MICROBIAL FLORA AND ORAL HYGIENE STATUS ASSOCIATED WITH ORAL POTENTIALLY MALIGNANT DISORDERS (OPMDS) AND ORAL SQUAMOUS CELL CARCINOMA (OSCC): A COMPARATIVE STUDY

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ABSTRACT

Background:Oral squamous cell carcinoma is a multifactorial disease and has been predominantly associated with betel quid chewing, smoking, alcohol consumption, and various other co-factors. The involvement of microorganisms in oral cancer is an area of research, where little attention has been paid to the relevance of these to overall patient morbidity.

Aim of the study:The study aims to assess the oral hygiene status and the type of microbial flora in subjects with Oral potentially malignant disorders (OPMDs) and Oral squamous cell carcinoma (OSCC) compared tohealthy subjects.

Materials and Methods:The oral hygiene index (OHI – index) of Green – Vermillion method was used and recorded. Collection of swabs from lesion sites of OPMDs and OSCC patientsandthe healthy controls. One oral swab was used for direct slide preparation for microscopic microbiological examination and the other swab was used for inoculating onto the culture plates.

Results:The most common oral microbes isolated were α-Haemolytic streptococcus, Staphylococcus aureus, and Staphylococcus epidermidis. Compare the oral microbial flora of the study groups with that of the control group, showing thatthe microbial flora was present in both relative frequencies varied. Other microbes likeKlebsiella pneumonia, Pseudomonas aeruginosa, Acinetobacter species,and Candida albicanswere isolated only in the OSCC group.

Conclusions:Only a restricted group of oral flora species thrive in the tumor environment and may contribute to cancer progression. These findings can aid clinicians in treatment and prognosis. Optimal oral hygiene and appropriate antimicrobial agents are crucial for oral cancer patients to enhance recovery and reduce post-operative complications.

KEYWORDS: Precancerous Conditions, Oral squamous cell carcinoma of Head and Neck, Microbiota, Oral hygiene.

INTRODUCTION:

The oral cavity which comprises of tongue, teeth, gingiva, and tonsils forms a niche for the microorganismsthat reside as commensals. This is indeed due to a delicate balance between this microbial flora and our immunesystem.During a disease, this balance is breached and these microbial florae become aggressive, giving rise to a host of diseases, some of which are inflammatory,while others are degenerative, transitory, or cancerous (1).

Oral cancer is the most prevailing type of cancer seen in the head and neckregion. Oral squamous cell carcinoma (OSCC) represents 95% of the head andneck cancer (2).Precancerous lesions of the oral mucosa, known as potentiallymalignant disorders in recent yearsconsist of a group of diseases, whichare characterized by an increased risk for malignant transformation (MT) to oralsquamous cell carcinoma (OSCC) (3,4). The use of tobacco, in any form (smoking and chewing), and increased alcohol consumption have been the major risk factors associated with oral cancer (5). Also, various other possible factors have beenimplicated to be contributing to oral cancer; these include poor oral hygiene, chronic mechanical trauma, diet andnutrition, viral infection, genetic and familial factors, and immune suppression (6).

Many studies have been carried out on the microbial flora of the mouth and theirassociation with various disease aspects. The role of microorganisms in causing oraldiseases, especially oral cancer has been proposed. Studies report shifts in bacterial colonization in the presence of OSCC lesions.Increased colonization of saliva or soft tissues in oral cancer subjects has alsobeen reported. It is seen that invasion of candidal hyphae into the oral epitheliumin chronic hyperplastic candidosis is associated with dysplastic changes leading to oral cancers (7). The relationship between bacteria and cancer is currentlyunclear. However, certain bacterial species are consistently linked to specificcancers.

Association of Helicobacter pylori in gastric cancer, Salmonellatyphi in gall bladder cancer, and a few other propositions has gained importance toresearch the possible role of bacteria in causing cancer, thus, the theory ofbacteria causing oral cancer raised (1,7,8). Studies have shown that poororal hygiene and lack of awareness of good oral health have also been relatedto the risk of development of oral cancer (9,10,11).Therefore, the present study will assess the relation of oral hygiene status and the trait ofthe microbial flora in subjects with oral squamous cell carcinoma (OSCC),

and Oral potentially malignant disorders (OPMDs), compared with healthy subjects.

MATERIAL AND METHODS:

The present study is a comparative observational study. The study group comprised patients who were clinically suspected of having oral squamous cell carcinoma and Oral potentially malignant disorders, which included leukoplakia, and erythroplakia and the control group consisted of healthy subjects

matched for age, sex, no habits, and dentition status, who were free of any oral lesions and systemic disease. Before being included in the study, all participants provided their written informed consent by signing an agreement. The study protocol received approval from the Institutional Ethical **Review Board**

The sample size consisted of 90 participants, with 30 in the control group, 30 with OPMDs, and 30 with OSCC. The demographic and clinical details of the participants wererecordedbefore the sample collection. The Simplified Oral Hygiene Index (OHI-S)of Green– Vermillion method was used and recorded for all the cases. The samples were collected before any surgical interventions. Two oral swabs were taken from the lesion site which one oral swab was used for direct slide preparation for microscopic examination of microbiological evaluationand the other swab was used for inoculating onto the culture plates. The culture swabs were placed insterile culture transporting tubes with written identification labels and transportedto the microbiological evaluation. Both gram stain and Periodic Acid Schiff stain was used for the smear slide for identification of organisms.

Culture plate inoculation:

The samples were inoculated onto sheep blood (5%) and MacConkey's agar plates respectively. The streaked culture plates were then incubatedin the bacteriological incubator at 370C for 24-48 hours. Subcultures wereperformedto identify clinically significant species of bacteria.

Statistical analysis:

Descriptive statistics to summarize the demographic details of the Oral potentially malignant disorders cases, oral squamous cell carcinoma cases, and the control group. Comparative analysis using chi-square tests to identify significant differences in microbial flora and oral hygiene status among the groups.

RESULTS:

Amongst a total of 90 cases, 69 were male and 21 were female in which a male-to-female ratio of 5:1was observed among the OPMDsand the OSCC groups.The distribution of OPMDs and OSCC cases was higher among individuals aged 50-60, as shown in Table 1.

The oral hygiene of all three groups was evaluated using the Oral Hygiene Index-Simplified (OHI-S) which showed that 73.3% of the cases in the OSCC group had poor oral hygiene. In contrast, the OPMDs group exhibited fair oral hygiene as shown in Table 2.

Microorganisms such as Candida albicans, Pseudomonas aeruginosa, Klebsiella pneumoniae, Neisseria species, Moraxella catarrhalis, β-hemolytic streptococci, Staphylococcus epidermidis, Staphylococcus aureus, and α-hemolytic streptococcus show statistically significant differences in their presence across the groups. The most common oral microbes isolated were α-Haemolytic streptococcus, Staphylococcus aureus, and Staphylococcus epidermidis. Compare the oral microbial flora of the study groups with that of the control group, showing that the microbial flora was present in both relative frequencies varied. Other microbes like Klebsiella pneumonia, Pseudomonas aeruginosa, Acinetobacter species, and Candida albicans were isolated only in the OSCC group as shown in Table-3.

There are no microorganisms that showed a statistically significant difference in their presence across the oral health condition groups (Good, Fair, Poor) where p-values are 0.05 as shown in Table 4.

DISCUSSION:

Oral squamous cell carcinoma (OSCC) is a disease with multifactorial etiology. This disease has a significant incidence worldwide and has a taxing prognosis, supporting further research on various factors that may revise the disease outcome (12).Studies have been done to see the association of the microbial flora with oral cancer, comparing significant alterations in the microbial flora, in premalignancy.There is no evidence suggesting that there is an association of oral hygiene status with microbial flora of oral precancer and cancer. Therefore, the present study had 30 participants of healthy persons as control,Oral potentially malignant

disorders, and oral squamous cell carcinoma as a study group. The present studyshowedmaleswerepredominate in the study group and the distribution of OPMDs and OSCC cases was higher among individuals aged 50-60yr. Similar results were found in a study by Milos Cankovic et al. (17)

The normal oral microbes in a physiological state could be altered, like thechanges during the development of an individual (age changes), hormonalinfluences, certain habits, environmental influences, etc. No statisticalassociations were found between bacterial presence and gender ($p>0.05$), age($p>0.05$), in the study groups. Similar results werefound in a study by Milos Cankovic et al. (17)

In the present study, we found a significant difference in the microbial flora present in the OSCC patients comparing them to the patients of OPMD and the control groups, similar to the previous studies where a comparison was made between the microbial content between the carcinoma site and the healthy oral mucosa (1, 17).Streptococcus (alpha hemolytic), Staphylococcus aureus, and Streptococcusepidermidiswere seen in all the studygroups. Klebsiella pneumonia, Pseudomonas aeruginosa, Acinetobacter species,and Candida albicans were isolated only in the OSCC cases and theseorganisms were isolated only in the carcinoma site, but not on the contralateralhealthy mucosa. This is under other studies where, a study by MilosCankovic et al. also had a similar finding, where Pseudomonas aeruginosa andKlebsiella pneumonia were isolated in the OSCC cases (17).RaghavendraByakodiet al. in his study also had Pseudomonas aeruginosa, Klebsiella pneumonia,Candida Albicans, Moraxella catarrhalis along with Proteus species, Citrobacterspecies, Streptococcus species, and Staphy lococcus species being isolated in highnumbers at the carcinoma sites (1). Hooper et al. also reported Acinetobacterlwoffii, Pseudomonas aeruginosa along with Citrobacter and Serratia species inthe deeper and superficial tumor specimens, where the study compared different microbial types within the OSCC tissue (18). Nagy et al. in their study reportedHaemophilus, Enterobacteriaceae, and Streptococcus species as microbial typesisolated at increased rates at the tumor sites (19). All of these studies reported thatthe microbial flora were unique to the tumor environment.

Poor oral hygiene was found significantly associated with OSCC cases in ourstudy, which has been frequently associated with the risk of oral cancer. It isgenerally agreed upon that poor oral hygiene poses as a risk factor, but lesscompelling than tobacco and alcohol. A study by Bloching et al. (20), concluded thatthe polymicrobial supragingival plaque could be a possible independent factorthat possessed a relevant mutagenic interaction with saliva and that individualoral health is a co-factor in the development of carcinomas in the oral cavity. Astudy by Sadia Minhas et al. in determining the clinic opathological and histological grading and their associations in OSCC patients concluded that poor oral hygiene was noted in 55.6% of the OSCC cases (21). Other studies byNeela Guha et al. (22), and Prabha Balram et al. (23) also concluded in their studies thatpoor oral hygiene was a risk factor in OSCC patients. Nevertheless, a dilemmaexists whether oral hygiene level was low before the development of OSCC andpremalignancy or if it is a consequence of OSCC and premalignancy.

The finding of Candida albicans on the lesions indicates a need for theirsuppression before any treatment for the tumor is taken up, or else severecomplications could occur. A study by Hashimoto et al. found that vertebralosteomyelitis and pneumonia were developed as a result of systemic candidiasisfollowing radiation treatment and surgery in oral cancer patients (24).

However, the present study did not find a significant correlation between themicrobial flora of the premalignant cases to that of the normal oral flora. In thepresent study, the microbial analysis was performed at the genera level ratherthan at the species and subspecies level; also, anaerobic culture and isolation of virus which could be part of commensals was not takenup because of lack of special enrichment media and required equipment and sensitive techniques.

The present study may support the hypothesis that the presence of pathogenicbacteria can contribute along with the existing factors in a tumor, and further helpin progression. The polymicrobial burden due to poor oral hygiene may alsopossess mutagenic interactions with saliva which may act as co-factors incarcinogenesis. Thus, optimal oral hygiene is important for oral cancer patientswhen scheduled for surgery, chemotherapy, or radiation therapy.

Limitations of the study

However, a study of a larger sample size with better facilities regarding the microbialculture may be required for further understanding of the complexities of microbialinteractions in the OPMDs and OSCC; and their role as aninitiator or a promoter, or a progressor. Confounding factors such as diet, smoking, and systemic health conditions were not fully accounted for. Geographic and demographic constraints further restrict the applicability of results. Variations in microbial identification methods and oral hygiene assessment criteria could affect reliability. Additionally, focusing on specific microbes might overlook the broader microbial community's impact. Future research should address these issues for more comprehensive findings.

CONCLUSION:

The present study assumes importance because, to our knowledge, no suchcorrelative study has been reported. Comparing and correlating factors betweenthe OSCC and OPMDs cases not only determined the significantdifferences in the alterations of microbial flora. The presence of these organismsexclusively in the tumor sites could implicate that these organisms canthrive in the tumor environment and could play a role in the progression ofthe cancer. Microorganisms mostcommonly associated with OSCC cases are different from the normalcommensals of the oral cavity. The significance of these findings may help theclinician in determining the treatment and prognosis.

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LEGENDS OF THE TABLES:

TABLE 1- Demographic details

		GROUP			
		Control	**OPMD**	**OSCC**	**TOTAL**
GENDER	Female	11 (36.7%)	5 (16.7%)	5 (16.7%)	21 (23.3%)
	Male	19 (63.3%)	25 (83.3%)	25 (83.3%)	69 (76.7%)
AGE	20-30	2 (6.7%)	3 (10.0%)	0 (0.0%)	5 (5.6%)
	31-40	14 (46.7%)	5 (16.7%)	5 (16.7%)	24 (26.7%)
	41-50	6 (20.0%)	5 (16.7%)	4 (13.3%)	15 (14.0%)
	51-60	6 (20.0%)	9 (30.0%)	15 (50.0%)	30 (33.3%)
	>60	2 (6.7%)	8 (26.7%)	6 (20.0%)	16 (16.4%)

TABLE 2- Oral hygiene status among the control, OPMD, and OSCC groups.

Oral hygiene status					
Groups	**Good N(%)**	**Fair N(%)**	**Poor N(%)**	**Chi-square value**	**p-value**
OPMD	1 (3.3)	19 (63.3)	10 (33.3)	40.740	0.001
OSCC	0 (0.0)	8 (26.7)	22 (73.3)		
CONTROL	7 (23.3)	23 (76.7)	0 (0.0)		

TABLE 3- Distribution of microbial generaamong the control group, OPMD, and OSCC group.

Microbial flora	OPMD		OSCC		CONTROL			
	Yes N(%)	No N(%)	Yes N(%)	No N(%)	Yes N(%)	No N(%)	Chi-square value	p-value
Candida albicans	0(0.0)	30(100)	4(13.3)	26(86.7)	0(0.0)	30(100)	8.372	0.015
Acinetobacter species	0(0.0)	30(100)	2(6.7)	28(93.3)	0(0.0)	30(100)	4.091	0.129
Pseudomonas aeruginosa	0(0.0)	30(100)	7(23.3)	23(76.7)	0(0.0)	30(100)	15.181	0.001
Klebsiella pneumonia	0(0.0)	30(100)	7(23.3)	23(76.7)	0(0.0)	30(100)	15.181	0.001
Neisseria species	4(13.3)	26(86.7)	3(10.0)	27(90.0)	10(33.3)	20(66.7)	6.237	0.044
Moraxella catarahalis	7(23.3)	23(76.7)	1(3.3)	29(96.7)	14(46.7)	16(53.3)	15.281	0.a001
β-haemolytic streptococci	12(40.0)	18(60.0)	6(20.0)	24(80.0)	25(83.3)	5(16.7)	25.205	0.001
Staphylococcus epidermidis	3(10.0)	27(90.0)	4(13.3)	26(86.7)	26(86.7)	4(13.3)	48.517	0.00a1
Staphylococcus aureus	20(66.7)	10(33.3)	30(100)	0(0.0)	30(100)	0(0.0)	22.500	0.001

Table 4:Distribution of the presence of various oral microorganisms in patients with different OHI-S oral health conditions

		Good		Fair		Poor			
		Count	N%	Count	N%	Count	N%	Chi square	p value
α haemolytic streptococcus	Negative	0	0.00%	11	40.70%	12	37.50%	0.697	0.706
	Positive	1	100.00%	16	59.30%	20	62.50%		
Staphylococcus aureus	Negative	1	100.00%	5	18.50%	4	12.50%	5.467	0.065
	Positive	0	0.00%	22	81.50%	28	87.50%		
Staphylococcus epidermidis	Negative	1	100.00%	24	88.90%	28	87.50%	0.162	0.922
	Positive	0	0.00%	3	11.10%	4	12.50%		
β-haemolytic streptococci	Negative	0	0.00%	19	70.40%	23	71.90%	2.389	0.303
	Positive	1	100.00%	8	29.60%	9	28.10%		
Moraxella catarahalis	Negative	1	100.00%	22	81.50%	29	90.60%	1.216	0.544
	Positive	0	0.00%	5	18.50%	3	9.40%		
Neisseria species	Negative	1	100.00%	24	88.90%	28	87.50%	0.162	0.922
	Positive	0	0.00%	3	11.10%	4	12.50%		
Klebsiella pneumonia	Negative	1	100.00%	26	96.30%	26	81.20%	3.351	0.187
	Positive	**0**	0.00%	1	3.70	6	18.80%		
Pseudomonas aeruginosa	Negative	1	100.00%	24	88.90%	28	87.50%	0.162	0.922
	Positive	0	0.00%	3	11.10%	4	12.50%		
Acinetobacter species	Negative	1	100.00%	27	100.00%	30	93.80%	1.81	0.404
	Positive	0	0.00%	0	0.00%	2	6.20%		
Candida albicans	Negative	1	100.00%	26	100.00	29	90.60%	0.83	0.66
	Positive	0	0.00%	1	0.00%	3	9.40%		