Evaluation of subgingival microbiota around single tooth implants

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ABSTRACT

Objectives: 1. To provide information about the subgingival microbiota around single tooth implants. 2. To assess the subgingival microbial flora around the teeth adjacent to single tooth implants. 3. To clinically evaluate the gingival health surrounding the single-tooth implants.

Methods: Patients undergoing the single-tooth implant replacements, were selected as subjects for the study. The natural teeth adjacent to implant sites were taken as control sites. Clinically each peri-implant gingival tissue health was evaluated. Subgingival plaque samples were removed with sterile curette and evaluated for microbial flora, by microscopic examinations. Bacterial cultures of samples studied. The similar procedure was followed for the control sites also. Finally the data collected were statistically analyzed and interpreted.

Results: The subgingival microbiota around single tooth implants was cultured and studied. *Enterobacter* species, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Streptococcus* species were predominantly found. *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* were found more frequently around implant sites than control sites. Anaerobic *Bacteroides* species were found in only one case around the implant site.

Conclusions: Prevention and control of bacterial infection in the peri-implant region are among the key factors in determining the long term success or failure of dental implant therapy. The thorough knowledge about the subgingival microbiota around the healthy and diseased peri-implant mucosa is needed to determine the overall outcome of implant therapy.

1. Introduction

Bacterial infection in the peri-implant region can jeopardize the long term success and survival of dental implant therapy. The information about the peri-implant subgingival microbial flora is needed to determine the outcome of implant therapy. While several factors are considered vital and crucial in determining the long-term success/survival or failure of dental implants, very little thought is given to the microbiological standing of the peri-implant tissues.¹ This clinical study was done mainly to focus on the significance of microbiological status of the peri-implant sulcus in determination of success/survival criteria in single-tooth implant therapy.

2. Materials and methods

Prior approval was obtained from the Ethical Committee and Research Committee of the institution. Informed written consents were obtained from all the participants in this study.

Armamentarium: Armamentarium used for the study is shown in the [Fig. 1]. Patients undergoing the single-tooth implant replacements,

in the Department of Prosthodontics, Mahatma Gandhi Post-Graduate Institute of Dental Sciences - Puducherry were selected as subjects for this study [Fig. 2].

Inclusion Criteria: 1. Patient should have undergone single-tooth implant therapy replacing the missing tooth, in Department of Prosthodontics, Mahatma Gandhi Postgraduate Institute of Dental Sciences, Puducherry. 2. Patient should be free of any systemic diseases which include diseases of endocrine origin, cardiovascular origin, hematological origin and any other debilitating diseases.

Diagnostic Phase: Medical history and dental history were taken. All the cases chosen for the study had a single-tooth implant replacing the missing tooth. The radiographic investigation that was conducted in this study included periapical radiography, panoramic radiography and radiovisuography. Blood investigation were done as it might influence the implant surgery protocol or long term success rate.

Microbial Sample Collection and Culture Procedures: Clinically each peri-implant gingival tissue health was evaluated, by using gingival index modified and plaque index modified. Then presence of any supragingival plaque was detected and removed with help of Hoe scaler. Later, subgingival plaque samples were removed with sterile

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Fig. 1. Armamentarium used for clinical examination and subgingival plaque sample collection.

Fig. 2. Single anterior tooth implant case [replacing missing 11-maxillary right central incisor].

Fig. 3. Subgingival Plaque Collection from implant site using implant curette.

implant curette from implant surfaces [Fig. 3] and placed in sterile normal saline placed on the microscopic glass slides. The subgingival bacterial plaque samples were placed in the anaerobic culture medium – Robertson Cooked Meat (RCM) medium in a screw capped tight glass container. Care was taken while collecting the sample and while transferring it to the medium in such a way that it was done as quickly as possible to avoid the exposure of the anaerobic organism to the air. The microscopic slides were stained by gram staining in the microbiological laboratory of the Department of Clinical Microbiology, Pondicherry Institute of Medical Sciences. These slides were evaluated for microbial flora, by microscopic examinations. The adjacent natural teeth to dental implants were taken as controls. Similar procedures

Fig. 4. Subgingival Plaque Collection from control site [adjacent tooth] using implant curette.

Fig. 5. Materials used for anaerobic bacterial culture.

were followed for the microbial sample collection for the adjacent natural teeth also [Fig. 4]. Then both aerobic and anaerobic bacterial cultures [Fig. 5] were done from this collected sample and bacterial culture growths (colonies) were studied and identified. Thus the subgingival microbiota around the immediately placed single tooth implants in anterior maxilla, around the conventionally placed single tooth implants in posterior mandible and around the natural teeth adjacent to the single tooth implants were evaluated and finally the data collected were statistically analyzed.

3. Results

Various variables in the present study, for the statistical evaluation are age of the patients, region of dental implant placement in the oral cavity, loading time considerations of dental implants, duration of time from implant placement to observation, gingival health of peri-implant area and adjacent natural tooth.

Comparison of the Aerobic and Anaerobic Bacteriological Culture from Sub-gingival plaque (Microbial Colonies Grown) between Implant sites and Control sites was made.

The main statistical tool used in the present study is Pearson's Chi-Square test. This statistical tool is used to find any correlations or associations between two or more variables. The same tool was used to determine Sensitivity, Specificity, Positive predictive value and Negative predictive value of presence of a particular microbial organism at implant site and at control site.

In the present study subgingival microflora associated with dental implants had been shown to be similar to that associated with natural teeth, which was in accordance with findings of previous studies.²⁻⁴

In the present study, subgingival microbial flora around single tooth

Table 1

Association between region and gingival health relative to gingival index modified (GIM). Figure in brackets are percentages to row total. Chi-square is not significant ($p > 0.05$).

REGION	Gingival Index Modified (GIM)		
	Healthy	Mild	Moderate
Anterior	1(20.0)	4(80.0)	0(0.0)
Posterior	4(66.7)	1(16.7)	1(16.7)
Both	5(45.5)	5(45.5)	1(9.1)
Pearson Chi-Square		4.55	$p = 0.10299$

implants was cultured and studied. *Enterobacter* species, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Streptococcus* species were predominantly found.^{3,4} Anaerobic *Bacteroides* species grown only one case out of twenty-two samples (eleven cases).

Microbial flora was correlated between immediately placed single tooth implants in anterior maxilla and around the conventionally loaded single tooth implants. No significant difference noted.

It was observed that there was no statistically significant association between the region in the oral cavity and the gingival health when it was correlated in terms of gingival index modified as well as in terms of plaque index modified (Table 1 & Table 2).

Microbial flora was compared between implant site and adjacent natural teeth (control site). *Klebsiella pneumonia* (Table 3) and *Pseudomonas aeruginosa* (Table 4) were found more frequently around implant sites than control sites.

The statistical evaluation of the data obtained from the present study shown that there was no significant association between the region in the oral cavity and the gingival health as well as loading time of single tooth implants and the gingival health when it was correlated in terms of gingival index modified as well as in terms of plaque index modified. No significant difference in the gingival health across different time duration (time from implant placement to observation).

4. Discussion

In the present study the single tooth implant cases were classified in terms of anterior and posterior teeth region of the oral cavity. The subgingival microbial flora was studied and compared between immediately placed single tooth implant in anterior maxilla and around the conventionally loaded single tooth implant in posterior mandible. Also this was correlated with the gingival health.

The oral hygiene status of all the cases studied was good to excellent (as measured through plaque index modified), which reflected upon gingival and peri-implant tissue health (gingival index modified) and this subsequently resulted in disease free subgingival area around single tooth implants and control teeth.

But the presence of Enterics like *Enterobacter* species, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* should be given attention. Enterics such as *Escherichia coli*, *Enterobacter* species, *Klebsiella* spp. and *Pseudomonas* spp. are infrequently found in the oral cavity and are

Table 2

Association between Region and Gingival Health Relative to Plaque Index Modified (PIM). Figure in brackets are percentages to row total. Chi-square is not significant ($p > 0.05$).

REGION	Plaque Index Modified (PIM)		
	Healthy	Mild	Moderate
Anterior	1(9.1)	3(60.0)	1(20.0)
Posterior	4(66.7)	1(16.7)	1(16.7)
Both	5(45.5)	4(36.4)	2(18.2)
Pearson Chi-Square		2.73	$p = 0.25518$

Table 3

Correlates the identification of *Klebsiella pneumoniae* between control site (adjacent natural tooth) and implant sites. It was observed that there was statistical significant difference in identification of *Klebsiella pneumoniae* between control site and implant sites. Chi-square is significant ($p < 0.05$).

		Control Site		Total (Implant)
		Identified	Not Identified	
Implant Site	Identified	3	2	5
	Not Identified	0	6	6
Total (Control)		3	8	11
Sensitivity				100%
Specificity				75%
Positive predicted value (Identified)				60%
Negative predicted value (Not identified)				100%

Pearson Chi-square 4.95 $p = 0.02610$.

Table 4

Correlates the identification of *Pseudomonas aeruginosa* between control site (adjacent natural tooth) and implant sites. It was observed that there is statistical significant difference in identification of *Pseudomonas aeruginosa* between control site and implant sites. Chi-square is significant ($p < 0.05$).

		Control Site		Total (Implant)
		Identified	Not Identified	
Implant Site	Identified	4	1	5
	Not Identified	0	6	6
Total (Control)		4	7	11
Sensitivity				100.0%
Specificity				85.7%
Positive predicted value (Identified)				80.0%
Negative predicted value (Not identified)				100.0%

Pearson Chi-square 7.54 $p = 0.00603$.

related to periodontitis as well as peri-implantitis cases.^{4,5} Few species of Enterics have developed resistance towards antiseptics as well as antibiotics.⁵

In the present study majority of the micro-organisms cultured were Gram negative, namely *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Enterobacter* species. The Gram positive organisms grown in bacterial culture were *Streptococcus* species (*Streptococcus viridans* also called as α -hemolytic *streptococci*).⁴

The microbial diversity found in the present study should therefore be considered in the long term maintenance of dental implants. These findings should be considered to prevent any kind of infection in the peri-implant tissues and also in treatment strategy of peri-implantitis.

Healthy peri-implant areas have smaller amounts of bacteria dominated by facultative Gram-positive cocci and rods, whereas diseased sites have a microbiota consists predominantly Gram-negative anaerobic rods and black-pigmented *Bacteroides*.^{6–11}

The key factor in the bacterial colonization of the internal part of screw retained implant fixed dental prostheses is the microbial leakage through the gap between the suprastructure and the abutment.¹²

Characteristics of microbiota around peri-implantitis cases have also been studied in depth by various researchers, which concluded that the peri-implantitis cases are result of a mixed anaerobic infection.^{13,14}

5. Conclusion

This clinical study was done mainly to focus on the significance and importance of microbiological status of the peri-implant sulcus in determination of success of single-tooth implant therapy. In the present study, subgingival microbial flora around single tooth implants was cultured and studied. The subgingival microbial flora was studied and compared between immediately placed single tooth implant in anterior

maxilla and around the conventionally loaded single tooth implant in posterior mandible. Also this was correlated with the gingival health.

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Declaration of competing interest

The authors declare that they have no conflicts of interest.

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