Time Dependent Oral Microflora Changes in Removable Partial Denture Wearers

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Abstract

Background: Denture prostheses are significant interventions in oral environment for restoring lost form and function due to tooth loss. Little is known about change in microflora in removable partial denture wearers with age and time since denture delivery. Therefore, the present study was conducted to evaluate and compare the oral microbial flora pre and post insertion of removable partial denture at different time intervals and age groups.

Materials and Method: A sample of 60 patients wearing removable partial denture was included in the study, 30 of age group 40-50 years (Group 1) and 30 of age group 50-60 years (Group 2).Sample of unstimulated saliva was collected from the patient's mouth from the distal most edentulous area using three sterile cotton swabs by one investigator. Colony count and identification of microorganisms was done.

Results: The difference among mean colony counts between group 1 and 2 were not statistically significant at any of the three timeintervals. A statistically significant difference in colony counts was seen between baseline, 1 month and 3 months in whole sample as well as both the age groups. Among various aerobic microorganisms, Streptococcus mitis, Diphtheroids and Candida Albicanswere the most prevailing aerobic micro organism after 3 months of denture use, while among anaerobic organisms, Prophyromonasgingivalis was the most prevalent microorganism after three months.

Conclusion: A significant difference in colony count as well as colonizing microorganisms has been found in the present study. This difference according to time is an important component in the affect of removable partial denture on oral health of denture wearers. Age group was not a significant factor affecting microbial health in RPD wearers.

Keywords: *Oral Microflora, Denture Stomatitis, Removable Partial Denture, Oral candidiasis.*

Introduction

Oral micro-flora plays an important role in both oral health and disease. The diversity of oral micro-flora and its role has been evident since the time when Antonie van Leeuwenhoek first examined the microbiome of dental plaque in 1700s[1]. Since then, the importance of oral micro-flora in oral cavity has been a matter of research. There has been a renewed emphasis on this study lately in wake of an evident and intricate connection of oral micro-flora with general health. At the same time, new frontiers are being reached everyday with role of oral micro-flora in its various aspects on oral health itself.

Oral micro-flora inhabits a unique ecosystem of oral cavity[2]. It may be present either in a suspended form in saliva or attached on the tissue surfaces of the oral cavity, either on mucosal surfaces or on the dental hard tissues[3]. Various areas of oral cavity are influenced by environmental factors differently, giving rise to different

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micro-floral combinations in different parts of mouth. Hence, dental micro-flora is different from the micro-flora found on the palate, gingival crevicular fluid or on tongue[3]. The combination of microbes found in each individual are reported to have interpersonal variations, so much so that some authors have described this uniqueness as being akin to that of fingerprints[3]. In-spite of this variation and diversity, normal micro-flora of the mouth has been determined in the past studies[4]. This has, in part, been possible as certain bacteria have been more commonly related to presence of a disease than others. Therefore, the number and types of microbes in oral cavity change with the presence of oral or systemic disease.

Salivary micro-flora may be an indicator of oral disease, but the mucosal micro-flora, more commonly on teeth and gingiva is more commonly associated with oral health and disease. Microbial flora of plaque biofilm has been found to have a causal relationship with dental caries[5] and periodontal disease[6]. Similarly, microorganisms associated with dental calculus are responsible for development of periodontal disease. Similarly, micro-flora present on denture prosthesis may indicate oral health and disease.

Denture prosthesis are significant interventions in oral environment for restoring lost form and function due to tooth loss. Change in oral environment owing to dentures may trigger a change in oral microflora, both quantitatively as well as qualitatively [7]. Certain types of bacteria have been shown to be associated with denture health and disease. Also, oral micro-flora on denture and teeth surfaces both has been found to be similar in both healthy and diseased oral cavity. Therefore, microbial changes associated with dentures are not only indicative of mucosal health, but may also lead to development of dental disease in remaining teeth[8]. Increase in microbial count has been seen after denture insertion. A recent study has shown that pathogenicity of Staphylococcus aureus increased post denture delivery as phagocytic efficiency against Staphylococcus aureus was demonstrably lesser against the strains present in plaque than in normal mouth.

Ecological change in oral cavity because of removable prosthesis is said to be disadvantageous. Investigations[8, 9,10,11] gives the idea that the physical presence of a denture brings a microbiological change, in localized region. Increased occurrence of caries and periodontal disease increases plaque formation caused by the denture in patients treated with removable partial prosthesis. This makes the person more prone to caries and periodontal disease.[5,6] Further, changes in oral micro-flora has also been demonstrated with age.

An estimated 21.4% prosthesis use has been observed in individuals aged 15 to 74 years. In 55 to 64 year-old group, 22. 2% were found to wear removable partial denture. Patients using removable partial dentures has been high in the past and is expected to continue in the future.[9] Therefore, prosthesis use among geriatric population remains high. This coupled with changing micro-flora and reducing immunity may make an more susceptible to oral disease. However, little has been known about change in micro-flora in removable partial denture wearers with age and time since denture delivery[12,13,14,15]. Understanding this relationship may be helpful in instating measures for preventing oral disease is geriatric removable partial denture users. Therefore there is a need to study and get clarity on changes in oral microbial flora with and without removable partial dentures in different age groups. Therefore, the present study was conducted to evaluate and compare the oral microbial flora pre and post insertion of removable partial denture at different time intervals and age groups.

Material and Method

Study design and setting

The present study was conducted among 60 patients who needed removable partial dentures as a part of their prosthetic treatment at department of prosthodontics in a teaching hospital in northern India. Ethical approval was taken from the institutional ethics committee (IEC letter no. MRDC/2017/IEC/12).

Selection Criteria: Inclusion criteria- Patients within age range from 40 to 60 years who required removable partial dentures for at least five missing teeth were included in the study.

Exclusion Criteria: Those who had systemic disease such as Diabetes Mellitus, Hyperthyroidism, Osteoporosis or who had consumed antibiotics at any time in six months preceding screening were excluded from the study.

Method of collection of data: Sample size- A sample of 60 patients wearing removable partial denture was included in the study. 30 were between age group 40-

50 years (Group 1) and remaining 30 were between age group 50-60 years (Group 2). The participation in study was voluntary and an informed consent was obtained before recruiting patient into the study. The participant was provided an information sheet explaining the aim of the study.

Study Procedure: The swab method was employed for collecting salivary samples [16]. Sample of unstimulated saliva was collected from the patient's mouth from the distal most edentulous area using three sterile cotton swabs by one investigator. Unstimulated saliva samples were collected from the patients at three time intervals-

T0 -At the time of denture insertion

T1 -After one month of use of denture

T2- After three months of use of denture.

The collected swab was shaken manually to permit the uniform distribution of microorganisms. The culture media used were blood agar, Sabouraud's dextrose agar (SDA) medium, mitis salivarius, brain heart infusion (BHI) broth andthioglycollate broth. First swab was inoculated in blood agar plate for the purpose of colony count using spread plate technique[11]. Inoculation was done using a sterilized spreader (bent glass rod). Second swab was inoculated in BHI broth for isolation and identification of aerobic microbes and third swab was inoculated in Thioglycollate Broth for isolation and identification of anaerobic bacteria. In the incubator, the culture plates were incubated at 37ºC for 24 hours.

Colony count & Identification of Micro organisms

The bacterial colonies in blood agar were seen and counted using Digital Colony Counter: After 24 hours of incubation the saliva sample from BHI broth was inoculated in Mitis salivarius agar medium and dextrose agar (SDA) medium for identification of aerobic microbes and from thioglycollate broth to blood agar for identification of anaerobic bacteria. The blood agar plates were put for incubation in an Mckintosh and Filde's anaerobic jar with anaerobic pouch for one week at 37ºC.

Depending upon the colony morphology, different types of colonies were marked in the culture plates. Gram staining, preliminary tests and conventional biochemical tests were performed on the marked colonies taken from the culture plates for identification of the microbe.

Gram positive and gram negative microbes were identified. Catalase test was done as preliminary test for identification of microbes. Conventional biochemical tests for microbial identification were also performed. These were Methyl Red and Voges-Proskauer test, Methyl red test, Voges-Proskauer test, Urease test, Bile esculin test, Sugar fermentation test, Germ tube test for identification of Candida albicans and Coagulase Slide and tube test.

Each of these tests was performed on the salivary samples obtained from the patients at three different intervals. Quantitative outcome measures used for comparison among age and time groups were colony counts, and percentage of Streptococcus mitis, Streptococcus mutans, Streptococcus salivarius, Enterococcus faecalis, Staphylococcus aureus,non pathogenic Staphylococcus,non pathogenicdiptheroids, Candida albicans and candida sp. for aerobes. For anaerobes, percentages for Actinomyces, Lactobacillus and Porphyromonas were determined.

The data was compiled in Microsoft Excel and analysed using SPPS software Version 23 (IBM Inc., Chicago). The Data was subjected to statistical analysis for calculations of mean, standard deviation and percentages. ANOVA was used to find the interaction effect between the age groups and time intervals. To find the significance of study parameters, t-tests and chi-square test were used to compare the mean values in different age groups and at different time intervals. P-value less than 0.05 considered as significant at 95% confidence level.

Results

The present study recruited 60 removable partial denture patients, 30 patients each in the age group of 40 to 50 years (Group 1) and 50 to 60 years (Group 2). The mean age of group 1 patients was 45.77+2.47 and 55.73+2.20 years for group 2 patients. The average age for all the study participants was 50.75+5.53.

Colony counts were performed for different aerobic and anaerobic microbes present in saliva. Mean colony count at baseline was 125.03+15.12 for 40 to 50 years age group and 126.57+19.69 for 50 to 60 yearsgroup . At first month, it was 220.00+32.44 for age group 1 and 215.67+28.70 for age group 2, while at three months, the colony count for group 1 was 288.00+25.48 and for group 2 was 281.17+21.64 (Table 1).

Table 1. Differences in Baseline, 1 month and 3 month colony count among age groups

	Mean Colony Count Group 1	Mean Colony Count Group2	T value	P value
Baseline	125.03	126.57	-0.388	0.736
1 month	220.83	215.67	1.307	0.196
3 month	288.00	281.17	1.120	0.268

The difference among mean colony counts between group 1 and 2 were not statistically significant at any of the three timeintervals. A Statistically significant difference in colony counts was seen between baseline, 1 month and 3 months in whole sample as well as both the age groups. The mean colony count was found to be higher in Group 1 as compared to group 2 except at the first month (Table 2).

Table 2. Colony Counts at baseline, 1 month and 3 months.

Total Sample						
		N	**Mean**	**Std. Deviation**	**t-value**	**p-value**
Colony Counts	Baseline	60	125.80	17.42	20.548	<0.001
	1 Month	60	220.83	30.81		
	1 Month	60	220.83	30.81	26.062	<0.001
	3 Months	60	284.58	23.69		
	Baseline	60	125.80	17.42	45.143	<0.001
	3 Months	60	284.58	23.69		
Group 1						
		N	**Mean**	**Std. Deviation**	**t-value**	**p-value**
Colony Counts	Baseline	30	125.03	15.12	15.056	<0.001
	1 Month	30	226.00	32.44		
	1 Month	30	226.00	32.44	18.445	<0.001
	3 Months	30	288.00	25.48		
	Baseline	30	125.03	15.12	31.807	<0.001
	3 Months	30	288.00	25.48		
Group 2						
		N	**Mean**	**Std. Deviation**	**t-value**	**p-value**
Colony Counts	Baseline	30	126.57	19.69	14.153	<0.001
	1 Month	30	215.67	28.70		
	1 Month	30	215.67	28.70	18.282	<0.001
	3 Months	30	281.17	21.64		
	Baseline	30	126.57	19.69	32.319	<0.001
	3 Months	30	281.17	21.64		

Among various aerobic microorganisms, Streptococcus mitis, Diphtheroids and Candida Albicanswere the most prevailing aerobic micro organism with 9% of prevalence, after 3 months of using denture. On the other hand among anaerobic organisms, Prophyromonasgingivalis was the most prevalent microorganism after three months. Tables 3 and 4 showed the number of colonies at three time intervals among two age groups for aerobic and anaerobic journals respectively.

Table 3. Number of aerobic colonies according to age groups and time since denture insertion

	Group 1 (N)	Group 2 (N)	Total (N)
Streptococcus Mitis1	16	19	35
Streptococcus Mitis2	18	15	33
Streptococcus Mitis3	27	25	52
Streptococcus Mutans1	10	11	21
Streptococcus Mutans2	11	14	25
Streptococcus Mutans3	10	17	27
Streptococcus Salivarius1	0	1	1
Streptococcus Salivarius2	1	1	2
Streptococcus Salivarius3	0	2	2
Enterococcus Faecalis1	0	1	1
Enterococcus Faecalis2	1	2	3
Enterococcus Faecalis3	2	2	4
Staphylococcus aureus1	8	3	11
Staphylococcus aureus2	2	5	7
Staphylococcus aureus3	3	5	8
CoNS1	20	15	35
CoNS2	17	15	32
CoNS3	10	15	25
Diphtheroids1	11	17	28
Diphtheroids2	15	17	32
Diphtheroids3	25	22	47
Candida Albicans 1	16	20	36
Candida Albicans. 2	18	19	37
Candida Albicans 3	28	26	54
Candida1	14	10	24
Candida2	12	11	23
Candida3	2	3	5

Table 4. Number of anaerobic colonies according to age groups and time since denture insertion

	Group 1 (N)	Group 2 (N)	Total (N)
Actino1	2	3	5
Actino2	9	11	20
Actino3	18	19	37
Lacto1	7	11	18
Lacto2	11	15	26
Lacto3	14	14	28
Porphyro1	18	18	36
Porphyro2	16	21	37
Porphyro3	26	25	51

Discussion

The present study is first of its kind that compares the oral micro-flora among RPD wearers of two age groups at three different time intervals. A significant difference in colony counts was observed in this study at all the three time intervals. The reason for this difference may be the presence of spaces between the prosthesis and tissue surface where and saliva accumulation may cause an increased microbial count. In this study, the number of colony forming units increased from pre insertion to 1 month and 3 months after denture insertion in both the groups. The colony counts in the total sample increased with time. The increase was greater from baseline to the first month as compared to increase from first to third month. The reason may be the balancing of oral micro-flora into a more stable environment as compared to an initial growth phase. Tales et al (2012) have observed in their study that redevelopment of microbial biofilm on both natural as well as denture teeth is faster after cleansing. The faster initial development phase may be connected to this phenomena[17]. The increase in colony count was highest in both the groups from baseline to the first month. However, among group 1 and group 2, the increase in colony count was lesser in group 2 as compared to group 1 from baseline to the first month. Increase was greater in group 2 as compared to group 1 from first month to third month post denture insertion. In contrast, a randomized controlled trial was conducted by Valentini et al. (2015), which concluded that time period did not cause a change in micro-flora associated with denture liners[18]. This indicates that there is a difference in growth of micro-flora on prosthesis according to difference in materials used.

Complete and removable partial dentures are the most common dental prosthesis given worldwide to rehabilitate missing teeth in form and function. Replacement of missing teeth is crucial for various physiological functions like chewing food, speaking, pronouncing words, swallowing food[19]. It is also important aesthetically as well as affects self-esteem of an individual. Whereas removable partial denture prosthesis has been found as an adequate treatment option for partially edentulous patients, these patients, being mostly geriatric need to be assessed for general health and other features while making a decision for removable partial denture prosthesis to be provided to them[20, 21, 22]. Geriatric patients have a greater tendency to changes in quantity, quality as well as flow rate of saliva[23,24]. Factors such as reduced immune responses,

susceptibility to infections and impaired host-defense mechanism should be considered before the prognosis of removable partial denture prosthesis is decided.

In partially edentulous mouths, the amount and type of microbes present and affect of denture on their quantity and quality should, therefore, be studied. The study by Tanaka et al (2010), determined that there was an increase in lactobaccilus count in saliva with increased number of missing teeth among geriatric RPD wearers[25]. Therefore, there a connection between RPD wear and change in oral micro-flora seems inevitable. However, there may be different aspects to it. O'Donnell et al (2015) also state that there is a difference in oral micro-flora in denture wearers according to the location of denture[26]. According to this study, *Bacilli and Actinobacteria* were the species that more commonly inhabited oral cavity in denture wearers. Moreover, the presence of natural teeth has a significant impact on the overall microbial composition, when compared to the fully edentulous. Furthermore, increasing levels of Candida spp. positively correlated with Lactobacillus spp. In the present study, increase was more prominently seen in *Porphyromonas* and *Actinobacteria* among anaerobic, and S.*mitis,* diptheroids and *Candida albicans*. This indicates that some microorganisms grow faster than others in a RPD environment. A study of microbiota at implant surface by Eick et al (2016) showed an increased number of Tannerella forsythia, Parvimonas micra, Fusobacterium nucleatum/necrophorum, and Campylobacter rectus on implant surface. These organisms were not found associated with RPD in the present study, therefore may be associated with growth of microorganisms associated with implant- gingival interface[27]. Also, a study conducted by Derafshi et al. (2017) observed that in addition to oral bacteria, certain non-pathogenic non oral bacteria were present in patients using removable dentures. These were Enterobacter cloacae, Raoultellaornithinolytica, Raoultellaplanticola, Kluyvera spp., and Enterobacter aerogenes[28]. Another study by Ella et al (2013) compares difference in predominance of oral streptococcus species in saliva of dentulous, edentulous, partial and complete denture wearers. The result of this study showed preponderance of Streptococcus milleri in removable partial denture group[29]. Therefore, a wide variety of pathogenic and non pathogenic microorganisms have been found to be associated with use of removable partial denture.

There is a lack of literature on affect of time on oral micro-flora of removable partial denture wearers. Effect of age on micro-flora of the oral cavity has been previously studied[30], however the effect of time since an individual has been wearing removable partial denture prosthesis is not known. The present study has been able to show that there is a significant difference in colony counts and microbial predominance with time. Further studies are recommended to study this aspect.

Conclusion

Health of teeth as well as oral soft and hard tissues has an inevitable association with oral micro-flora. Any change in micro-flora is bound to affect oral health. A significant difference in colony count as well as colonizing microorganisms has been found in the present study. This difference according to time may be an important component in the affect of removable partial denture on oral health of denture wearers and should be considered in cases with denture stomatitis and other infections of oral cavity associated with RPD wear.

Ethical Clearance: Ethical approval was taken from the institutional ethics committee (IEC letter no. MRDC/2017/IEC/12).

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