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Microbiological Analysis Of The Saliva Of Chronic Periodontitis Patients Before And After Scaling And Root Planing For Selected Pathogens - A Clinical Study

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Abstract

Aim: The purpose of the study was to analyse the microbiological count of selected pathogens in saliva of chronic periodontitis patients before and after scaling and root planing using Multiplex Polymerase Chain Reaction.

Materials & Method: Twenty patients in the age group of 20-50 years, suffering from generalized chronic periodontitis, were included in the study. At baseline, gingival index (GI-Loe and Silness), probing pocket depth (PPD) and clinical attachment level (CAL) were recorded and saliva samples were collected. After collecting baseline data, the scaling and root planing was completed and the patients were recalled after 4 weeks. All the clinical parameters were again recorded and saliva samples were again collected. The saliva samples thus collected were sent to the laboratory for microbiological analysis.

Results: The results showed that the count of the periodontopathogens in saliva of chronic periodontitis patients was decreased after scaling and root planing. The count of bacteria that were found after treatment was also less as compared to the pretreatment count. With the decrease in count of bacteria there was decrease in probing depth and increase in the clinical attachment level. Thus, the clinical conditions of the gingiva were improved.

Conclusion: It was concluded that saliva may be used as a diagnostic method for the detection of Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Fuobacterium nucleatum and Tannerella forsythia in chronic periodontitis patients.

Key Words

Saliva, Polymerase Chain Reaction, Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Fuobacterium nucleatum and Tannerella forsythia

Introduction

Periodontal disease is a multifactorial disease and strong associations have been reported between periodontal disease and certain microorganisms in the literature. The most important periodontal pathogens associated with periodontal disease are Tannerella forsythia, Porphyromonas gingivalis, Treponema denticola, Prevotella intermedia, Fusobacterium nucleatum and Aggregatibacter actinomy cetemcomitans [1].

Recent advances in molecular biology have enabled the identification of specific bacteria in large numbers of periodontitis subjects. The polymerase chain reaction (PCR) is the most accepted technique and is commonly used for the identification of periodontopathic bacteria in many laboratories. Polymerase Chain Reaction (PCR) assay has the potential for being an ideal detection method of periodontal microorganisms. It is relatively easy to perform and demonstrates excellent detection limit and very little cross reactivity under optimal conditions[2].

Detection of periodontopathic microorganisms in the oral cavity can be done by taking samples of GCF, plaque and saliva. But as the microbiota may vary significantly from site to site, a large number of pockets may have to be examined to confirm or exclude the presence of specific periodontopathic species. As the periodontal pocket bacteria are continuously washed into saliva by gingival crevicular fluid, a whole saliva sample may offer a rapid and easy alternative to individual pocket samples for determining subgingival bacterial presence.

Periodontal therapy aims to prevent, arrest, control or eliminate periodontal disease. It includes scaling, root planing and surgery, which are aimed at improving clinical conditions by lowering the microbial load either by physical removal of plaque or by radical alteration of the subgingival habitat.

The aim of the present study was to analyze the microbiological count of selected pathogens i.e Porphyromonas gingivalis (Pg), Fusobacterium nucleatum (Fn), Tannerella forsythia (Tf) and Aggregatibacter actinomycetemcomitans (Aa) in saliva of chronic periodontitis patients and the comparison of count of bacteria in unstimulated saliva before and after scaling and root planing using multiplex Polymerase Chain Reaction (PCR).

Materials & Method

A total of 20 patients, aged between 20-50 years, suffering from generalized chronic periodontitis were selected amongst those reporting to the Out Patient Department of Periodontology, Guru Nanak Dev Dental College and Research Institute, Sunam.

Systemically healthy people suffering from generalized chronic periodontitis with probing pocket depth of 4-6 mm, atleast at one site in each quardrant were included in the study.

Pregnant and lactating women, patients who received periodontal treatment or antibiotics atleast 6 months prior to participation in the study and smokers were excluded from the study.

Method

All patients were informed of the purpose of the study. At baseline, gingival index (GI), probing pocket depth (PPD) and clinical attachment level (CAL) were recorded. Patients were instructed to avoid alcohol for 12 hours before sample collection, and foods with high sugar or acidity, or high caffeine content, immediately before sample collection, since they may compromise the assay by lowering saliva pH and increasing bacterial growth.

Method Of Saliva Collection

The subjects were asked to rinse mouth with 15 ml of water to wash out exfoliated cells. The subjects were then asked to sit comfortably in dental chair with eyes open, head tilted forward slightly. They were asked to rest for 5 minutes before commencing unstimulated saliva collection and minimize orofacial movements. Saliva was collected in the mouth while patient kept his/her lips closed. The subjects were then asked to expectorate in a sterile vessel by using spitting method given by Navazesh M in 1993[3]. About 0.5 ml of unstimulated mixed saliva was transferred in an Eppendorf tube containing 1.5 ml of tris EDTA.

Then scaling and root planing was done. All patients were instructed not to use any mouth rinse and only normal oral hygiene procedures were permitted. The patients were recalled at the end of 4 weeks and all the clinical parameters were recorded and saliva samples of the same patients were again collected.

The saliva samples thus collected were sent to the laboratory for detection of Aggregatibacter actinomycetemcomitans , Porphyromonas gingivalis, Tannerella forsythia and Fusobacterium nucleatum by Multiplex Polymerase Chain Reaction. The data thus obtained was compiled and put to statistical analysis.

Data Analysis

Quantitative data was presented as mean ± SD or median and interquartile range, as appropriate. Normality of quantitative data was checked by measures of Kolmogorov Smirnov tests of normality. For skewed data Wilcoxon signed rank test was applied for pre to post comparisons. For normally distributed data Paired t-test was applied. All calculations were two sided and performed using SPSS version 17 (Statistical Packages for Social Sciences, Chicago, IL). A p-value of <0.05 was considered to indicate statistical significance.

Table I : Comparison Of Gingival Index, Probing Pocket Depth And Clinical Attachment Level At Baseline And After Four Weeks

	At Baseline (Mean) ± Standard Deviation (Mm)	After Four Weeks (Mean) ± standard Deviation (Mm)	P –value	Significance (S)
Mean Gingival Index (Gi)	1.94 ± 0.36	1.13 ± 0.17	< 0.001	Hs
Mean Probing Pocket Depth (Ppd)	4.03 ± 0.68	2.63 ± 0.77	< 0.001	Hs
Mean Clinical Attachment Level (Cal)	8.28 ± 1.53	6.92 ± 1.69	< 0.001	Hs

HS- Highly Significant

Table II : Count Of Different Bacteria In All The Subjects At Baseline And After Four Weeks

S No.	Fn Baseline	Fn After 4 Weeks	Aa Baseline	Aa After 4 Weeks	Pg Baseline	Pg After 4 Weeks	Tf Baseline	Tf After 4 Weeks
1	-	-	40 × 103	2 × 103	-	-	18 × 103	-
2	300 × 103	0.8 × 103	-	-	8 × 103	6 × 103	-	-
3	20 × 103	1 × 103	-	-	55 × 103	-	-	-
4	1.5 × 103	-	-	-	2000 × 103	3.6 × 103	-	-
5	20 × 103	-	-	-	-	-	10 × 103	-
6	1.3 × 103	-	-	-	-	-	3.1 × 103	2 × 103
7	8 × 103	0.3 × 103	-	-	50 × 103	1.5 × 103	-	-
8	-	-	2 × 103	-	-	-	-	-
9	1.2 × 103	-	-	-	90 × 103	5 × 103	-	-
10	-	-	-	-	-	-	4 × 103	-
11	28 × 103	1.2 × 103	-	-	600 × 103	2.3 × 103	-	-
12	-	-	180 × 103	3 × 103	2000 × 103	-	-	-
13	30 × 103	1 × 103	-	-	73 × 103	-	800 × 103	3 × 103
14	-	-	-	-	20 × 103	-	9 × 103	2 × 103
15	26 × 103	1.2 × 103	39 × 103	0.6 × 103	210 × 103	4.2 × 103	-	-
16	-	-	10 × 103	3 × 103	31 × 103	-	-	-
17	-	-	1.2 × 103	-	-	-	-	-
18	1.8 × 103	-	-	-	480 × 103	1.3 × 103	-	-
19	220 × 103	0.8 × 103	-	-	2 0 × 103	-	-	-
20	1.3 × 103	-	3 × 103	1 × 103	-	-	2.2 × 103	-

Observations And Results

For the study presence of particular bacteria was evaluated as positive or negative and numbers of copies were calculated pretreatment and post-treatment.

The comparison of mean gingival index (GI) at baseline and after four weeks was done with the application of Wilcoxon Signed Ranks test. The pre and post treatment mean value and standard deviation was found to be 1.94 ± 0.36 and 1.13 ± 0.17 mm respectively and this was found to be highly significant (p-value <0.001) when compared. Comparison of mean probing pocket depth at baseline and after four weeks was done by applying t-Test. It was seen that there was significant reduction (p-value <0.001) in the mean post treatment probing pocket depth as compared to the mean pre treatment probing pocket depth. Similarly when comparison of mean clinical attachment level (CAL) at baseline and after four weeks was done, the results were found to be highly significant (HS) with the pre and post treatment mean clinical attachment level ± standard deviation (S.D) 8.28 ± 1.53 and 6.92 ± 1.69 mm respectively **(Table I)**.

It was observed that the number of patients having all the four bacteria was more before treatment than after treatment. Also the mean pretreatment count of bacteria was more than after treatment **(Table II)**.

Pretreatment prevalence rate of four bacteria (Fn, Aa, Pg and Tf) were 65%, 35%, 65% and 35% respectively. After treatment their prevalence rates were reduced to 35%, 25%, 35% and 15% respectively **(Table III)**.

Using Wilcoxon Signed Rank test the baseline mean value of bacteria was compared with after four weeks value. The results showed significant decrease in all the four bacteria with highly significant (p- value 0.001) decrease in Fn and Pg bacteria whereas Aa and Tf bacteria showed significant difference

Table III : Percentage Prevalence Of Different Bacteria In All Subjects At Baseline And After Four Weeks

Bacteria	At baseline	After four weeks
Fn	65%	35%
Aa	35%	25%
Pg	65%	35%
Tf	35%	15%

Table IV : Comparison Of Count Of Different Bacteria At Baseline And After Four Weeks

Bacteria	At Baseline (Mean) ± Standard Deviation	After Four Weeks (Mean) ± Standard Deviation	P -value	Significance (S)
Fn	32.96× 103 ± 79.44	0.32× 103 ± 0.47	0.001	Hs
Aa	13.76× 103 ± 40.94	0.48× 103 ± 0.99	0.018	S
Pg	281.85× 103 ± 609.85	1.20× 103 ± 1.95	0.001	Hs
Tf	42.32× 103 ± 178.40	0.35× 103 ± 0.88	0.018	S

HS- Highly Significant, S- Significant

(p- value 0.018) **(Table II and IV)**.

Discussion

Periodontal disease encompasses a group of biological phenomenon that occur within the gingival sulcus. The World Workshop on Clinical Periodontics (1996) described Aggregatibacter actinomycetemcomitans (Aa), Porphyromonas gingivalis (Pg) and Tannerella forsythia (Tf) as relevant microorganisms associated with periodontal breakdown4. Fusobacterium species (Fn) are associated with coaggregation of initial colonizers and late colonizers resulting in plaque formation. Aggregatibacter actinomycetemcomitans is a gram negative, capnophillic, coccobacillus bacterium that produces a number of virulence factors. Porphyromonas gingivalis is a gram negative, rod shaped anaerobic, black pigment forming bacterium. Tannerella forsythia is a gram negative, anaerobic, spindle shaped bacterium which has been reported to express a number of enzymatic and proteolytic activities that contribute to its ability to compete effectively in the complex biofilm.

The worsening or improvement of periodontal status is accompanied by a shift in bacterial composition of subgingival plaque. It has therefore been suggested that microbial testing can be used for diagnosis, to optimize periodontal therapy and to assess its outcome[5],[6].

The difficulties in culturing fastidious oral pathogenic bacteria had led to the development of new approved technologies with faster and more accurate detection. A multiplex Polymerase Chain Reaction (PCR) was used in this study to analyze the presence of putative periodontopathic bacteria due to its improved ability to detect these microorganisms.

The sample materials like subgingival plaque, saliva and GCF have been used for the detection of periodonto pathogenic bacteria. Subgingival plaque samples and GCF samples have to be collected from particular sites. So, these are difficult to collect as compared to saliva[7],[8]. Also, saliva is an easily and inexpensively accessible biological fluid which provides an excellent sample for large scale studies of oral microbial profiles. During the collection of stimulated saliva, mastication increases the total number of bacteria shedding from all oral surfaces including buccal mucosa, cheeks and tongue so, the exact count of the periodontal bacteria is not determined[9]. Moreover, the stimulated saliva mainly consists of saliva from parotid gland (major salivary gland) and it is not representative of the subgingival bacterial profile. Therefore, unstimulated saliva was collected in the study.

According to Lowenguth RA and Greenstein G in 1995, assessment of the periodontium to mechanical non-surgical therapy should be performed no sooner than 3-4 weeks following treatment to allow for soft tissue healing and maturation. So, post treatment data and saliva samples in the present study were collected 4 weeks after scaling and root planning[10].

There was a statistically significant improvement in gingival index 4 weeks after scaling and root planing. The reason behind the decrease in the mean gingival index was the improvement in the gingival conditions after the non surgical periodontal therapy by alteration in the subgingival ecological environment through disruption of microbial biofilm and suppression of inflammation[11]. Bleeding on probing is present during active phase of periodontitis which means sulcular or pocket epithelium is no longer intact being more permeable to lipopolysaccharides and other bacterial products[12]. As quoted by Badersten A et al in 1984 that bleeding on probing was encountered more often in pockets with deeper probing depths than in the shallow depths so, bleeding on probing was decreased after the therapy[13].

A significant decrease in the mean probing pocket depth and gain in the clinical attachment level after scaling and root planning was seen. Wennstrom JL et al in 2005 stated that intention for SRP is to remove microbial biofilm, calculus and contaminated root cementum or dentin to prepare root surface which is biocompatible for soft tissue healing. After instrumentation, inflammation in the pocket epithelium and underlying connective tissue is reduced and a new dentogingival junction forms within 2 weeks. This results in the marked reduction in clinical signs and symptoms of disease[14]. So, the periodontal sites showed improved gingival conditions as a result of decreased inflammation, decreased probing pocket depth and gain in attachment level following treatment.

The pretreatment percentage prevalence of Aa was 35% and it reduced to 25% after scaling and root planing (Table III). SRP reduced the levels of microorganisms, by removing the plaque and calculus. Various studies such as by Takamatsu N et al (1999), Mombelli et al (2000) and Doungudomdacha S et al (2001) showed similar results[15],[16],[17]. At baseline Aa was found in 7 subjects whereas after treatment 5 subjects were found to have Aa and in those 5 subjects the count was significantly reduced (p-value = 0.018) than it was before treatment (Table IV). This may be due to the high tissue penetrating ability of Aa, that it is not completely eliminated after SRP. Aa produces a pathogenic product, epitheliotoxin which is capable of directly causing tissue destruction. It may assist Aa in penetrating the sulcular epithelium and gaining access to the underlying connective tissue[18]. Incomplete elimination of Aa after mechanical instrumentation has provided the rationale for the adjunctive use of antimicrobial agents in Aa associated periodontitis[10]. The prevalence rate of P.gingivalis before treatment was 65% (13 subjects) which was reduced to 35% (7 subjects) after the treatment **(Table III and Table IV)**. More than half of the subjects initially positive for this organism were also positive after treatment, although the number of bacteria significantly reduced (p-value = 0.001) **(Table IV)**. The fimbrae of P.gingivalis are a critical factor for mediation of interaction of this bacterium

with host tissues, as they promote both bacterial adhesion and invasion of targeted sites. So, it was not completely eliminated after the treatment. Sandros J et al in 1993 and Karima MM in 2010 stated that P.gingivalis exhibits multiple adhesive properties and co-exists in large numbers in the deep pockets. They have shown that P.gingivalis is related very strongly to clinical parameters such as bleeding on probing. Similarly, we observed that as pretreatment P.gingivalis was present in more number of subjects, the pockets were found to be deeper but as the count of the organism was decreased post treatment there was improvement in the gingival conditions with decrease in PPD and GI and gain in CAL[19].

T.forsythia was present in 35% (7 subjects) of patients before the treatment and 15% (3 subjects) of the patients after the treatment **(Table III)** but the count was significantly reduced (p-value = 0.018) in all the three subjects **(Table IV)**. The overall detection frequency of T. forsythia in periodontal pockets was significantly correlated to the probing pocket depth. Klein MI and Goncalves RB in 2003 found that T. forsythia was detected more frequently and with higher numbers in deeper periodontal pockets. So, its prevalence was lower at shallow sites and in successfully treated individuals[20].

In our study percentage prevalence of F.nucleatum was 65% (13 subjects) pretreatment and it reduced to 35% (7 subjects) **(Table III)**. The comparison of mean count of Fn before and after treatment was found to be highly significant (p-value = 0.001) **(Table IV)**. It is found in the shallower pockets after the treatment and it acts as a bridge between the early and the late colonizers. It can also secrete serine proteases that provide nutritional requirements to other organisms (like P.gingivalis and T.denticola). These proteases are found to degrade elements of the periodontal connective tissue and host defense systems such as immunoglobulins and complement. Proteases can inactivate key components of the plasma proteinase cascade, blood clotting systems and degrade serum protease inhibitors[21].

The prevalence rate of all the four bacteria reduced in unstimulated saliva after the mechanical therapy which was in accordance with study conducted by Takamatsu N et al in 1996. They used a DNA probe and polymerase chain reaction to accurately measure levels of Aa, Pg and Bf in subgingival plaque samples before and after 1 month of initial therapy. The results showed the prevalence of Aa as 30.8%, Pg as 84.6% and Tf as 100% before the treatment and after treatment their prevalence rates reduced to 19.2%, 50.0% and 65.4% respectively[15].

In our study, a positive correlation has been found between the clinical parameters i.e GI, PPD and CAL and the detection frequencies of the organisms in the unstimulated saliva. The relation of these microorganisms with the increasing probing pocket depth may be due to higher levels of anaerobiosis (decreased oxygen tension) at deeper sites, difference in subgingival temperature or requirement of other substances (such as hemin) for their growth thereby, providing a more conducive environment for their growth[22].

The sampling of saliva is considerably simple and has many advantages in terms of collection, storage, shipping and voluminous sampling providing an excellent biological medium for large scale studies of oral microbial profiles. According to Zhang L et al in 2009 saliva is widely regarded as a microbial reservoir. The bacterial community in saliva correlates clearly with that of other niches in the oral cavity. It has been suggested that detection of certain bacterial species in saliva reflects their presence on the tongue, within dental plaque and periodontal pockets. Due to significant transit of biomarkers from GCF to saliva, the latter has been considered a relevant medium for monitoring periodontal status[23].

Saliva reflects the presence of microorganisms in diseased condition and after SRP the count of bacteria was reduced and with this reduction there was improvement in the gingival conditions and achievement of periodontal health. Thus, it seems reasonable to employ unstimulated saliva samples to screen for the presence of periodontal pathogens in the human oral cavity. Also, in search for non-invasive, inexpensive and easily run sampling method for the detection of periodontopathogens unstimulated saliva may prove useful.

Conclusion

The four target periodontopathogens were found in the saliva of chronic periodontitis patients and their number reduced after resolution of inflammation and return of periodontal tissues to health.

It may be concluded that unstimulated saliva may be used as the diagnostic fluid for the detection of periodontal pathogens and to prognosticate the periodontal treatment thereby providing excellent biological medium for large scale studies of oral microbial profiles.

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