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Comparison of Oral Aerobic Bacterial Profile In Patients with Oral Squamous Cell Carcinoma and Patients with Oral Pre Cancerous Lesions, Attending Tertiary Care Centre In Rajasthan

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ABSTRACT

Background

More than 90 percent of oral cancers are squamous cell carcinomas, making them the most frequent kind of malignant tumor in the oral and maxillofacial area. Oral cancer rates are rising rapidly over the globe. Oral malignant tumors may develop when there is an incongruity between the microbiome and the host.

Material & Methods

This prospective study was carried out in microbiology department, Tertiary care centre,Jaipur.A total of 200 oral swab samples have been collected 100 for control group and 100 for case group and processed for bacterial identification according to standard microbiological guidelines.

Results

Among the 100 samples studied from the oral squamous cellcarcinoma patients, *Pseudomonas* species (n = 45) were the predominant isolate, followed by *Klebsiella* species (n = 26)and *Staphylococcus* species (n = 15). Of the 100 samples studied among the control group, *Micrococci*(30) and *Diptheroids* (23) is the predominant bacteria isolated.

Conclusion

Patients with precancerous lesions had a more varied and abundant oral microbiome compared to those with

oral cancer and healthy controls but that there was no difference between the groups in terms of overall microbiota composition. When comparing the oral bacterial profiles of those with cancer and those without, there were clear distinctions. Therefore, bacteria may be used as biomarkers in the diagnosis of oral cancer and as therapeutic targets in its therapy.

Keywords

Oral microbial profile, oral squamous cell carcinoma, oral pre canceous lesions

INTRODUCTION

Squamous cell carcinomas are the most common kind of oral and maxillofacial malignant tumour, accounting for more than 90% of all cases of oral cancer. Oral cancer is becoming more common across the globe, and despite significant advances in cancer therapy, it still has a high death rate and a low 5-year survival rate (Allavena, 2008). [(1)]When a person is diagnosed with oral cancer, the disease quickly spreads to other parts of the head and neck, threatening the patient's life and causing severe impairment in areas like swallowing, speech, pain, numbness, and breathing. Although tobacco use, betel nut chewing, and excessive alcohol use are known to increase the likelihood of developing oral squamous cell carcinoma, this only accounts for around 85% of all cases (Berkovits, 2016). [(2)]

Helicobacter pylori was originally implicated as a causative factor in stomach cancer, and its carcinogenicity was shown in the 1990s. [(3)] Subsequently, other analyses examined the link between microorganisms and secondary malignancies. Specifically, it has been established that *Epstein-Barr virus* (EBV) infection is connected to both Burkitt lymphoma and nasopharyngeal carcinoma, that *Salmonella typhi* infection is associated with an increased risk of gallbladder cancer, and that HPV infection is linked to cervical cancer (Perera, 2018). [(4)] These results offer a foundation for future study of the link between microbes and oral cancers and point the way towards potential new avenues of inquiry.

Oral bacteria have been linked to tumour formation in several studies; however, the exact mechanism is unclear. From culturing bacteria in the lab through PCR molecular techniques to next-generation sequencing for the identification of 16S rRNA genes and on to metagenomicsequencing, the research process has come a long way. [(5)] Alterations to the oral microbiota have been linked to an increased risk of cancer in certain studies, whereas in others no such link has been found. Increasing evidence suggests that the mucosal surface, tumour tissue, and saliva of patients with oral squamous cell carcinoma vary considerably from those of people with healthy oral cavities (Eckert, 2018). [(6)] In many circumstances, the aggressiveness of the cancer patient's medication may change the oral microbiota; the appearance of potential pathogens may result in opportunistic infections in immune-compromised people, increasing morbidity and death. Since only handful of comparative studies have been undertaken in the past to compare the bacterial profile in precancercancerous and cancer patients so we aimed to carry out this research to correlate the bacterial isolates in these patients.

MATERIAL AND METHODS

This Hospital based comparative type of cross-sectional study was conducted in the Bacteriology Lab, Department of Microbiology, SMS Medical College, and Jaipur.Data has been collectedfor about

one and half year after taking approval from Research Review board i.e. from Jan 2021 to June 2022. A total of 200 oral swab samples were collected,100 each for both the study groups.

The study population was grouped as:

Group 1: Clinically and Histo-pathologically diagnosed patients with oral squamous cell carcinoma.

Group 2: Patients with clinically diagnosed oral premalignant lesions (Leukoplakia, orallichen planus, erythroplakia, oral submucous fibrosis) which were taken as control for this study.

Inclusion Criteria for Both Study Groups

1. Patients 20 -50 years of age.
2. Either of the sex.
3. Patients with their consent to participate in the study.

Exclusion Criteria for Both Study Groups

1. Patients with a history of existing chemotherapy, radiotherapy and surgery for their condition.
2. Patients receiving antibiotic treatment currently or who had received antibiotic treatment within the last 4 weeks.
3. Patients receiving any medications or surgery for their oral precancerous lesions.
4. Patients diagnosed with HIV, HBV, HCV, and TB.

Microbiological Analysis

Method

1. Sample Collection

Standardized aseptic conditions were followed for collecting the swabs for all patients. In Group 1, the swabs were taken from the tumour site and in Group 2 (Control)swabs were taken from the lesion site. All specimens were collected and transported within a maximum of two hours to the microbiology laboratory of SMS Medical College, Jaipur and inoculated immediately.

2. Isolation and Identification of Bacterial Species

For possible isolation of aerobic bacterial pathogens, each swab sample were inoculated onto Blood agar, MacConkey agar and Thioglycollate broth then these plates were cultured aerobically at 37˚C for 24 hr.Each bacterial isolate was then be identified on the basis of morphology, gram staining and biochemical testsi. e conventional and by advanced VITEK II system as per standard lab protocol.

RESULTS

Out of 200 samples studied from both the groups**,** most cases were studied in the control i.e Group 2 (32) with an average age of 41-50 years, followed by (31 cases) with an average age of 41-50 years.In the Group 1, a similar pattern was observed; the greatest number of cases (41) 41% were in the age range of 41-50 years, followed by (27) 27% in the age range of 51-60 years as shown in Table 1.

Table2 shows the distribution of the cases according to **sex**.In group 2 there were 72 % malessimilar patterns were found in Group 1, with 79% of them being male and the 21% being female.

A total of 8.82% of those in the control (pre-cancer) group consumed BIDI/cigarettes, 70.59 % gutka, and 20.59% of themconsumedsupari, whereas 21.05 % of those in the cancer group consume BIDI/cigarettes, 73.68 % took gutka, and 22.81% of them consumedsupari. Among the control group the maximum lesions (46.08%) were observed onthe buccal mucosa, followed by 26% on the tongue. Similar patterns were observed in cancer group

(group1); the buccal mucosa had the highest number of cases 42.11%, followed by the tongue 10.53% as depicted in table 3.

Table 4 shows the distribution of bacteria in both the groups according to gram staining. In the cancer group; the gram negative bacteria were (79) 69.30%, and the gram positive bacteria were (35) or 30.7% while in the control (18) 17.65 % were Gram negative bacteria rest 84 (82.35%) were gram positive bacteria.This observation was statistically significant.

In the cancer group the most common organism isolated was *Pseudomonas* (39.47%), followed by *Klebsiella*(22.81%), *Escherichia coli* (5.26%), and *Acinetobacter* (3.51%) while *Staphylococcus*(13.16%) was the most common pathogen found in Gram positive cocci followed by *Streptococcus* sp. (7.89%) in the control group. *Streptococcus* sp. (16.67%)was the most common pathogen found in Gram positive cocci followed by *Staphylococcus* sp which were (11.76%.) gram negative bacilli were statistically higher in cancer group cases as compared to control group as depicted by table 5.

DISCUSSION

More than 700 different kinds of microbes call the oral cavity home, making it one of the most diverse and complicated microbial ecosystems in the human body. Ecological dysbiosis, often known as an imbalance in the microbiome, has been researched extensively in both human and animal subjects. These are characterized by a decline in the variety of microorganisms and an increase in the prevalence of harmful bacteria. Evidence suggests that ecological dysbiosis may play a role in carcinogenesis. Previous research has shown a link between oral cavity bacteria and other types of cancer.

In this study,most of the patients were males in both the study groups i.e 72% males in control and 79% males in cancerous group,similar results are also mirrored in previous studies ofAnjali K et al (2019)where 72.9% (70) of cancer patients were males and 27.1% (26 cases) were females, with a male-to-female ratio of 2.7:1. Also the cancer group in previously conducted studied were mostly in the age – group between 41-50 years. Ashreen et al (2020). Gutkha chewing habit is seen in both the study groups.This observation is similar to Ashreen et al (2020)study. The distribution of predisposing factors in oral cancer patients has shown that almost 90% of patients had regular uptake of betel nut and betel leaf.

In this study *Pseudomonas* had a prevalence of 27.31% whereasa previous study ofAshreen et al (2020) [7] reported *Pseudomonas* prevalence as 36%. Our findings are similarto another study conducted in western European hospitals where *P. aeruginosa* was one of the most common organisms, constituting 29% of all Gram-negative isolates.However, in regards to isolates from pre-operative patients, the percentage of *Pseudomonas* was higher in this study. Further studies are also required to rule out the potential of these pathogenic bacteria in role of carcinogenesis.

The microbial population and the host often maintain a state of dynamic equilibrium, but there are exceptions such as specific helpful bacteria that may efficiently combat foreign infections and boost tissues and the immune system.The metabolic activities and the uncontrolled growth of cells might both contribute to tumor development. A previous study has observed that *Capnocytophagagingivalis, Prevotellamelaninogenica, and Streptococcus mitis* counts were significantly increased among the

oral cancer patients and these species could be used as diagnostic markers for oral cancers with 80% sensitivity and 82% specificity, respectively .[8] In cancerous patients an optimized antibiotic prophylaxis is one clinical focus to limit postoperative infections. A combination therapy is most often recommended, and broad-spectrum antibiotics are the drugs of choice because of increased pattern of resistance in these patients.[9]

Microbial markers' significance in making the tumor-disease connection demonstrates their utility as a noninvasive diagnostic tool for oral cancers.[10,11] Using such a small sample size is the study's main flaw. Oral malignant tumors have been linked to changes in the microbial microbiome,[12] and further testing will confirm their diagnostic utility. Also we studied only the aerobic bacteria in both the study groups which is another limitation, further studies could be conducted in future studying complete microbiota so that their role in malignant transformation can be linked.

CONCLUSION

The oral microbiota of persons with oral cancer and precancerous lesions was shown to have significant morphological changes across a wide variety of species. Our research shows that metabolic pathway changes brought about by dysbiosis of the oral microbiota have a direct and detrimental effect on dental health. More research is needed to better understand the relationship between “oral microbiota imbalances and oral cancer”, so that new data may be gathered in support of using microbiome-targeted therapy for disease prevention.

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Table 1: Distribution of the Patients according to age group

Age	Group 1 (Control)		Group 2 (Cancer group)		P-value
	number	percentage%	Number	percentage%	
≤20	1	1	0	0	0.038
21-30	11	11	1	1	
31-40	31	31	26	26	
41-50	32	32	41	41	
51-60	20	20	27	27	
>60	5	5	5	5	
Grand Total	100	100	100	100	

Chi-square = 11.924 with 5 degrees of freedom; P = 0.038

Table 2: Distribution of the Patients according to Sex

Sex	Group 1 (Control)		Group 2 (Cancer group)		P-value
	No	%	No	%	
Female	28	28	21	21	0.324
Male	72	72	79	79	
Grand Total	**100**	100	**100**	100	

Chi-square = 0.973 with 1 degree of freedom; P = 0.324

Table 3: Distribution of the patients according to location of lesion

Location	Group 1 (Control, N=100)		Group 2 (Cancer, N=100)		P-value
	No	%	No	%	
Buccal Mucosa	47	46.08	48	42.11	1.000
Floor of mouth	5	4.9	11	9.65	0.193
Hard palate	4	3.92	7	6.14	0.535
Lower alveolus	4	3.92	10	8.77	0.166
Retromolartrigone	10	9.8	7	6.14	0.612
Tongue	26	25.49	12	10.53	0.019
Tongue&Floor of Mouth	1	0.98	0	0	1.000
Upper alveolus	3	2.94	5	4.39	0.718
Grand Total	**102**	100	**114**	100	

Table 4: Comparison of isolated organisms according to gram staining

Gram Staining	Group 2 (Cancer group)		Group 1 (Control)		P-value
	Number	Percentage%	Number	Percentage%	
Gram Positive	35	**30.7**	84	**82.35**	
Gram Negative	79	**69.3**	18	**17.65**	<0.001
Total	114	**100**	102	**100**	

Table 5: Distribution of isolated organism between the groups according to gram staining

	Group 2 (Cancer N=114)		Group 1 (Control , N=102)		P-Value
	No.	%	No.	%	
Gram negative bacilli	**81**	**71.05**	**20**	**19.61**	**<0.001**
Escherichia Coli	6	5.26	0	0.00	0.053
Acinetobacter species	4	3.51	0	0.00	0.160
Klebsiella species	26	22.81	6	5.88	<0.001
Pseudomonas species	45	39.47	14	13.73	<0.001
Gram positive cocci	**24**	**21.05**	**30**	**29.41**	**0.206**
Saphylococcus species	15	13.16	12	11.76	0.918
Streptococcus species	9	7.89	17	16.67	0.077
Enterococcus faecalis	0	0.00	1	0.98	0.956
(NORMAL COMMENSAL)	**9**	**7.9**	**52**	**50.98**	**<0.001**
Micrococci	6	5.26	29	23.53	<0.001
Diptheroids	3	2.63	23	22.55	<0.001