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Conglomeration of Pathology, Oncology and Microbiology from Oral and Maxillofacial Perspective

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ABSTRACT
Ecological community of commensals, symbiotic and pathogenic organisms that share our body space. Alteration in the ecologic population of the microflora results in dysbiosis and are critical determinants of systemic health and diseases, especially in the scenario of immunosuppression. Oral microbiome and chronic inflammation may have a role in carcinogenesis.

INTRODUCTION

The oral cavity has the second largest and diverse microbiota with over 700 species of bacteria. It contains bacteria, fungi, viruses and protozoa. Oral cavity with its various niches is a complex habitat. The microbes colonize the hard surfaces of the teeth and the soft tissues of the oral mucosa[1]. Recent studies indicate that the oral microbiome has essential functions in maintaining oral and systemic health and the emergence of 16S rRNA gene next-generation sequencing (NGS) has greatly contributed to revealing the complexity of its bacterial component[2]. Few studies have characterized the OSCC related oral microbiome. Features of the oral microbiome associated with OSCC have been explored by comparing OSCC patients with healthy controls, or by comparing tumor sites with the surrounding normal tissue[3] . Pushalkar *et al*. studied the saliva microbiome of OSCC patients and proposed their potential application as a diagnostic tool to predict oral cancer[4] Candida auris has emerged as a multi-drug resistant ascomycete yeast. It is easily transmissible and well as persistent on environmental surfaces. It is associated with high mortalities, persistent candidaemia, inconsistencies in testing results, misidentification and treatment failure. This leads to complications in management and prognosis[5]. The aim and objective of this article is:

- To analyze the prevalent microbial population in healthy individuals and patients with oral squamous cell carcinoma using 16s rRNA sequencing and qPCR
- To study, Candida auris in patients with immune suppression (HIV seropositive), denture wearers (diabetics and non-diabetics) and healthy individuals by using qPCR in the Department of Oral and Maxillofacial Pathology, Ragas Dental College and Hospital
- To study the oral microbiome in HIV seropositive and HIV seronegative individuals using 16srRNA sequencing and Qpcr

MATERIALS AND METHODS

- Analysis of oral microbiome was done using 16srRNA sequencing in the following groups

Group A-Healthy individuals (n = 10)
Group B-OSCC (n = 10)

- We further analysed C.auris in HIV seropositive patients (n = 10), denture wearers (n = 10) and healthy individuals (n = 10)

- Analysis of oral microbiome was done using 16srRNA sequencing in the following groups

Group A-HIV seropositive (n = 11)
Group B-HIV seronegative (n = 11)

RESULTS AND DISCUSSIONS

RESULTS I

Distribution of Overall Phyla of Microbes Present in all the Samples:

- PHYLA-Proteobacteria (39%), Firmicutes (22%), Actinobacteria (15%) and Bacteroidetes(12%)
- OSCC-Bacillus, Buchnera, Caulobacter, Clostridium, Corynebacterium, Desulfutomaculum, Enterococcus, Flavobacterium, Gemmata, Hymenobacter, Lactobacillus, Listeria, Lysinibacillus, Marinifilum, Ruminococcus, Streptococcus, Streptomyces and Thermoanaerobacter
- HEALTHY-Bacillus, Enterococcus, Lactobacillus, Massilia, Paenibacillus, Streptococcus
- COMMON-Bacillus, Enterococcus, Lactobacillus and Streptococcus
- Saccharolytic-Bacillus, Buchnera, Clostridium, Corynebacterium, Desulfutomaculum, Enterococcus, Flavobacterium, Gemmata, Hymenobacter, Lactobacillus, Listeria, Ruminococcus, Streptococcus, Streptomyces and Thermoanaerobacter.
- Aciduric-Bacillus, Caulobacter, Clostridium, Corynebacterium, Desulfutomaculum, Enterococcus, Lactobacillus, Listeria, Lysinibacillus, Ruminococcus, Streptococcus
- Aerobic-Buchnera, Caulobacter, Clostridium, Corynebacterium, Gemmata, Hymenobacter, Lysinibacillus and Streptomyces
- Anaerobic-Bacillus, Desulfutomaculum, Enterococcus, Flavobacterium, Lactobacillus, Listeria, Marinifilum, Ruminococcus, Streptococcus

Proteobacteria was the major phyla present in both OSCC patients and healthy individuals. In the OSCC group 22% were obligate anaerobes whereas in the control group only facultative anaerobes were present.

Distribution of Phyla of all the Microbes Present in the Oscc Group: In OSCC patients, Streptomyces was seen both in alveolus (20%) and tongue (20%) whereas Bacillus and Listeria were seen only in the alveolar lesions (30%). Based on the site of the lesion, Streptococcus was the predominant bacteria present in all the sites (tongue (10%), buccal mucosa (20%), alveolus (10%), palate (20%).

D**istribution of Phyla of all the Microbes Present in the Control Group** Proteobacteria was the most common (35%).

RESULTS II

Average Copy Numbers of Candida Auris: The average copy numbers of C. auris was highest in denture wearers with a value of 548401.1 followed by HIV seropositive patients with a value of 474966.4. Candida auris was not detected in 4 out of 10 controls while the rest had a low copy number value of 9792.71.

RESULTS III

Oral Microbiome in Hiv Seronegative Individuals: 102 species were observed including Legionella

Oral Microbiome in Hiv Seropositive: 30 species were observed including Neiserria

Oral Microbiome Common in Hiv Seropositive and Seronegative: Aphanizumen, Betaproteobacterium, Methylococcus.

The oral microbiome is complex and our study shows that there are differences in the microbiome of OSCC subjects and healthy individuals. The data from this study will help us to identify the species which need to be studied further to ascertain their role in oral carcinogenesis. The 16S r RNA is a very powerful tool for comparative microbiome analysis. The BLAST results derived using 16S r RNA, gene DNA sequences was used to identify the evolutionary relationship by a phylogenetic tree. The limitations being it identifies only the Shine-Dalgarno domain, a domain common in Bacteri and Archaea with an overlap with mitochondrial and chloroplast RNA.

CONCLUSION

16S rRNA sequencing using Metagenomic Sequencing is a viable and powerful tool to study the oral microbiome. There are variations in the microbiome in OSCC group compared to the control group. The present study was able to identify the bacterial species that further need to be studied. C. auris an azole resistant fungal pathogen, which is capable of causing invasive fungal infections has been only isolated from blood and has not been identified rom salivary samples from South India or other parts of the world. In this study we have isolated C.auris from HIV seropositive patients, denture wearers and controls and the mean copies/µl was different across the three groups with statistical significance. Though none of our patients with HIV and denture wear showed clinical candidiasis, they had elevated copies of C.auris as compared to controls. So, C.auris could be a salivary commensal, in the South Indian population. In this study, we could not establish a definitive association between the organisms identified in the context of specific HIV infection with oral lesions is probably due to predominant organisms involved in HIV infection being fungal organisms. We conclude by stating the limitation of this preliminary study is small sample size, the sensitivity of the technique and huge number of microorganisms that constitute the oral microbiome.

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