

## SALIVARY COUNT OF *STREPTOCOCCUS MUTANS* IN CARIES PREDICTION

\*JAVERIA UMAR, \*AMBREEN MUNIR, \*A HAMEED AND \*SAFIA AHMED

### ABSTRACT

*The purposes of this study were to give the characteristics of Streptococcus mutans isolated from saliva, and the salivary level of S. mutans and its relationships, with dental caries. Dental caries is a common infectious disease worldwide. The aetiology of the disease is multifactorial, life habits and streptococcus mutans infection being the most important factors. Paraffin stimulated saliva was taken from 40 subjects of three age groups in sterilized glass tubes. The saliva sample was serially diluted on GSTB agar plates. The plates were incubated anaerobically at 37 °C for 48 hours in candle extinction jars. S. mutans were identified on the basis of morphologic and biochemical characteristics. Viable cell count of S. mutans (CFU I ml) was also done on GSTB agar plates. S. mutans were found in all samples in significant percentage. The results indicated that subjects that had dental caries harboured high level of S. mutans  $10^6 > \text{CFU I ml}$ . In age group III, the percentage of subjects with high level of S. mutans and dental caries was found to be higher as compared to age group I and age group II.*

### INTRODUCTION

Dental caries disease includes a breakdown of enamel, the hardest material in the human body, and a subsequent breakdown of the underlying dentin. The disease is the most prevalent of the chronic diseases affecting the human race. The aetiology of dental caries disease is multifactorial in that simultaneous participation of multiple factors is required for caries to occur. Today, mutans streptococci are considered to be the main aetiological microorganisms in caries disease, with lactobacilli and other microorganisms participating in the disease progression<sup>(1)</sup>. The fact that dental caries is a world-wide disease requiring vast economic resources and causing a great deal of discomfort has called upon attempts aimed at developing an accurate screening method for detection of the 5-20% of subjects comprising the high-caries-risk group<sup>(2)</sup>. Because mutans streptococci are considered to be the predominant pathogens of dental caries disease, indi-

viduals heavily colonized by mutans streptococci were thought to automatically be at high risk for caries. Indeed, in young children, early mutans streptococcal colonization on tooth surfaces has been recognized as an indicator of later high scores of decayed, missing and filled surfaces in deciduous teeth (dmfs index)<sup>(3,4)</sup>.

Epidemiological studies show that human populations worldwide carry mutans streptococci<sup>(5)</sup>. Mutans streptococci have been demonstrated in nearly all subjects in populations with high, low and very low prevalence of caries<sup>(6)</sup>. These microorganisms are harboured by 33-75% of 4-year-old children<sup>7</sup>. The time period when most children gain mutans streptococci in their oral flora is when the primary teeth are erupting, i.e., between 8 months and 3 years of age<sup>(8)</sup>. The probability of colonization with mutans streptococci is high when inoculation with mutans streptococci is frequent and microbial cell count is at least  $10^6$  per ml saliva<sup>9</sup>.

\* Microbiology Research Laboratory, Department of Biological Sciences, Quaid-i-Azam University, Islamabad

**Corresponding Author:** Dr. Safia Ahmed, Department of Biological Sciences, Quaid-i-Azam University, Islamabad. Phone: 92-51-2876245, Fax: 92-51-9218999, [E-mail: safiamrl@yahoo.com](mailto:safiamrl@yahoo.com)

Selective media culturing either in a microbiological laboratory can assess levels of these cariogenic bacteria in the mouth. In the future, monoclonal antibody technology is expected to be available routinely for rapid in-office assessments of cariogenic bacterial levels. Saliva that is stimulated by chewing can be used as a sampling method to collect bacteria from the teeth and around the mouth and quantify them as colony forming units, except in very young children (about 3 years or younger). Levels of mutans streptococci of  $10^6$  CFU/ml and lactobacilli levels of  $10^3$  cfu/ml and above in stimulated saliva are considered high risk. The combination of the two groups of bacteria is particularly damaging<sup>(10)</sup>.

## MATERIAL AND METHODS

The study was carried out on the 40 individuals of different age groups, AGE group I (11-19 years), age group II (20-34 years), age group III (35-50 Years). Paraffin stimulated saliva samples of about 2ml were collected from 40 subjects of Rawalpindi and Islamabad city, in sterilized glass tubes. Each subject chewed Paraffin wax (block form) for 2-3 minutes by each subject. Consent was taken from the subject before sampling and each individual, including questions about their age, dietary habits, and oral hygiene, filled a questionnaire.

The samples were brought to Microbiology Laboratory of Quaid-i-Azam University, within one hour of sampling and samples were processed immediately. Glucose, Sucrose, Potassium tellurite, bacitracin agar (GSTB) medium" was used for the isolation and to count the salivary level of *Streptococcus mutans*. Serial dilution and spread vplate technique was employed for viable cell count. For anaerobic growth, the duplicated GSTB plates of each dilution were incubated in candle extinction jars at 37 °C for 48 hours.

Identification of *S. mutans* was done by observing its colonial morphology on the GSTB agar and by applying the biochemical tests that include gram staining, Voges proskaur test, sugar fermentation tests (mannitol, sucrose, raffinose, sorbitol, salicin) and by hemolysis. The number of colonies were counted to calculate the original concentration of *Streptococcus mutans* in the saliva (the number of colony forming units /ml (CFU/ ml).

## RESULTS

*S. mutans* was isolated from the subject's saliva on GSTB agar medium and was grown under anaerobic

TABLE 1: MORPHOLOGICAL AND CULTURAL CHARACTERISTICS

Morphological identification on GSTB agar	<i>Streptococcus mutans</i>
Size	0.02 to 0.7 mm
Pigmentation	Beige to brown, dark gray, slate gray
Form	Rough frosted, though frosted, circular, irregular
Margin	Entire to undulate
Elevation	Pulvinate to umbonate

condition. Identification of *S. mutans* was done on the basis of colonial morphological characterization on GSTB agar plates. The colonies of *S. mutans* were 0.02-0.7 mm in size, dark gray to slate gray in color. It showed rough frosted, irregular form with entire to undulate margins (Table 1).

Biochemical results confirmed that the *S. mutans* are gram-positive streptococci; give positive results for Voges proskaur test, able to ferment sugars (mannitol, sucrose, raffinose, sorbitol, salicin). It showed adhesive growth on the walls of the test tubes when ferment sucrose and raffinose. It showed alpha (α) hemolysis (no clearing) on the blood agar plates (Table 2). Identification was based upon Bergyes Manual Determinative Bacteriology<sup>(12)</sup>.

Three levels of *S. mutans* i.e. high level with CFU/ml >  $10^6$ , medium level with CFU/ml >  $10^5$ , low level with CFU/ml >  $10^3$  were compared among different age

TABLE 2: RESULTS OF BIOCHEMICAL TESTS FOR *S. MUTANS* ISOLATES

Biochemical tests	<i>Streptococcus mutans</i>
Grams staining	Gram positive streptococci
Voges proskaur Test	+
<b>Sugar Fermentations</b>	
Sucrose	+
Mannitol	+
Raffinose	+
Sorbitol	+
Salicin	+
Hemolysis Test	α (Hemolysis)
Bacitracin resistance	+
Catalase Test	—

groups including males and females subjects. In age group I (11-19 year's) 40% of subjects had high level of *S. mutans*. The percentage of subjects for medium and low level of *S. mutans* was same i.e. 30%. The occurrence of dental caries was found in 60% subjects of this age group. In age group II (20-34 years), 35% subjects had high level of *S. mutans*. 25% subjects had medium

level, whereas 40% subjects of this age group had low level of *S. mutans*. 50% subjects of this age group experienced dental caries. In age group III (34-50 years) 50% subjects had high level of *S. mutans* than 30% for medium and 20% subjects for low level of *S. mutans*. The occurrence of dental caries was found in 80% subjects of this age group (Table 3, Fig, 1,2,3,4).

TABLE 3: PERCENTAGE DISTRIBUTION OF MEAN DIFFERENCE OF *S. MUTANS* LEVELS AMONG DIFFERENT AGE GROUPS

Age Groups	Levels of <i>S. mutans</i>			Occurrence of Dental Caries
	High	Medium	Low	
I (11-19) Years N=10	4 (40%)	3 (30%)	3 (30%)	6 (60%)
II (20-34) Years N=20	7 (35%)	5 (25%)	8 (40%)	10 (50%)
III (35-50) Years N=10	5 (50%)	3 (30%)	2 (20%)	8 (80%)

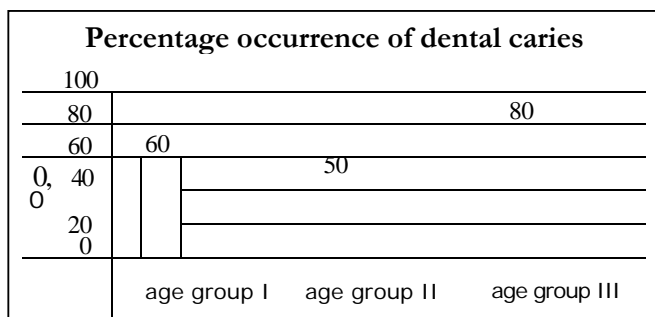


Fig. 1: The percentage occurrence of dental caries in three age groups, age group I (11-19 years), age group II (20-34 years) and age group III (35-50 years).

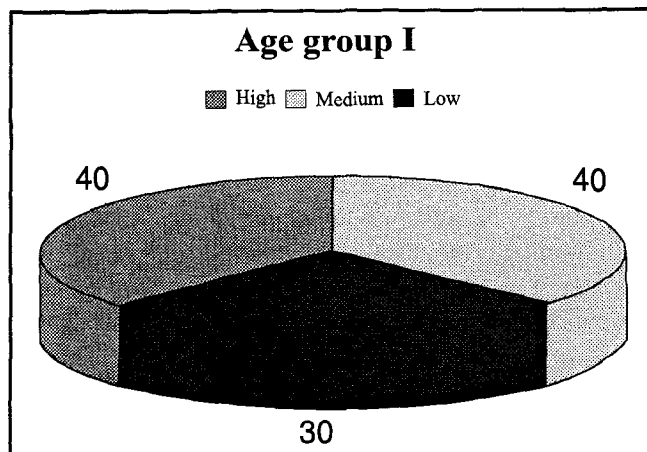


Fig 2a: Percentage level of *S. mutans* in age group I (11-19 years).

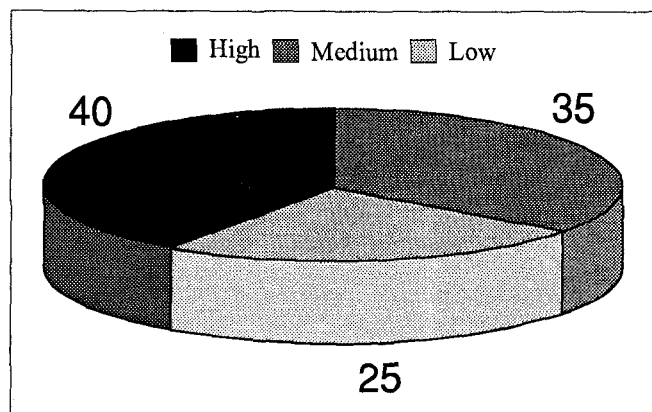


Fig 2b: Percentage level of *S. mutans* in age group II (20-34 years).

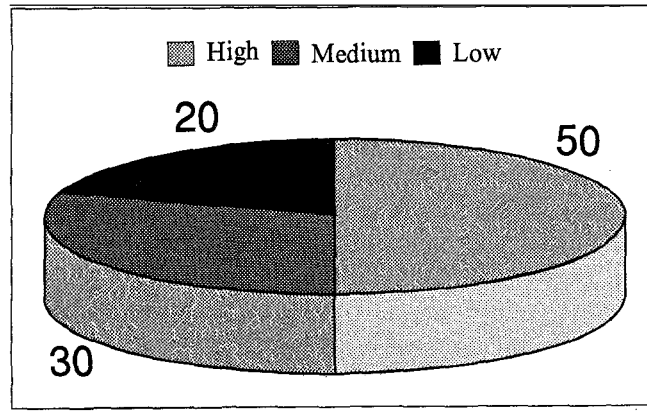


Fig 2c: Percentage level of *S. mutans* in age group III (35-50 years).

The results showed that in all age groups subjects with high level of *S. mutans*  $10^6 >$  CFU/ml experience dental caries. But percentage of subjects with high level of *S. mutans* and occurrence of dental caries were found to be higher in age group III (35-50 years)

## DISCUSSION

Mutans streptococci have been found in the mouth of all populations sampled so far, indicating that these bacteria have a global distribution. In each population, individuals with very high numbers can be found; some have medium counts and still other persons very low, or are in fact without these bacteria. A high count, which is a relative estimation, corresponds to about  $10^6$  CFU. Several studies that have compared sound and carious roots have detected mutans streptococci more frequently and as a higher proportion of the microbiota in lesions than on control caries free surfaces<sup>(13)</sup> Several factors such as tooth brushing, diet, acidogenic potential of the mutans streptococci strain and site of the carious lesion can modify the salivary *Streptococcus mutans* (Sm) counts<sup>(14)</sup>.

In the present study when Salivary level of *S. mutans* was compared in three age groups (Table 3), Percentage of salivary level of *S. mutans* was found to be higher in age group III (35-50 years) i.e. 50% as compared to age group I (11-19 years) with 40% of *S. mutans* level and 35 % in age group II (20-34 years). In all three age groups individuals with high level of *S. mutans* CFU/ml  $> 10^6$  experienced caries. This result is in accordance with the studies (15). They made a cross sectional study of 167 school children (12-13 years) to evaluate the relationship between dental caries and CFU/ml of *S. mutans* and Lactobacillus. Saliva sample was collected for qualitating the *S. mutans* and *Lactobacillus*. The prevalence and levels of *S. mutans* and *Lactobacillus* in saliva and its possible correlation with dental caries and periodontal condition was investigated in 473 Italian school children, 9-13 years of age. Stimulated saliva samples were collected. Salivary *S. mutans* and Lactobacillus were identified in 52% and 21% of children, respectively. The prevalence of *S. mutans* was higher among children of 13-year. A positive correlation between the levels of *S. mutans* and Lactobacillus to dental caries was found<sup>(18)</sup> (Brambilla et al., 1999). The salivary levels of mutans streptococci and lactobacilli, the percentage of surfaces harboring plaque and the frequency of carbohydrate intake best explained the variation in the frequency of root surface caries. Other contributory factors were the saliva secretion rate and the buffer capacity<sup>(17)</sup> (Fure and Zickert, 1990).

A 5 years incidence of dental caries in random samples of 60, 70-, 80- years old individuals of Goteborg were observed for salivary and microbial condition, by Fure (1998m). He found that over all salivary counts of Lactobacillus and *S. mutans* increases during the period in the respective group of 60, 70, 80 years, 15, 39 and 39% had a *S. mutans* count  $> 10^6$  CFU/ml in saliva. So it was found that age, salivary level of *S. mutans* and Lactobacillus were best predictor of the incidence of root caries.

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