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Determination of Streptococcus Mutans in Healthy Versus Chronic Periodontitis Patients – A Clinicomicrobiology Study.

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Abstract
Aim: *The aim of study is to examine whether MS persist within saliva and subgingival environment of periodontitis patients and to determine whether there is any association between MS colonisation and periodontal disease.*
Methods and Material: *The study was conducted on 40 patients who reported to Department of Periodontology at College of Dental Sciences & Research Centre in January to March 2017. Male/Female non-smokers aged between 20-50 years were randomly allocated to two groups: Group A (n=20) were Healthy patients & Group B (n=20) were patients with CGP (Chronic generalised periodontitis). Unstimulated saliva was collected in sterile containers and subgingival plaque samples were collected from four deepest pockets in CGP and from first molar in Healthy patients. All samples were cultured on TYCSB agar media with bacitracin for quantification of MS.*
Results: *Increased colonisation of MS were seen CGP in both samples and also a positive correlation seen with periodontal parameters.*
Conclusion: *More severe forms of periodontal disease may create different ecological niches for proliferation of MS. Therefore, root caries or proximal caries will increase the risk of CGP.*
Keywords: *Chronic periodontitis, Colonisation of MS, healthy, saliva, subgingival plaque.*

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I. Introduction

Dental plaque is also known as dental biofilm and mass of microbes grow on surface within the mouth and it is etiologic factor for periodontal disease.[1]

Periodontal disease is defined as an inflammatory disease of supporting tissues of teeth caused by specific microorganisms or groups of specific microorganisms, resulting in progressive destruction of the periodontal ligament and alveolar bone with periodontal pocket formation, gingival recession or both.[2] It is now widely accepted that periodontal diseases are infections in which specific bacteria play an important role. The continuous deposition of dental plaque accumulation may result in an imbalance between pathogenic species and the host defence mechanisms, which may lead to gingival inflammation.[3-5]

The bacterium streptococcus mutans (MS) is generally accepted to be the principal etiological agent of dental caries due to its high cariogenic potential.[6] Higher levels of MS have been associated with a higher risk for dental caries.[7] Thus, microbiological diagnosis of some of these specific pathogens should improve our ability to identify individuals or sites at risk for developing oral diseases.

According to Banting et al (1980)[8], root surface caries is a common disease in adults. Hix & O'Leary (1976)[9] also suggested that gingival recession, in most instances caused by periodontal disease, is considered to be a prerequisite for the development of carious lesions on root surfaces, although root surface caries is not necessarily a sequel of recession in all individuals. The development of root surface caries may be found among the etiological factors, the oral microflora and the diet. Other factors known to affect the coronal caries process, such as the salivary secretion rate, salivary buffer effect and oral sugar clearance time, should also receive due attention in patients who have exposed root surfaces and thus, may be at risk for root surface caries development.

A positive correlation is seen between root caries prevalence and a diseased periodontium.[10] Root caries lesion is initiated on a root surface exposed to the environment; such exposure is, therefore, a prerequisite for this type of decay. Exposure may occur as a result of periodontal disease or vigorous tooth brushing or as a side effect of periodontal treatment. The relationship of root caries to periodontal health have used different definitions of root caries and different ways to describe a subject's periodontal condition.[11]

According to Ravald & Birkhed (1992)[12], it was proved that exposed root surfaces after active periodontal treatment are at risk for root caries. Based on multiple regression analysis it has been reported that root caries occurrence before periodontal treatment, root plaque scores and the number of teeth, but not salivary MS counts, were good predictors for new root caries lesions.

The aim of study is to examine whether MS persist within the saliva and subgingival environment of periodontitis patients and to determine whether there is any association between MS colonisation and periodontal disease.

II. Material and Methods

The study was conducted in Department of Periodontology at College of Dental Sciences & Research Centre, Ahmedabad, Gujarat in January to March 2017. Samples processing and all other laboratory procedures were done at Department of Oral Pathology and Microbiology at the College of Dental Sciences and Research Centre, Ahmedabad.

Sources and Samples: Samples were collected from forty patients out of which 20 were of control group A (Healthy) and 20 were of test group B having (chronic generalised periodontitis (CGP)) seeking periodontal treatment at College of Dental Sciences and Research Centre, Department of Periodontology and Implantology, Ahmedabad.

Inclusion Criteria for the study were based on the following condition:
Healthy patients were selected on basis of absence of gingival inflammation and loss of attachment, probing depth < 3 mm. CGP patients were selected as having loss of attachment, periodontal pocket depth > 5mm. Patients were informed about the procedure and an informed consent form was signed by them.

Exclusion criteria for the study were based on the following condition:

Individuals included in the study were systemically healthy and had not undergone any periodontal therapy in last six months, antibiotic therapy within last three months. Pregnant or lactating woman, smoking, patients on corticosteroids and chemotherapeutic were excluded from the study.

Clinical parameter recorded:

- Plaque index (Quigley and Hein 1962): presence/absence of Plaque scored by running a probe along the tooth surface.
- Gingival index (Loe H and Sillnes 1963): to assess the severity of gingivitis.

Armamentarium for procedure:

Gloves, face mask, periodontal probe, mouth mirror, green cloth, autoclave, saliva container, disposable petri plates, laminar air flow, pre-calibrated round loop-2mm (Hi-media), microbial incubator, and stereomicroscope.

III. Methods

1). Saliva sample collection:

10 µl of unstimulated saliva was collected during morning (Figure 1). Individuals were instructed not to drink, eat, smoke, chew chewing gum or brush their teeth for at least 30 minutes before examination. Subjects were comfortably seated and after few minutes of relaxation, they were trained to avoid swallowing saliva and asked to learn forward and spit all saliva they passively produced into a saliva container. After 2 minutes of rest, saliva was passively flowed into saliva container. Saliva samples were plated on culture plates comprising mitis salivary agar culture media with bacitracin for quantification of the MS colonies.

Figure 1: Saliva sample collection

2). Subgingival plaque samples:

After careful removal of supragingival plaque, subgingival plaque samples in periodontitis patients (Group A) were taken from four deepest periodontal pockets, one in each quadrant, using 1 sterile curette per site. Plaque samples from healthy participants (Group B) were taken from the mesial aspect of all the four first molars. The curette was transported in 2 ml reduced transport fluid media. These samples were transferred to the Laboratory of Microbiology and processed within 24 hours at 37 °C. Each samples were plated TYCSB agar with Bacitracin for quantification of MS colonies. All the culture plates were incubated for 24 h. Mutans streptococci were identified based on their growth and characteristic colony morphology on TYCSB. (Figure 2 to 7)

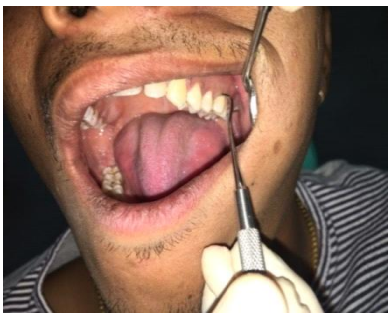

Figure 2: Subgingival plaque sample collection in Healthy patients.

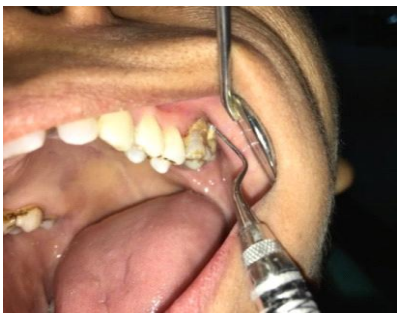

Figure 3: Subgingival plaque sample collection in CGP patients.

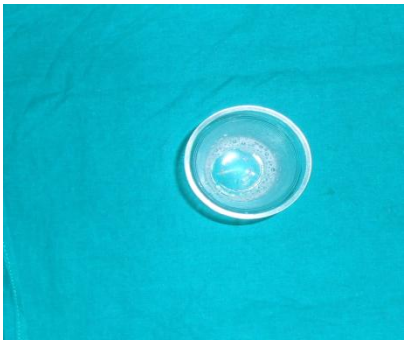

Figure 4: Samples mixed in normal saline.(Both unstimulated saliva and subgingival plaque samples were mixed into 0.9% normal saline).

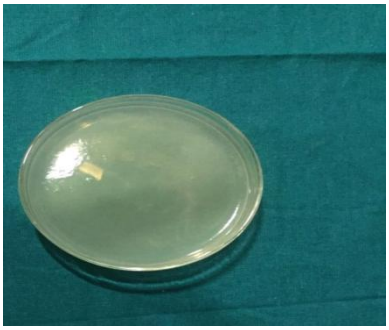

Figure 5: TYCSB agar with bacitracin culture media.

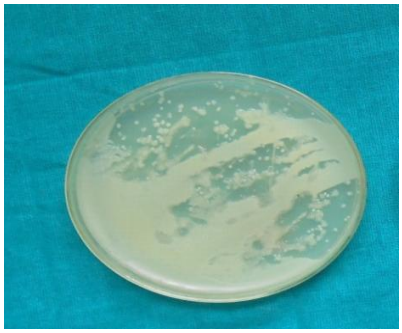

Figure 6: Colonisation of MS under specific aerobic condition.

Figure 7: Smear stained with gram stain prepared for gram positive MS and identification of under microscopy.

MS (On TYCSB agar media): Rough colonies were identified by their irregular margin. Surface of culture plated showed frosted glass like appearance. (Figure 8)

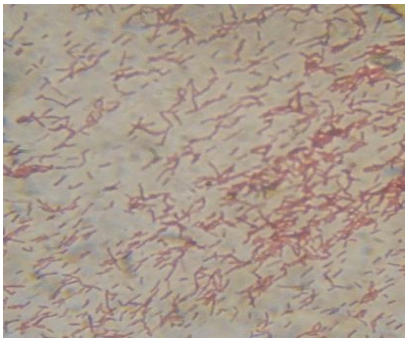

Figure 8: MS colony morphology.

IV. Data analysis

Clinical data from all patients were entered in an excel format and unpaired student't' test was used to compared the saliva and subgingival plaque samples in Healthy and CGP patients. *P*- value was 0.05, which was statistically significant.

V. Results

The study groups comprised a total of forty participants, categorized into two groups – healthy and CGP. Inter and intra group comparison of MS count in saliva and subgingival plaque sample was done. The highest colonies of MS were seen in CGP patients followed by control patients. Similar to saliva samples, the highest colonies were seen in CGP patients followed by control patients in subgingival plaque sample.

Table1: Summarizes the intra-group comparison of streptococcus mutans counts in saliva and subgingival plaque samples of patients with Group A (Healthy) and Group B (CGP).

	Group	n	Mean	Std. Deviation	**P* value
MS in saliva	Group A (Healthy)	10	657.50	199.359	0.001
	Group B (CGP)	10	726.95	300.377	
Ms in subgingival plaque	Group A (Healthy)	10	66.80	17.606	0.000
	Group B (CGP)	10	428.90	179.831	

Intragroup comparison in saliva and subgingival plaque samples, MS count was statistically significant.
MS: Streptococcus mutans, CGP: Chronic generalized periodontitis.

Bar chart representing the influence of the suggested bacterial colony count in saliva and subgingival plaque samples in both group A (Healthy) and group B (CGP).

Table 2: Inter group comparison of MS count in group A (Healthy) and group B (CGP) patients.

Group		Mean	SD	*p value
Group A (Healthy)	MS in Saliva	657.50*	199.36	0.394
	MS in plaque	66.80*	17.606	
Group B (CGP)	MS in saliva	726.95*	300.78	0.395
	MS in plaque	428.90*	179.831	

Inter group comparison in MS was not statistically significant.
MS: Streptococcus mutans;
CGP: Chronic generalized periodontitis.

Bar chart representing the influence of the suggested bacterial colony count in both group A (Healthy) and group B (CGP) patients.

Table 3: Summarizes comparison of periodontal parameters between two groups Healthy and CGP.

	n	Mean	*p value
PI			0.000
Group A(Healthy)	10	0.440	
Group B(CGP)	10	2.040	
GI			0.000
Group A(Healthy)	10	0.765	
Group B(CGP)	10	2.605	

PI and GI between two groups were statistically significant.
PI: Plaque index;
GI: Gingival index.

Bar chart representing the comparison of plaque index (PI) and gingival index (GI) in both groups Healthy and CGP.

Mean MS of saliva in CGP group was 726.95 mm and mean MS of subgingival plaque in CGP group was 428.90 mm while mean MS of saliva in healthy group was 657.50 mm and mean MS of subgingival plaque in healthy group was 66.80 mm. On intragroup comparison between plaque and saliva samples within each group, there was a statistical significant difference seen in Healthy and CGP patients. More colonies of MS were detected in saliva samples. (Table 1)

Intergroup comparison between plaque and saliva samples, show no statistically significant difference. (Table 2)

The mean plaque index in chronic periodontitis group was 2.040 mm and mean gingival index was 2.605 mm while the mean plaque index in healthy group was 0.440 mm and mean gingival index was 0.765 mm. Plaque index and gingival index between two groups were statistically significant. (Table 3)

VI. Discussion

Dental caries and periodontitis are two of the most common oral diseases. Furthermore, both diseases share many social and behavioural background factors in common, which have been related to their etiology.[13] In present study, mean **MS of subgingival plaque** in CGP group was higher compared to Healthy group in both intra and inter group (Table 1 and 2) because if frequency of fermentable dietary carbohydrates intake were to increase, so plaque would production in subgingival area in CGP patients. Such conditions would favour the proliferation of MS lead to faster rates of acid production, enhancing demineralization further (Loesche 1986).[14] A higher presence of MS in periodontitis may derives from low oxygen tension within the periodontal pocket, which favours growth of MS (Dekeyser 2005).[15]

Immunoglobulin A (IgA) is the predominant immunoglobulin isotope in unstimulated saliva. Salivary IgA has been shown to absorb and affect the adhesion of oral microorganism. Mean **MS of unstimulated saliva** in CGP group was higher compared to Healthy group in both intra and inter group (Table 1 and 2). Because of salivary IgA antibodies reacting with antigen from specific bacteria such as MS. Some such reactions could be more advantages from a protective aspect, as they could interfere with important virulence or adhesion factors of the bacteria. However, the studies by KollKlais (2005)[16] suggested that a strong inverse relationship in proportion of aerobic and anaerobic as well as gram-positive and gram-negative microorganisms was found in periodontally diseased sites of CGP patients and in healthy sites of healthy subjects. So, the oral commensals may play an important in suppression of microbiological balance in the oral cavity. De Soete (2005)[15] observed that MS to produce large amount of acids, which decrease the pH. Bacteria generally have a relatively narrow pH range for growth that may influence their intraoral distribution. One might speculate that the changes in

microbial composition after an initial periodontal therapy could resulted in more favourable growth condition for MS. Thus, there occurs a positive relationship between MS and incidence and/or prevalence of root caries.

Rickard et al. (2006)[17] suggested that bacteria growing in microbial communities adherent to tooth surface demonstrate the regulation of expression of specific genes than those present in biofilm. The quorum sensing system of MS in biofilm may enable them to survive obligatory periodontal pathogens that are present in periodontal pockets. Thus, in the oral biofilm, multiple species can coaggregate to colonize the tooth surface by metabolisation of substrates. A central gram – negative filaments core supports the out coccal cells, which are firmly attached by interbacterial coaggregation. After cleaning teeth, MS compete for colonizing saliva coated enamel or dentin mediated by the expression of adhesion. Periodontitis associated microorganisms can coexist with MS and survive acidic conditions constrained by interspecies interactions.[2] On correlating the counts with periodontal parameters, both healthy and CGP groups showed statistically significant between MS in saliva and plaque with gingival index (GI) and plaque index (PI). The possible explanation that the depth of the periodontal pocket increases, there is larger surface area for bacterial colonisation. Another study observed that plaque index score and gingival index score were at highest in periodontitis patients who still have to receive initial periodontal therapy. So, mean high proportion of PI and GI in periodontitis patients because of this suggested that MS increase in subgingival area.[18]

VII. Conclusion

In this study, a higher colonization of MS was seen in saliva and subgingival plaque samples of periodontitis patients. In addition, a positive correlation was seen between periodontal parameters and MS count in chronic periodontitis. Further study to elucidate the relationship between the proportional shift of subgingival mutans streptococci and the progression of root caries is needed to implement a root caries prevention protocol in periodontal practice.

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