ORIGINAL RESEARCH

A QUANTITATIVE EVALUATION OF STREPTOCOCCUS MUTANS IN DENTAL CARIES AT DIFFERENT SITES

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Running Title: Quantitative evaluation of streptococcus mutans in dental caries

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ABSTRACT

BACKGROUND: *Streptococcus mutans* have been strongly associated with the initiation of dental caries (DC). Their quantification and identification may be helpful for early intervention measures. **OBJECTIVES**: We conducted the study with an aim to evaluate the

colony counts of *S. mutans* with respect to the location of DC and correlate their prevalence with the age of the patient. **METHOD**:The study population comprised of 120 patients with DC. They were divided into two groups according to age, each comprising of 60 patients. The swab samples were collected and organisms were isolated using Mitis Salivarius Bacitracin (MSB) Agar. Manual counting of colonies on plates illuminated by transmitted light was done. Results were summarized and analysed statistically. **RESULTS**: The prevalence of caries was found to be more in children, with an increased incidence in females. The posterior teeth and occlusal/incisal surface were found to be affected more commonly in both the groups. The mean colony count of *S. mutans*was significantly higher in Group I as compared to Group II. **CONCLUSION**:Bacterial colony counts may help in taking early intervention measures against specific organisms and thereby prevent the development of new carious lesions.

Keywords: Dental caries, Streptococcus mutans, occlusal caries, smooth surface caries

INTRODUCTION

Streptococcus mutans are major cariogenic organisms because of their ability to produce significant amounts of glucan and acid that may alter the pH of saliva. They may survive at low pH. Their ability to modulate sugar metabolic pathways with irreversible binding to teeth is an important mechanism of dental caries (DC) pathogenesis[1].

Taking into account the important role of *S. mutans*in the etiopathogenesis of DC, their quantification and identification may be helpful for epidemiological and early intervention measures. We conducted the study with an aim to evaluate the colony count of *S. mutans* with respect to the location of DC and to correlate the prevalence of *S. mutans* colony count with the age of the patient.

MATERIALS AND METHODS

The study population comprised of 120 patients divided into 2 groups according to their age. Group I comprised of 60 patients aged between 5-15 and Group II comprised of 60 patients aged 16 years and above. The informed consents were obtained after the patients and their parents were informed of the study and related procedures. Approval from the Institutional EthicsCommittee had been obtained prior to the study.

Dental caries was diagnosed clinically by means of visual and tactile methodssupplemented by the use of Intra Oral Periapical Radiographs. Patients having signs and symptoms of DCwere included in the study and those with evidence of periapical infections, periodontal infections, systemic diseases and individual undergoing fluoride treatment were excluded from the study.

Mitis Salivarius Bacitracin (MSB) Agar Base (Himedialabs, Mumbai) was used for *S. mutans*. 0.2 units/ml bacitracin was added to Mitis Salivarius Agar. 90.07 grams of agar powder was suspended in 1000 ml of distilled water and was heated till boiling to dissolve the medium completely. Autoclaving at 15 lbs pressure (121°C) for 15 minutes was done for sterilization. Then the solution was cooled to 50-55°C and 1 ml of sterile 1% Potassium Tellurite Solution was added. The mixture was mixed well and poured into sterile petri dishes for bacterial culture.

Salivary samples along with caries debris were collected under sterile conditionfrom patients having signs and symptoms of DC using sterile cotton swabs. The swabs were inserted at carious sites, kept for 1 to 2 minutes, taken out and placed in a sterile vial containing 1 ml saline for transportation. A loopful (10 µl) of the saliva along with caries debris was streaked on different isolation media. The inoculated plates were incubated at their respective temperature *i.e.S. mutans*for 48 hours at 37^0 C. After the bacterial cultivation, the bacterial count of *S. mutans* was done in Colony Forming Units (CFUs) and was recorded as CFU/ml x 10^3 for each sample. Manual counting of colonies on plates illuminated by transmitted light was done.

Theresults weretabulated and statistical analysis was done. Data was summarized as Mean ± SE (standard error of the mean). Comparison of groups was done usingStudent's t test and two way analysis of variance (ANOVA). Tukey's post hoc test was used to determine the significance of mean difference within and between the groups after ascertaining normality by Shapiro and Wilk test and homogeneity of variances by Levene's test. Categorical groups were compared by chi-square (χ^2) test. A two-tailed (α=2) p value less than 0.05 ($p<0.05$) was considered statistically significant.

RESULTS

The demographic characteristics of the study population are summarized in Table 1. The age of Group I and group II subjects ranged from 5-15 years and 16-52 years, respectively. In

both groups, females were found to be more affected with caries than males. In both groups, posterior teeth were found to be more affected with caries than anterior teeth and occlusal/incisal surface caries was more common than smooth surface caries.

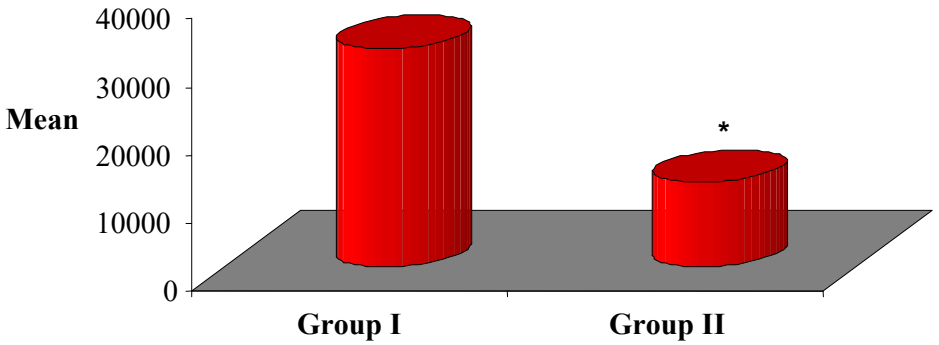
Table 1: Demographic characteristics of two groups

Demographic characteristics	Group I (n=60) (%)	Group II (n=60) (%)
Age (yrs):		
Mean ± SE	10.20 ± 0.65	31.25 ± 1.60
Sex:		
Male	26 (43.33%)	22 (36.67%)
Female	34 (56.67%)	38 (63.33%)
Teeth involved:		
Posterior (%)	36 (60%)	40 (66.67%)
Anterior (%)	24 (40%)	20 (33.33%)
Surface:		
Occlusal/incisal	36 (60%)	32 (53.33%)
Smooth surface	24 (40%)	28 (46.67%)

Bacterial colony count according to groups

The mean (±SE) colony count of *S. mutans* in Group I was 34256±4289 CFU while in Group II, it was 14854±2970 CFU. Comparing the mean colony count of *S. mutans* of two groups, t test (t=2.34, p=0.029) revealed significantly different and higher colony count of *S. mutans* in Group I as compared to Group II [Figure 1].

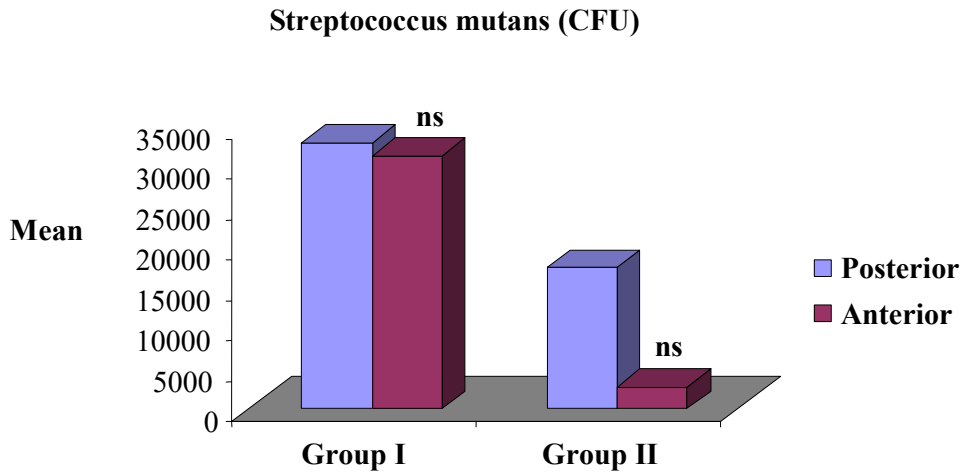
Figure 1: Mean colony count of *S. mutans* of two groups in CFU



*p<0.05- as compared to Group I

Bacterial colony count according to group and tooth involved

In both the groups, incidence of DC was found to be higher in posterior teeth. The mean colony counts of *S. mutans* were higher in posterior (In Group I 33756±4682, in Group II 17675±3467) than anterior teeth (In Group I 31362±4533, in Group II 2784±486) and higher in Group I than Group II. Comparing the mean colony counts (Tukey test) of *S. mutans* within the teeth involved (posterior vs. anterior), [Figure 2] and between the groups (Group I vs. Group II) the difference was found to be statistically insignificant ($p>0.05$).

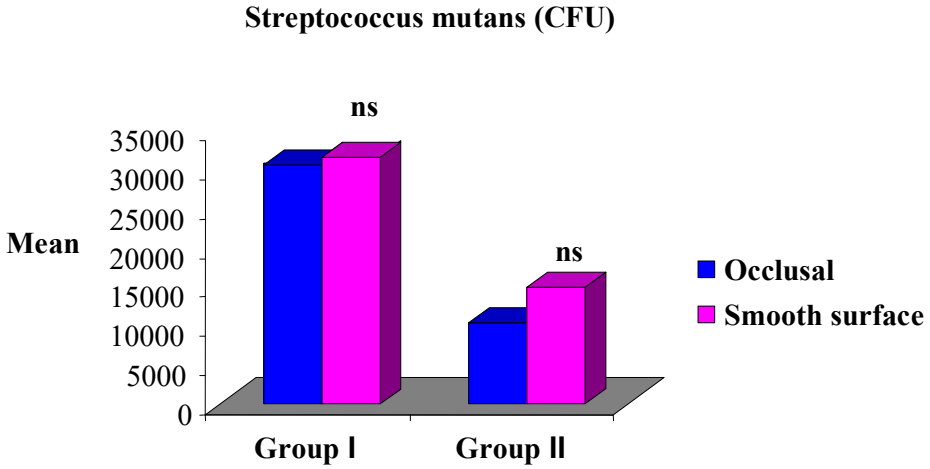


nsp>0.05- as compared to Posterior

Figure 2: For each group, mean colony count of *S. mutans* between the tooth involved

Bacterial colony count according to group and tooth surface

DC was found to be more on occlusal/incisal surface in both Group I and Group II. The mean colony counts of *S. mutans* were higher in smooth surface (In Group I 32964±4889, in Group II 15221±2948) than occlusal/incisal surface (In Group I 31412±4274, in Group II 10434±2212) and higher in Group I than Group II. Comparing the mean colony counts (Tukey test) of *S. mutans* within tooth surface (occlusal/incisal vs. smooth surface), [Figure 3] and between the groups (Group I vs. Group II), the difference was found to be statistically insignificant($p>0.05$).



[ns]p>0.05- as compared to Occlusal/ incisal

Figure 3: For each groups, mean colony count of *S. mutans* between the tooth surface

DISCUSSION

Dental caries results from interactions of three factors, which include the causative organism i.e. the bacteria with the ability to produce acid, a substrate that the bacteria can metabolise, and multiple host factors including teeth and saliva. The ecological imbalance in the physiological equilibrium between tooth minerals and oral microbial biofilms results in DC [2].Streptococci are the pioneer organisms that colonize the tooth surfaces [3]. *S. mutans* produce acid from carbohydrate metabolism that lower the pH of saliva and they have the ability to survive in this low pH environment[4].

As reported in other studies, we too found a higher prevalence of DC in children [5]. The prevalence of caries was also found to be higher in females in both the groups (Group I 56.67% and Group II 63.33%)as also reported in other studies.[6],[7],[8].However, some other studies reported no statistically significant difference in the caries prevalence between the two sexes[9],[10],[11].

The frequently affected teeth with caries were found to be posterior teeth in both the groups, which might be due to their surface nature that helps in food retention at the supplemental grooves present on the posterior teeth as well as the larger surface area that help in bacterial adhesion and multiplication. Similar findings were reported in other studies as

well[6],[12],[13] . Correlating with the microorganisms, *S. mutans*counts were higher in posterior than anterior teeth.

DC was found to be more on occlusal/incisal surface in both Group I and Group II in our study. This was reported in other studies also [14],[15],[16]. This could be due to more numerous and deeper developmental grooves, pits and fissures present on the occlusal surface that may cause food accumulation, pooling of saliva and bacterial colonization.

Comparing the number of microorganisms, the mean colony counts of *S. mutans* were higher on smooth surface than occlusal/incisal surface and higher in Group I than Group II.

S. mutans play a central role in the etiology of DC as they have the ability to adhere to the enamel salivary pellicle and to other plaque bacteria and to produce acid lowering the pH of saliva and creating the risk for cavities [17].

A few limitations of our study include sample size, manual counting procedure which might incorporate counting error during the colony counting. Better results could be yielded with larger sample size and by use of automated colony counter.

CONCLUSION

Streptococci are the pioneer organisms that colonize the tooth surfaces and are associated with the initiation of caries. The incidence of caries can be reduced by taking control measures such as reduction in consumption of carbohydrate in diet and by impeding development of biofilm with proper oral hygiene care. Bacterial colony counts may help in early intervention, may help in taking specific measures against specific organismsand thereby prevent development of new carious lesions.

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