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Original Research

Salivary streptococcus mutans level in patients before, during and after fixed orthodontic treatment in Bengalee population

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ABSTRACT:

Total 50 patients from Bengalee populations of both sexes and 12 to 20 years age group were studied in the present study. To evaluate the levels of salivary streptococcus mutans before during and after fixed orthodontic treatment in Bengalee population. It was seen that the average value significantly increased from 2.53 x 10^6 CFU/ml before fixed orthodontic treatment to 8.73 x 10^6 CFU/ml during fixed orthodontic treatment. Again it was significantly decreased to 1.67 x 10^6 CFU/ml after fixed orthodontic treatment. Therefore the increase was temporary. Careful attention would be necessary during fixed orthodontic treatment to prevent occurrence of enamel demineralization and development of white spots (early carious lesion).

Key words: S.mutans, saliva, fixed orthodontic treatment.

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INTRODUCTION

The oral cavity is a reservoir for vast numbers of bacteria and other microorganisms. More than 250 species of microorganisms have been identified as part of the flora of gingival crevice. In a healthy oral cavity, these microorganisms coexist in a balanced state with their host. But when changes occur in the normal oral environment, the balanced flora changes and imbalance may result. Such changes can be brought about by the introduction of orthodontic appliances. Metallic orthodontic brackets and bands provide increased retention sites for plaque forming microorganisms. Also fixed orthodontic treatment makes maintenance of oral hygiene more difficult. Together these induce specific changes in the oral environment such as decreased pH and increased plaque accumulation, 1–3 elevated S mutans levels which may be responsible for decalcification of teeth and dental caries.[1,4,9,11]

If the elevated level of S.mutans remains unchanged after the orthodontic appliances have been removed from the patient's mouth. The patients will continue to be at increased risk for development of caries following orthodontic treatment. It will be considered as a hazard of orthodontic treatment. So, this type of study is required in Bengalee population to evaluate the S.mutans level in saliva before, during and after orthodontic treatment. It will help to determine the caries risk level and the necessary of preventive measurements.

The aims and objectives of the present study was

1. To measure the streptococcus mutans level in patients, before during and after fixed orthodontic treatment in Bengalee population.
2. To evaluate the probability of developing caries in those patients following orthodontic treatment

MATERIAL AND METHOD

The present study was carried out in the Department of Orthodontics of Dr. R. Ahmed Dental College and Hospital, Kolkata. The microbiological studies were done in the

Department of Microbiology of N.R.S. Medical College and Hospital, Kolkata.

Selection Criteria

1. Patients having malocclusion and requiring fixed orthodontic treatment.
2. No prosthodontic appliances in the mouth.
3. Absence of severe systemic disease.
4. No history of any antibiotic or antimicrobial therapy within the past three months.

10 subjects were considered as control.

Preparations advised to take before sample collection

The patients were advised not to brush their teeth in the morning on the day in which the samples were collected, and not to take any foods.

Collection of Sample

The samples were collected in the Department of Microbiology of N.R.S. Medical College & Hospital, Kolkata. Morning sample of saliva before brushing of teeth were obtained, following salivary stimulation by chewing a piece of paraffin for one minute, in a sterile glass test tube. After collection each sample was sonicated for one minute and vortexed for 30 seconds to disperse the microorganisms present in the sample. Each sample was then serially diluted from 10^1 to 10^6 in 0.5 mol/lit phosphate buffer (pH 7). From each dilution 25 µl was spoted with the help of a micropipette on one third of a selective growth medium, mitis salivarious agar, which was contained 20% sucrose and was supplemented with 0.2 U/ml bacitracin.**(Fig.1)**

Inocculation was done following the standard procedure as mentioned in Mackie and McCartney[7]. Each plate was then incubated in CO_2 enriched environment (candle jar) at 37°C for 24 to 48 hours. The dilutions having from 20 to 100 colony forming units were counted with the aid of 20 times magnification. As the mitis salivarious agar was supplemented with bacitracin which can only permit the growth of S. mutans, so only the presence of typical colony following incubation was almost confirmatory for the above mentioned organism. No further biochemical tests were done to confirm the isolated Streptococcus mutans.

Composition of Mitis Salivarious Agar Base

Ingredients	Gms/Litre
Casein enzymic hydrolysate	15
Peptic digest of animal tissue	05
Dextrose	01
Sucrose	50
Dipotassium Phosphate	04
Trypan Blue	0.075
Crystal violet	0.0008
Agar	15
Final pH (at 25°C)	7.0 ± 0.2

Composition of Phosphate Buffer[7]

Stock Solution:

A. 0.2M solution of monobasic sodium phosphate (31.29 NaH_2PO_4, $2H_2O$ in 1000ml).
B. 0.2 M solution of dibasic sodium phosphate (28.39 gm of Na_2HPO_4 or 71.79 of $Na_2HPO_4.12H_20$ in 1000 ml)

39 ml of A solution + 61 ml B solution diluted to total 200 ml give rise to disisered phosphate buffer solution of pH7.

Inoculating loop: Nichrome wire loop of 4 mm diameter was sterilized by red hot flaming.

Colony morphology of Streptococcus mutans

On sucrose containing media like mitis salivarious agar Streptococcus mutans produces extracellular polysaccharide, giving characteristic opaque rough white colonies usually not strongly adherent to the agar and sometimes surrounded by 'wet' (water-soluble) glucan polymer. **(Fig.2)**

The smear was prepared from the typical colony and was stained with gram stain.

Gram-stain morphology

S.mutans cells are gram positive 0.5 to 0.75 µm in diameter. Sometimes elongated with short reds, occurring in pairs or in short chains. **(Fig.3)**

Figure 1: mitis salivarious agar

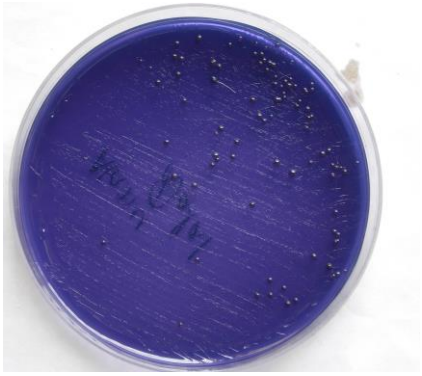

Figure 2: water-soluble glucan polymer

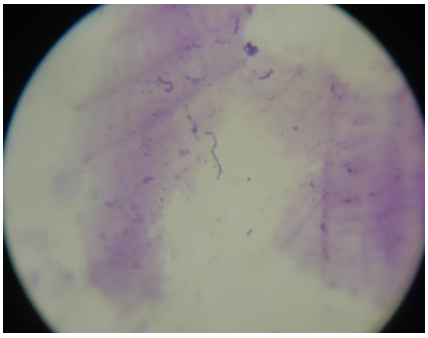

Figure 3: S.mutans cells are gram positive

RESULTS

The data were collected after microbiological examination and statistical analysis were performed. The results and observations were as follows:-

Table 1: **Comparison of salivary S.mutans levels before, during and after fixed orthodontic treatment, in Bengalee population.**

Stages of treatment	Sample Size (n)	Average Salivary S.mutans level (x) x 10^6	S.D.	S.E.	Range	95% confidence interval
Before treatment	15	2.53	0.247	0.064	2.2 to 2.9	2.40 – 2.67
During treatment	15	8.73	0.753	0.194	8.0 to 10.0	8.32 – 9.15
After treatment	15	1.67	0.195	0.050	1.4 to 2.0	1.56 – 1.77

Table 2: **Comparison of salivary S.mutans level at different stages of fixed orthodontic treatment**

Salivary S.mutans level at different stages of treatment (Average x 10^6 CFU/ml)			Comparison by 't' test		
Before treatment (I)	During treatment (II)	After treatment (III)	(I) Vs. (II)	(I) Vs. (III)	(II) Vs. (III)
2.53	8.73	1.67	t = 30.31	t = 10.67	t = 35.16

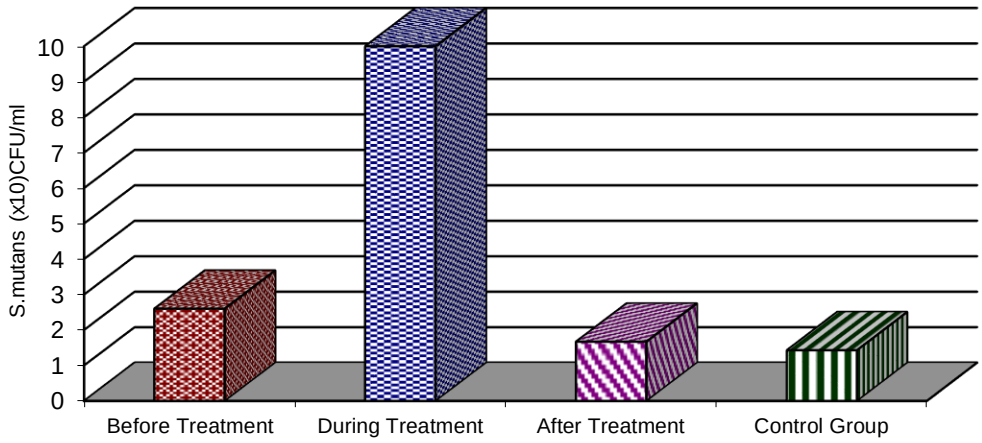

Fig. 4: **Average salivary S.mutans level before, during and after fixed orthodontic treatment per milliliter of saliva.**

In control group the range (minimum to maximum) of salivary S.mutans lies with $1.2x10^6$ CFU/ml to $1.6x10^6$ CFU/ml. From table-1, it could be seen that 95% confidence interval of the average salivary S.mutans at all the three stages of treatment (i.e., before, during and after fixed orthodontic treatment) are higher than the average control values. So it can be said that the average salivary S.mutans level of the experimental group at all the three stages of fixed orthodontic treatment are all significantly higher than the control values ($p<0.05$).

DISCUSSION

Role of Streptococcus mutans in initiation and progress of dental caries is well known.**12,13 (Underwood and Miller[32]Clark[34])** Saliva has been used to monitor the total oral load of these micro-organisms[2,10] **Richardson L. et al.[30]** Br Dent J. 1987 Feb 7; 162(3):103-6.

A marked increase in salivary level of S. mutans therefore imposes greater caries risk.

Previous studies have shown that patients who receive orthodontic treatment were more susceptible to white spot lesions (early carious lesion) adjacent to brackets.[15]**Boyd R.L., Leggott P.Q. and Quinn R.S. et al[3]** wrote a literature that, orthodontic appliances simultaneously make maintenance of oral hygiene more difficult and more important because improper oral hygiene may initiate dental caries.

Williams R.A.D., Bowden, G.H., Hardie, J.M. and Shah, H.[16] in the year **1975** said that the identification of S.mutans was based largely on colonial morphology on selective culture media suchas mitis salivarius agar, supplemented by one or two tests like fermentation of mannitol.

In this study the salivary levels of S.mutans before fixed orthodontic treatment in Bengalee population have been found out with the range of 2.2×10^6 CFU/ml to $2.9x10^6$ CFU/ml with S.D. of 0.247 (Table 1).During fixed orthodontic treatment the range was 8.0×10^6 CFU/ml to 10×10^6 CFU/ml and the mean value was 8.73×10^6 CFU/ml with S.D. of 0.753 (Table 1).After fixed orthodontic treatment the value was within the range of 1.4×10^6 CFU/ml to 2.0×10^6 CFU/ml. The mean value was 1.67×10^6 CFU/ml with S.D. of 0.195 (Table 1).

In the control group the salivary level of S.mutans were within the range of 1.2×10^6 CFU/ml to 1.67×10^6 CFU/ml and the mean value was 1.43×10^6 CFU/ml.

Similar study was done by Richard G. Rosenbloom, DMD, and Norman Tina off, DDS, MS; Farmington, Cone in the year 1991.[11] In their study the average value of S.mutans during fixed orthodontic treatment was $8.3x10^6$ CFU/ml and after fixed orthodontic treatment, the average value was 1.9×10^6 CFU/ml.

From the above findings it could be seen that the average value of S.mutans in saliva during fixed orthodontic treatment was increased markedly when compared with pre treatment value or with the control group. These elevated levels of S.mutans (due to increased retention sites for S.mutans and their adhesive property) during fixed orthodontic treatment might be responsible for the formation of white spot (early carious lesion) or demineralization. Corbett J.A. et al[5] in the year 1981 have done the comparison of S.mutans concentrations in

banded and non-banded orthodontic patients. They observed higher value in banded orthodontic patients. Mattingly J.A. et al[8] have also shown the enhancement of S.mutans colonization by direct bonded orthodontic appliances in the year 1983. This increased level of S.mutans during fixed orthodontic treatment indicates that careful attention should be given to prevent development of white spot (early carious lesion) or occurrence of enamel demineralization. Particularly in fixed orthodontic patients who have poor oral hygiene or increased carious activity.

After fixed orthodontic treatment the average value of S.mutans was less than that during fixed orthodontic value and even less than pretreatment value. The possible explanation of these findings might be after removal of the fixed appliances and correction of malocclusion (e.g. crowding) the maintenance of oral hygiene becomes more easier. It was also a significant finding that though the salivary level of S.mutans increased markedly during fixed orthodontic treatment it was temporary.

The preventive measurement that might be taken during fixed orthodontic treatment:-

1. Proper brushing.
2. Mouth rinsing with 0.2% Chlorhexidine Gluoconate to prevent plaque accumulation.
3. Prevention of decalcification could be done by daily self treatment with 0.4% SnF_2 gel.[13,14]
4. Mouth rinsing with SnF2 and NaF.
5. Interproximal plaque formation could be reduce by topical application of acidulated phosphate fluoride.
6. Treatment time should be as short as possible.

CONCLUSION

In the studied population of Bengalee subjects, requiring fixed orthodontic treatment, Streptococcus mutans level in saliva during treatment period (with bands and brackets) is significantly more than pre treatment value. However this raise is temporary and post treatment salivary level of S mutans is even lower than pre treatment value suggesting improved condition for better performance of oral hygiene practice. If adequate measures are not taken to combat the raise in S mutans level with the fixed appliance in mouth, the treatment period is sufficient to cause enamel demineralization (early carious lesion or white spot lesions).

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