, the frequency of epithelial dysplasia was high in OSMF and a higher incidence of leukoplakia among OSMF patients. [14] The molecular pathways for carcinogenesis is distinct for OSCC cases which are transformed from OSMF as they have different morphology and histological changes compared to OSCC without OSMF. [15, 16] Previous studies suggest that the OSCC arising with the background of OSMF patients were predominantly in young males with good prognoses as they have a better grade of tumor differentiation along with a lesser incidence of lymph node metastasis, and other studies reported more invasive and a higher rate of metastasis in OSCC arising with the background of OSMF.[16-18,] As conflicting statements put forth in the literature, the present study considered the OSCC arising from OSMF as a distinct entity. The clinical characteristics of the disease and its progression need to be studied. Thus the present study was undertaken to study the differences in OSCC cases with OSMF and the OSCC cases without OSMF.

2. MATERIALS AND METHODS

The medical records/files of the oral pathology department were reviewed from 2017 to 2022. A total of 40 cases were selected, of which 20 cases were of OSCC and 20 cases of OSCC with OSMF. The cases with full clinical profiles, habit history, and available paraffin tissue blocks for histopathological confirmation of the cases were included in the study. The cases with missing clinical data, habit history, and demographic data were excluded from the study. After obtaining the institutional ethical clearance, the data was collected from the Department of Oral Pathology, Panineeya Mahavidyalaya Institute of Dental Sciences, Hyderabad, and Government Dental College, Hyderabad. (IEC No: PMVIDS&RC/IEC/MEDICAL/PR/487-21) The clinical parameters for the study were the anatomical location of the tumor, number of lesions, lymph node metastasis, and restriction in mouth opening. The OSMF cases associated with other premalignant conditions, such as leukoplakia and lichen planus, were excluded from the study. The habit history included the type of habit and duration of the habit. The type of habit was categorized into areca nut, areca nut with tobacco, and tobacco. The duration of the habit was noted in the number of years. Histopathological grading was done based on Broder's grading and Bryne's grading. The Haematoxylin and eosin-stained slides of 20 OSCC with OSMF were compared with 20 OSCC without OSMF cases. In each case, all the parameters of Broder's grading and Bryne's grading were analyzed in detail, and scored [19]

3. STATISTICAL ANALYSIS

The data were tabulated and analyzed by SPSS 20.0 statistical software. Frequency tables of categorical data were analyzed using the Pearson Chi-square test. The probability level was fixed at <0.05.

4. RESULTS

Out of 20 OSCC with a background of OSMF cases, 70% were male, and 30% were females. The average age of the subjects was 45 ± 11.82 years. 50% of the cases belonged to the 40s, and 10% were below 30 years. Out of 20 cases of OSCC without OSMF, 55% were male, and 45% were females. The average age of the OSCC cases was 52.14 ± 13.09 years. 40% of the cases belonged to 50s, and only 5% were below 30 years. There was a statistically significant difference between the ages of the 2 study groups (P <0.023). The OSCC cases with OSMF were much younger than the OSCC cases without OSMF.

Table 1: Showing age distribution of study groups.

Age group	OSCC with OSMF	OSCC without OSMF
18 -29	2	1
30 – 39	2	1
40 – 49	10	5
50 – 59	4	8
60 and above	2	5

Table 2: Showing the comparative analyses of demographic data, risk factors, and clinical characteristics between OSCC with OSMF and OSCC without OSMF.

Parameter	Category	OSCC with OSMF	OSCC without OSMF	Total	Level of significance
Gender	Male	14 (70%)	11 (55%)	25 (62.5%)	**0.96**
	Female	6 (30%)	9 (45%)	15 (37.5%)	
Age	≤ 45	14 (70%)	6 (30%)	20 (50%)	**0.01**
	>45	6 (30%)	14 (70%)	20 (50%)	
Type of habit	Areca nut	3 (15%)	6 (30%)	9 (22.5%)	**0.42**
	Tobacco + areca nut	11 (55%)	8 (40%)	19 (47.5%)	
	Tobacco	2 (10%)	4 (20%)	6 (15%)	
	Combination	4 (20%)	2(10%)	6 (15%)	
Duration of habit	0-1	1 (5%)	0 (0%)	1 (2.5%)	**0.71**
	1-10	8 (40%)	11 (55%)	19 (47.5%)	
	11- 20	6 (30%)	7 (35%)	13 (32.5%)	
	21-30	4 (20%)	1 (5%)	5 (12.5%)	
	31-40	1 (5%)	1 (5%)	2 (5%)	

There was no significant difference between the two study groups concerning the type of habit and the duration of the habit. Both groups consumed tobacco with areca nut predominantly (Table 2).

Table 3: Showing the comparative analysis of clinical and pathological characteristics between OSCC with OSMF and OSCC without OSMF.

Parameter	Category	OSCC with OSMF	OSCC without OSMF	Total	Level of significance
Type of tumor	Exophytic	3 (15%)	5 (25%)	8 (20%)	**0.42**
	Endophytic	17 (85%)	15 (75%)	32 (80%)	
Extension of tumor	Single	14 (70%)	13 (65%)	27 67.5%)	**0.73**
	Multiple	6 (30%)	7 (35%)	13 32.5%)	
Anatomical site of tumor	Buccal mucosa	13 (65%)	11 (55%)	24 (60%)	
	T + BM	2 (10%)	0 (0%)	2 (5%)	
	MG, LV and SP	0(0%)	2 (10%)	2 (5%)	
	RMT + BM	2 (10%)	0 (0%)	2 (5%)	
	AR + BM	0 (0%)	1 (5%)	1 (2.5%)	
	AR, BM, & SP	0 (0%)	3 (15%)	3 (7.5%)	
	AP and mandible	0 (0%)	1 (5%)	1 (2.5%)	
	RMT	1 (5%)	1 (5%)	2 (5%)	
	Tongue	2 (10%)	0 (0%)	2 (5%)	
	Alveolobuccal sulcus	0 (0%)	1 (5%)	1 (2.5%)	
Size of the tumor	1 (<2cm)	5 (25%)	2 (10%)	7 (17.5%)	**0.16**
	2 (2-4cm)	7 (35%)	4 (20%)	11(27.5%)	
	3 (>4cm)	5 (25%)	5 (25%)	10 (25%)	
	4 (>4cm + involving bone and skin)	3 (15%)	9 (45%)	12 (30%)	
Palpable lymph nodes	Yes	8 (40%)	14 (70%)	22 (55%)	**0.056**
	No	12 (60%)	6 (30%)	18 (45%)	

The most common type of tumor observed in both study groups was endophytic in the form of ulceroproliferative growth. The most common anatomical site involved was observed as buccal mucosa in both study groups. The tumor size was observed to be larger in OSCC without OSMF group compared to OSCC with OSMF group, but a statistically significant difference was not found. Palpable lymph nodes were observed predominantly in OSCC without OSMF group compared to OSCC with OSMF group. There was no significant difference between the groups concerning clinical characteristics (Table 3, 4).

Table 4: Showing the correlation between clinical TNM early to moderate stage and advanced stage in OSCC with and without OSMF groups.

TNM staging	OSCC with OSMF (n=20)	OSCC without OSMF (n=20)	Total (n=40)	Level of significance
Early to moderate stage	13 (65%)	8 (40%)	18 (45%)	0.11
Advance stage	7 (35%)	12 (60%)	22 (55%)	
Total	20	20	40	

The histopathological grading was done based on Border's and Byner's grading systems. In the OSCC with OSMF cases, collagen bundles were observed in the submucosa (Figure 1). As per Broder's grading system, the incidence of poor and moderately differentiated grades was higher in OSCC without OSMF compared to OSCC with OSMF (Figure 2). Well-differentiated is predominantly seen in OSCC with OSMF cases (Table 5). There was no statistically significant difference between the two study groups. As per Bryne's grading system, predominantly poorly differentiated cases were observed in both study groups. There was no significant difference observed among the groups (Table 5,6).

Table 5 shows the comparative analyses of histopathological grading between OSCC with OSMF and OSCC without OSMF.

Parameter	Category	OSCC with OSMF (n=20)	OSCC without OSMF (n=20)	Total (n=40)	Level of significance
Broder's grading	Well-differentiated	10 (50%)	7 (35%)	17(42.5%)	0.61
	Moderately differentiated	10 (50%)	12 (60%)	22 (55%)	
	Poorly differentiated	0 (0%)	1 (5%)	1 (2.5%)	
Bryne's grading	4-8 (Well differentiated)	1 (5%)	2 (10%)	3 (7.5%)	0.71
	9-12(Moderately differentiated)	8 (40%)	6 (30%)	14 (35%)	
	13-16 (Poorly differentiated)	11 (55%)	12 (60%)	23(57.5%)	

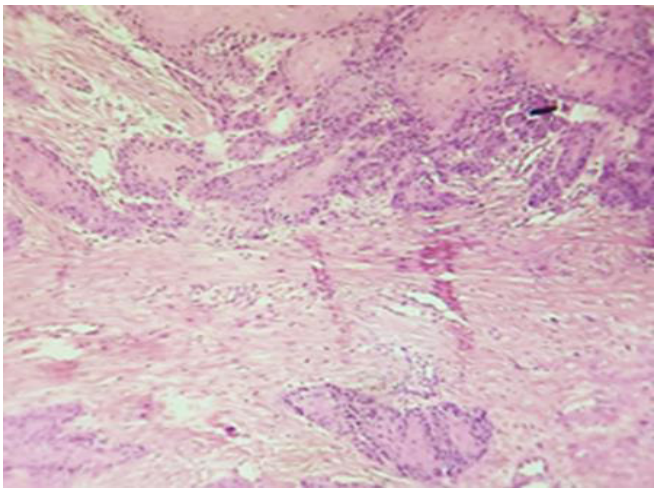

Fig 1: Showing oral squamous cell carcinoma arising with the background of oral submucous fibrosis. Presence of dense collagen fibers in the submucosa.

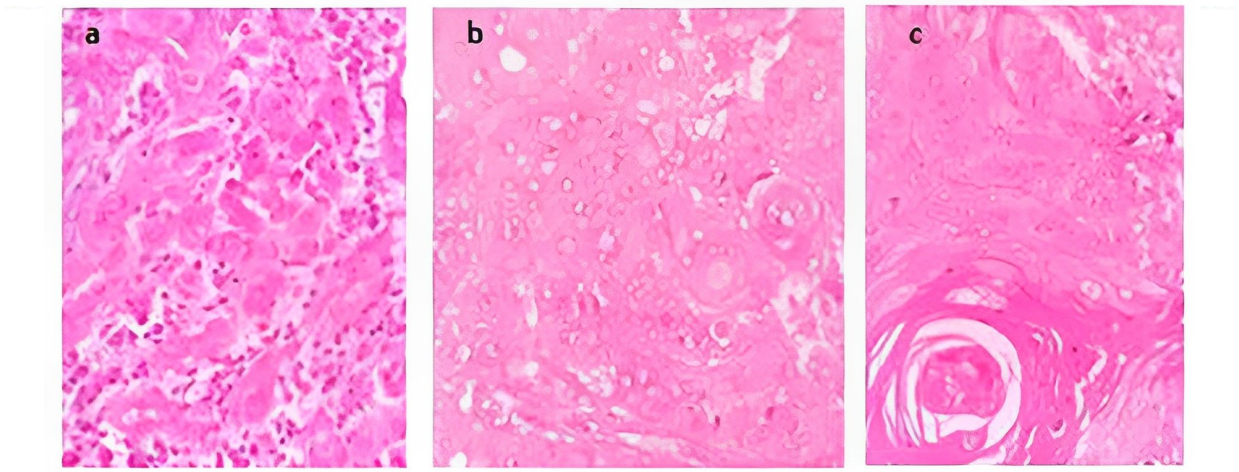

Fig 2: Showing different grades of OSCC. A - Poorly differentiated OSCC, B- Moderately differentiated OSCC, C- Well differentiated OSCC (As per Border's grading system).

The intravascular invasion (Figure 3) and perineural invasion were observed in 27.5% and 50%, respectively. There was no significant difference between the two study groups concerning the pattern of invasion, degree of keratinization, inflammatory infiltration, and nuclear polymorphism (Table 5 (Figure 4).

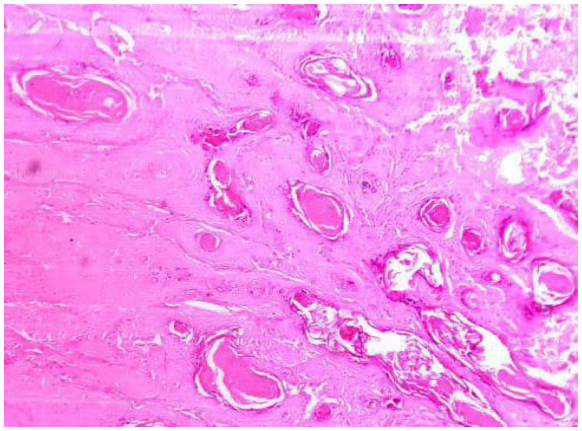

Fig 3: Showing intravascular invasion

Table 6: Showing the comparative analyses of histopathological characteristics between OSCC with OSMF and OSCC without OSMF.

Parameter	Category	OSCC with OSMF (n=20)	OSCC without OSMF (n=20)	Total (n=40)	Level of significance
Intravascular invasion	Positive	4 (20%)	7 (35%)	11 (27.5%)	0.28
	Negative	16 (80%)	13 (65%)	29 (72.5%)	
Perineural invasion	Positive	9 (45%)	11 (55%)	20 (50%)	0.52
	Negative	11 (55%)	9 (45%)	19 (47.5%)	
Inflammatory infiltration	Moderate to High	12 (60%)	13 (65%)	25 (62.5%)	0.74
	Low	8 (40%)	7 (35%)	15 (37.5%)	
Degree of keratinization	1 & 2	8 (40%)	10 (50%)	18 (45%)	0.52
	3 & 4	12 (60%)	10 (50%)	22 (55%)	
Nuclear polymorphism	1 & 2	3 (15%)	6 (30%)	9 (22.5%)	0.25
	3 & 4	17 (85%)	14 (70%)	31 (77.5%)	
Pattern of invasion	1 & 2	6 (30%)	5 (25%)	11 (27.5%)	0.72
	3 & 4	14 (70%)	15 (75%)	29 (72.5%)	

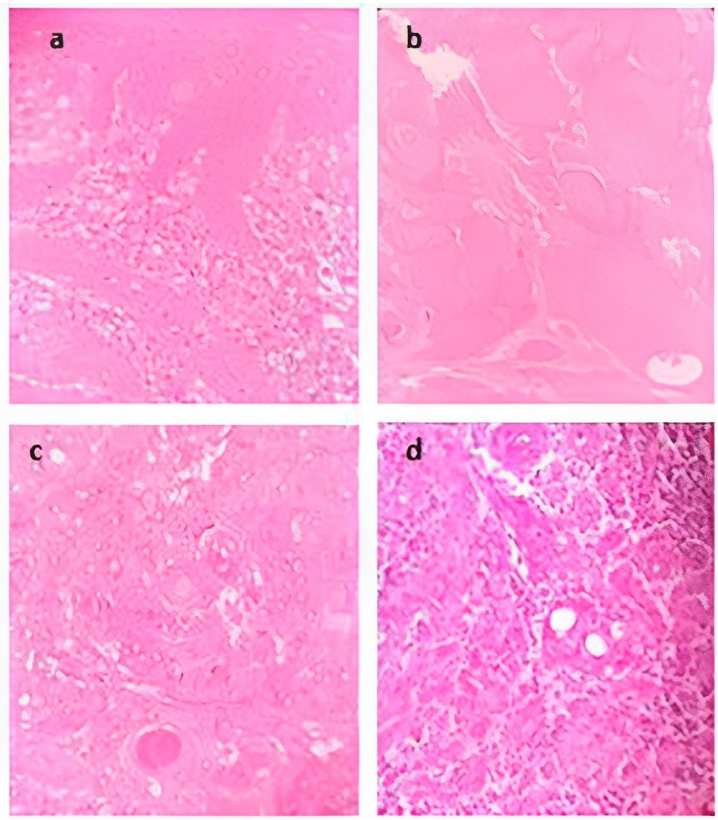

Fig 4: Showing a pattern of invasion a – Pushing, well-defined invasive front, b - Invasion by large cords of cells, c – Invasion by a small group of cells, d- Widespread cellular dissociation

5. DISCUSSION

Oral submucous fibrosis is a potentially malignant condition as it is more likely to develop into oral cancer. Literature suggests that OSMF patients are 19.1 times more likely to develop oral cancer than those without it. [20] Few studies reports stated that OSCC arising from OSMF was more invasive and had a higher rate of metastasis. Along with that, they also observed a higher rate of recurrence than the OSCC without OSMF.[18] Among all the other risk factors, areca nut has been proven as a causative agent for OSMF, and not only that it is also considered a Class I carcinogen. Areca nut might contribute to the early development of OSCC in younger age groups. [15, 21] In the present study, the incidence of OSCC and OSCC with OSMF was higher in males at 55% and 70%, respectively. These findings were similar to other studies by Rangaswamy S et al. and Siriwardena B. S. M. S. et al., which also found a higher incidence in males. [15, 22] Mohiuddin S et al. found that the incidence of OSCC with OSMF was higher in females, which is contrary to the results of the present study.[23] The OSCC with OSMF group was younger compared to OSCC without OSMF in the present study. There was a significant difference observed among the groups concerning age. Most OSCCs with OSMF cases were in the 40s age group, and OSCCs without OSMF were in the 50s. Swetha Acharya et al. findings were similar to the results of the present study, where they found a statistically significant difference between the 2 study groups.[24] Siriwardena B. S. M. S. et al. reported that the incidence was high in both groups above 50 years, but the incidence of the cases below the 50s was more in OSCC with OSMF, and the mean age of OSCC with OSMF was lesser than the OSCC without OSMF. They did not find any significant difference between the two groups concerning age, contrary to the results of the present study where the OSCC with OSMF cases were mostly in the 40s, and a statistically significant difference was observed concerning the present study age between the 2 study groups.[22] Arecoline is a component of areca nut with carcinogenicity, mutagenicity, and genotoxicity. The risk of developing OSMF is 32 to 109.6 times more in areca nut chewers.[25] Arecoline interferes with extracellular matrix formation and degradation and induces fibrosis. [7] Studies suggest that areca nut extract and arecoline trigger Reactive oxygen species (ROS) generation in fibroblasts and keratinocytes and also induced tumor-promoting mediators such as IL6, EGFR, ERK, TGF-β, and Ras [26-28] In the present study the most common habit observed in both groups was OSCC with OSMF and OSCC without OSMF cases was tobacco with areca nut. The incidence was 55% and 40%, respectively. The exclusive tobacco usage was higher in OSCC without OSMF group compared to OSCC with OSMF. There was no significant difference between the duration and type of habit in both groups. These results are similar to Swetha Acharya et al.; they also found a higher incidence of tobacco with areca nut in OSCC with the OSMF group than those without the OSMF group. Also, they found no difference in the type and duration of habit between both groups.[24] OSCC arises from the mucous membrane of the oral cavity, and the anatomical locations most commonly involved are the buccal mucosa and labial mucosa, including lips, gingiva, retromolar trigone, the floor of the oral cavity, palate, and the tongue.[29] In the present study, the buccal mucosa was both groups' most common anatomical location of tumor development. The study by Schmidt Jensen et al. reported that the tumor's most common anatomical site was the mouth's floor. [30] Lahore by Rakia et al. and Abdul et al. reported that the tongue is the most commonly involved site [31, 32] These findings were contrary to the present study's findings. Siriwardena B. S. M. S. et al., and Shruthi Rangaswamy et al., reported the buccal mucosa as the most common site, similar to the present study's results.[15, 22] In the present study, multiple site involvement was reported more frequently in OSCC without OSMF compared to those with OSMF.Lymph node metastasis (LNM) was observed in 40% of OSCC with OSMF cases and 70% of OSCC without OSMF cases. The incidence of lymph node metastasis was much higher in OSCC cases without OSMF compared to OSCC with OSMF. These results are supportive of Gadbail et al. Siriwardena et al. also found that regional lymph node enlargement was significantly higher in OSCC compared to OSCC with OSMF [22, 33] In another study by Singh et al. reported that lymph node metastasis was significantly less in OSCC with OSMF compared to those without OSMF where the percentages were 28.6% and 81.1% respectively which is contrary to the results of the present study [34] The fibrosis or dense extracellular matrix causes the blockage of the submucosal lymphatics in OSMF cases which could be a reason for the lesser incidence of lymph node metastasis in OSCC with OSMF cases. The reduced and blocked submucosal vascularity and lymphatics may benefit the overall prognosis in OSCC with OSMF cases compared to those without OSMF. [25,35] In the present study, the tumor type was predominantly endophytic in both groups. The tumor size and multiple site involvement were predominant in OSCC without OSMF compared to OSCC with OSMF, but a statistically significant difference was not observed. Gadbail et al., and Swetha Acharya et al., found similar findings in their studies concerning tumor type, size, and extension. [24,33] The well-differentiated carcinoma was predominant in OSCC with the OSMF group, and moderately differentiated was predominant in OSCC without the OSMF group. No significant difference was observed between the study groups regarding tumor differentiation. Gadbail et al., and Zhou et al., reported that well-differentiated carcinoma was significantly more in OSCC-OSF compared to OSCC. [33,36]

6. CONCLUSION

The OSCC with OSMF can be considered a different entity as the age of onset of the disease is significantly early compared to OSCC without OSMF. The OSCC with OSMF cases has a better prognosis as the lymph node involvement is less than those without OSMF. The type and duration of habits did not differ much among the groups. This study is retrospective. Thus only the available data from the medical records were considered. Within the study's limitations, there were no significant differences concerning histological differentiation and clinical characteristics between the OSCC with and without OSMF cases. Further studies on the molecular pathways involved in the malignant transformation of OSMF can be carried out to understand the OSCC arising with the background of OSMF.

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8. AUTHORS CONTRIBUTION STATEMENT

B H Shiny Vinila conducted the research in data collection, literature review, and manuscript preparation under the supervision of Dr. N Vishali and Dr. Ashalata Gannepalli. Dr. N Vishali and Dr. Ashalata Gannepalli guided the whole study with methodology, result analysis, discussion of the study, and manuscript editing.

9. CONFLICT OF INTEREST

Conflict of interest declared none.

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