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Original Research Article

A comparative evaluation of microorganisms around dental implants and in subgingival plaque of patients with chronic periodontitis

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ABSTRACT

Introduction: This study was done to compare microorganisms around dental implants inserted in site of missing mandibular first molars and in subgingival plaque of patients with chronic periodontitis.
Materials and Method: This study comprised of 20 chronic periodontitis patients and 20 patients who received dental implants. Subgingival plaque and peri-implant biofilm were sampled in both groups which were evaluated using 454-prosequencing of bacterial V1 to V3 regions of 16S rDNA.
Results: The mean probing depth in group I was 6.8 mm and in group II was 2.4 mm, clinical attachment level in group I was 7.3 mm and in group II was 0 and bone loss in group I was 6.7 mm and in group II was 0. The most predominant microorganisms in group I was Catonella, Desulfovibrio, Mogibacterium, Peptostreptococcus, Actinobacillus actinomycetemcomitans, Porphyromonas gingivalis, Tannerella forsythensis and Treponema denticola. The three most abundant OTUs linked with the implant were Pseudomonas, Leptotrichia hongkongensis and Granulicatella adiacens.
Conclusion: Comparison of subgingival biofilms in patients with chronic periodontitis and biofilms around dental implants revealed significant diversity. It was found that dental implants may alter the composition of microbiome.
Clinical significance: Knowledge about comparison of subgingival biofilms in patients with chronic periodontitis helps in taking preventive precautions for successful out cone of dental implants.

1. Introduction

Most of the dental diseases such as dental caries, gingivitis and periodontitis are caused by bacteria. Dental caries involves dental hard tissue (enamel, dentin, pulp) whereas gingivitis is an inflammation of gingiva and periodontitis is resulting in breakdown of connective tissue surrounding the tooth.[1] A marked vertical or horizontal bone loss and secondarily loss of tooth is hallmark of the disease. The etiology of periodontitis is multifactorial. Apart from role of micro- organism in the disease process, genetic factors and social modulation also play an important role.[2]

The occurrence of bacterial species such as Porphyromonas gingivalis, Treponema denticola, Actinobacillus actinomycetemcomitans (AA) and Tannerella forsythensis are considered to be present in patients with periodontitis. Clinically mandibular first molars are first one to be affected and supposed to be refractory during treatment. Periodontitis is the main reason for early loss of molars. It has been revealed in numerous studies that micro- organisms in cases of refractory periodontitis are different from that seen in cases of chronic

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periodontitis.[3]

Peri- implantitis and peri-implant mucositis is the leading causes of dental implants failure. Several techniques are used in assessing bacterial profile in Peri- implantitis and peri-implant mucositis.[4] Deep-sequencing of 16S rDNA, PCR amplification and DNA-hybridisation tests helps in identification bacterial ecology. Deep-sequencing of 16S rDNA can be useful in detection of multiple samples per sample.[5]

It has been observed that dental implants are the potential site for plaque accumulation. Bacterial accumulation around dental implants results from subgingival plaque.[6] There is variation in occurrence of peri- implant bacterial species around implants in healthy subjects and patients of periodontitis.[7] The presents study compared microorganisms around dental implants inserted in site of missing mandibular first molars and in subgingival plaque of patients with chronic periodontitis.

2. Materials and Methods

This study was done in department of peridontology and oral implantology. This study was conducted among 20 patients of chronic periodontitis of both genders. Inclusion criteria were patients age ranged 18-60 years, non- diabetic, non- hypertensive and patients not on systemic medications. Exclusion criteria were patients above 60 years of age, patients on systemic steroids, smokers and those not giving consent. We also involved 20 subjects who received dental implant in missing mandibular third molar not less than 1 year.

Ethical clearance was obtained before staring the study from ethical committee of the institute. All enrolled patients were informed regarding the study and their consent was obtained. Patients were divided into 2 groups. Group I included chronic periodontitis patients and group II were those who received dental implants.

Periodontal patients were subjected to scaling and root planning, however, mandibular first molar were extracted due to extensive periodontal pockets around it. Peri-implant biofilm around dental implants and subgingival plaque at the first permanent molar before tooth extraction were collected. Samples were stored in 2 ml sterile tube. 100 μl of phosphate-buffered saline was added in all tubes. They were frozen at -80°C prior to sample processing.

All the samples were subjected to DNA isolation with QiaAmp DNA mini kit. DNA numbering was done with NanoDrop 8000 spectro- photometer. The V1 to V3 regions of the primers of the bacterial 16S rDNA were designed to perform pyrosequencing using 454 GS FLX Titanium platform. The forward primer was 5'-AGAGTTTGATCCTGGCTCAG-3' and the reverse primer was 5'-TTACCGCGGCTGCTGGCAC-3'.The Polymerase chain reaction (PCR) test was performed.

Operational taxonomic units (OTUs) revealed and the number of tags per sample, alpha diver sity containing richness estimators (Chao and Ace) and diversity estimators (Shannon and Simpson) were evaluated using QIIME with default parameters. Results of the study were tabulated for statistical analysis which was performed using fishers exact test with level of significance be < 0.05.

3. Results

Table 1 Shows that mean probing depth in group I was 6.8 mm and in group II was 2.4 mm, clinical attachment level in group I was 7.3 mm and in group II was 0 and bone loss in group I was 6.7 mm and in group II was 0.Table 2 shows estimates of sequences and the alpha diversity found with tags, operational taxonomic unit (OUT), Chao, Ace, Shannon and Simpson. The difference between both the groups was significant (P< 0.05).

Figure 1 shows that the microorganisms in group I was Catonella in 65%, Desulfovibrio in 54%, Mogibacterium in 47% and Peptostreptococcus in 38%, Actinobacillus actinomycetemcomitans in 24%, Porphyromonas gingivalis in 20%, Tannerella forsythensis in 21% and Treponema denticola in 15%. Figure 2 shows that the three most abundant OTUs linked with the implant were Pseudomonas in 58%, Leptotrichia hongkongensis in 42% and Granulicatella adiacens in 24%.

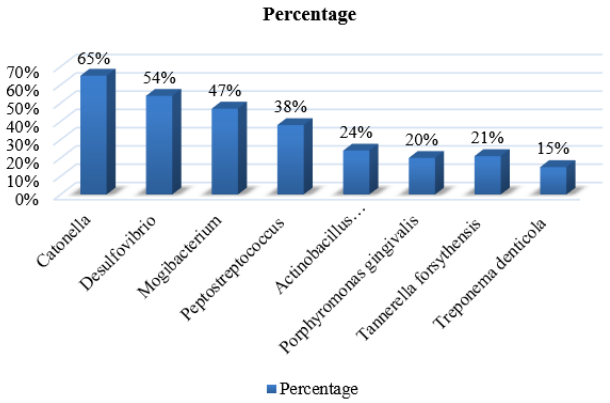

Fig. 1: Assessment of microbiomes in group I

4. Discussion

Periodontitis is most prevalent microbial disease characterized by significant destruction of attachment loss and ultimately loosening and loss of teeth.[8] Microbial species commonly seen in patients with chronic periodontitis is Catonella, Desulfovibrio, Mogibacterium, Peptostreptococcus, Actinobacillus actinomycetemcomitans, Porphyromonas gingivalis, Tannerella forsythensis etc.[9] Numerous periodontal microorganisms may be seen in healthy sites apart from

Table 1: Assessment of parameters in both groups

Parameters (Mean± SD)	Group I	Group II	P value
Probing depth (PD)	6.8± 2.1	2.4± 2.1	0.01
Clinical attachment levels (CAL)	7.3± 3.2	0	0.001
Bone loss (BL)	6.7± 1.4	0	0.001

Table 2: ssessment of estimates of sequences and the alpha diversity

Variables	Group I	Group II	P value
Tags	9846.2± 3412	12120± 4246	0.01
Operational taxonomic unit (OUT)	252.4± 46	210± 52	0.001
Chao	360.8± 58	292± 76	0.001
Ace	378.2± 90	326± 92	0.05
Shannon	390.2± 110	324± 116	0.05
Simpson	0.062±0.12	0.16± 0.02	0.001

Significance, P< 0.05, fishers exact test

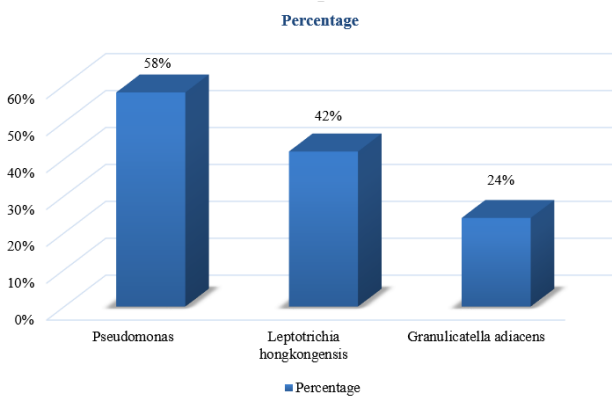

Fig. 2: Assessment of microbiomes in group II

unhealthy site in chronic periodontitis patients which can initiate and propagate periodontitis.[10]

Dental implant therapy has revolutionarized the field of dentistry and it has gained importance in last few years in Periodontics, Oral surgery and Prosthodontics owing to high survival rates. Thus, it is considered to be best treatment option for replacing missing one or multiple teeth. Recent data mentioned difference in occurrence of periodontal microorganisms in diseased as well as healthy individuals.[11] Similarly there in variation in presence of microbial flora in chronic periodontitis patients and in patients with dental implants. It has been observed that dental implants frequently manipulate the bacterial microenvironment. Moreover, studies have mentioned presence of low anaerobic and aerobic bacteria and periodontal pathogens around implants in healthy subjects.[12] The present study was conducted to assess microorganisms around dental implants inserted in site of missing mandibular first molars and in subgingival plaque of patients with chronic periodontitis.

In this study, we found that mean probing depth in group I was 6.8 mm and in group II was 2.4 mm, clinical attachment level in group I was 7.3 mm and in group II was 0 and bone loss in group I was 6.7 mm and in group II was 0. Zhang et al[13] determined the microbia composition of 10 healthy dental implants and 10 chronic periodontitis patients using 454-prosequencing of bacterial V1 to V3 regions of 16S rDNA. There was significant bacterial diversity in chronic periodontitis patients in comparison to implant subjects. The genera Catonella, Desul- fovibrio, Mogibacterium, Peptostreptococcus and Propionibacterium were present in higher abundance in chronic periodontitis subjects, while implant subjects had higher proportions of Brevundimonas and Pseudomonas species.

We found that estimates of sequences and the alpha diversity found with tags, operational taxonomic unit (OUT), Chao, Ace, Shannon and Simpson. The difference between both the groups was significant (P< 0.05). The microorganisms in group I was Catonella in 65%, Desulfovibrio in 54%, Mogibacterium in 47% and Peptostreptococcus in 38%, Actinobacillus actinomycetemcomitans in 24%, Porphyromonas gingivalis in 20%, Tannerella forsythensis in 21% and Treponema denticola in 15%. The three most abundant OTUs linked with the implant were Pseudomonas in 58%, Leptotrichia hongkongensis in 42% and Granulicatella adiacens in 24%.

Huntin et al[14] assessed microbial profile in 17 partly edentulous patients (98 implants) around implants and teeth in patients with peri-implantitis with 19 subjects as controls. Results showed a putative periodontal microflora at teeth and implants in patients and controls as done with microbiological DNA-probe analysis. Patients with peri-implantitis had high levels of periodontal pathogens, Actinobacillus actinomycetemcomitans, Porphyromonas gingivalis, Prevotella intermedia, Bacteroides forsythus and Treponema denticola. These findings indicate a site-specific inflammation rather than a patient-associated specific host

response.

Ong et al[15] examined and assessed microbial flora in 19 patients with periodontitis. Patients were given dental implants (osseointegrated titanium implants). It was found that Actinobacillus actimomycetemcomitans was present in 1 site, and Prevotella intermedia was found in 7 sites. 22 of 37 sites had a greater proportion of anaerobes than aerobes. Authors suggested that the submucosal plaque of implants must be monitored regularly for the presence of these periodontitis-associated species. The drawback of this study is little tested size.

5. Conclusion

Comparison of subgingival biofilms in patients with chronic periodontitis and biofilms around dental implants revealed significant diversity. It was found that dental implants may alter the composition of microbiome.

6. Source of Funding

None.

7. Conflict of Interest

None.

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