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Frequency of Streptococcus mutans in patients having dental caries; a data from clinical setting in rural area of Balochistan

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ABSTRACT

To assess the frequency of *streptococcus mutans (S. mutans)* and its association with socio-demographic factors in patients having dental caries presenting at a DHQ hospital in rural area of Balochistan. It was a cross-sectional study conducted at the clinical setting or DHQ hospital of a rural area in Balochistan from April 2020 January 2021. About 120 patients of age 18 to 40 years of either gender presenting dental caries were included in the study using non-random consecutive sampling technique. Data regarding age, gender, ethnicity, addictions (like gutka and smoking) pH level and oral hygiene was obtained from all the participants by researcher himself. The number of *S. mutans* colonies (CFU) was counted using a self-illuminating binocular microscope after plates were incubated for 48 hours at 37°C. All findings were entered into a pre-designed proforma. SPSS version 23 was used to analyze data. The median age of patients with dental caries was 28 years (IQR: 21-35 years) and 83.3% of the patients were males. The median *S. mutans* count of all the patients with dental caries was estimated as 35 with IQR as 32 to 38. Mann-Whitney statistics showed significant difference between ethnic groups for *S. mutans* count (p=0.049). A weak correlation was observed between *S. Mutans* count and pH level (r=0.206) with statistically insignificant difference (p=0.206). Frequency of *S. mutans* is 100% in individuals with dental caries. *S. mutan* counts have a significant association with ethnicity and weak correlation with pH in saliva.

Keywords: Dental caries, dental cavity, dental health, saliva, salivary pH, *Streptococcus mutans*.

INTRODUCTION: Dental caries (tooth decay) is a complex and chronic infectious disease that affects people all over the world (WHO, 2017) According to the WHO, approximately 36% of the individuals globally have dental caries in their permanent teeth, and over 530 children have dental caries in their primary teeth. In developing countries, it is one of the most common diseases (WHO, 2020; Shitie *et al.*, 2021). In Pakistan, the overall prevalence of dental caries was estimated as 57% (Siddiqui *et al.*, 2021). Dental caries caused due to many factors like diet, host factors (saliva compositions as viscosity, flow rate, and salivary pH) and the presence of acidogenic bacteria in the tooth biofilm, primarily *Streptococcus mutans (S. mutans)* (Oda *et al.*, 2015; Al-Mahmood *et al.*, 2020) *S. mutans* can stick to the bacteria in the enamel plaque and the salivary pellicle. They are a powerful acid originator, resulting in an acidic environment that increases the risk of cavities. *S. mutans* commonly develops in tooth cavities after 6 to 24 months of dental caries (Forssten *et al.*, 2010). According to an study, acidicity appears to be the most persistent feature of *S. mutans* and is linked to its cariogenicity (McDonald *et al.*, 2004). Another study found that *S. mutans* counts vary in biofilm formation, acid sensitivity, and other features that allow them to colonize sucrose-rich environments (Lembo *et al.*, 2007). Literature reported high frequency of *S. mutans* in children with caries than children without caries (Ghasempour *et al.*, 2013). As a result of the close link between microbe counts in plaque and saliva, determining *S. mutans* in saliva has been proposed as a tool for detecting high risk group for dental caries (Ghasempour *et al.*, 2013; Sánchez-Acedo *et al.*, 2013) because saliva is in constant touch with all of the teeth, it provides a more accurate depiction of *S. mutans* colonization over the whole dentition (Ghasempour *et al.*, 2013; Sánchez-Acedo *et al.*, 2013).

OBJECTIVES: The objective of the current study was to assess the frequency of *S. mutans* and its association with socio-demographic factors in patients having dental caries presenting at a DHQ hospital in rural area of Balochistan.

MATERIAL AND METHODS: It was a cross-sectional study conducted at the clinical setting or DHQ hospital of a rural area in Balochistan from April 2020 to January 2021. Sample size of 117≈120 patients was estimated using Open Epi online sample size calculator by considering frequency of *S. mutans* as 56% (Chhabrani *et al.*, 2020) in individuals with dental caries, margin of error as 9% and 5% level of significance. Patients of age 18 to 40 years of either gender presenting dental caries were included in the study using non-random consecutive sampling technique. Patients with history of antibiotic therapy or pregnant females were excluded from the study.

Ethical approval of the study was obtained from ethical review committee before conduct of study. Informed consent was obtained from all the eligible participants. Data regarding age, gender, ethnicity, addictions (like gutka and smoking), pH level and oral hygiene was obtained from all the participants by researcher himself. Passive drooling was used to collect 2ml unstimulated salivary samples from the individuals, which were kept at 0 C. The saliva samples were then sent to a lab to be tested for *S. mutans*. The microbial content was determined using the dilution and spread plate method. All of the patients' salivary microbial analyses was done by diluting each salivary

sample 1:10 in distilled water. After that, each sample was streaked onto a plate containing Mitis salivarius agar media (MSA). The number of *S. mutans* colonies (CFU) was counted using a self-illuminating binocular microscope after plates were incubated for 48 hours at 37°C. All findings were entered into a pre-designed proforma. SPSS version 23 was used to analyze data. Median and interquartile range were reported for age and *S. mutans* count (because distribution was not normal). Frequency and percentage were reported for gender, ethnicity, addictions (like gutka and smoking) and oral hygiene. Association of *S. mutans* count with age, gender, ethnicity, addictions, oral hygiene and pH level was checked using Mann-Whitney U test or Kruskal Walis test. A p-value≤0.05 was considered as statistically significant.

RESULTS: Total 120 individuals with dental caries were included in the study, with the median age of 28 years (IQR: 21-35 years). Most of the patients were males (83.3%) and had ethnicity lasi (58.3%). Of 120 patients, 55.8% were gutka chewers and 32.5% were smokers. Almost 45.8% of the patients had never brushed their teeth and 29.2% of the patients were maswaak users (table 1).

Age in years	28 (21-35)
Gender	
Male	100 (83.3)
Female	20 (16.7)
Ethnicity	
Balochi	41 (34.2)
Lasi	70 (58.3)
Brahui	4 (3.3)
Pashtoon	5 (4.2)
Addiction	
Gutka	67 (55.8)
Smoking	39 (32.5)
Oral hygiene	
Brushing not at all	55 (45.8)
Maswaak	35 (29.2)
Brushing once a day	8 (6.7)
Brushing twice a day	6 (5)
Brushing once a week	7 (5.8)
Brushing once a month	9 (7.5)

Data expressed as Median (IQR)/ n(%)

Table 1: Baseline characteristics of study variables (n=120).

The median *S. mutans* count of all the patients with dental caries was estimated as 35 with IQR as 32 to 38 (figure 1). The median pH level of all the patients with dental caries was estimated as 7.1 with IQR as 6.9 to 7.2. Most of the patients had pH>7 (65.8%), followed by pH<7 (28.3%) and only 5.8% had neutral pH level (figure 2). The mean rank of *S. mutans* count was compared with socio-demographic factors. Mann-Whitney statistics showed significant difference between ethnic groups for *S. mutans* count (p=0.049) (table 2).

The Spearmen correlation was applied between *S. Mutans* count and pH level among patients with dental caries. A weak correlation was observed between *S. Mutans* count and pH level (r=0.206) with statistically insignificant difference (p=0.206) (figure 3).

DISCUSSION: In many countries, *S. mutans* has been isolated from the oral cavity (Salman *et al.*, 2017). The frequency and distribution of isolation are substantially varied. Although various complicated aspects such as brushing and eating habits, sucrose content in the food, and probable immune reactions of the host and environment all influence the prevalence of microorganisms, the isolation technique used is equally significant (Marcotte and Lavoie, 1998; Babaeekhou *et al.*, 2020).

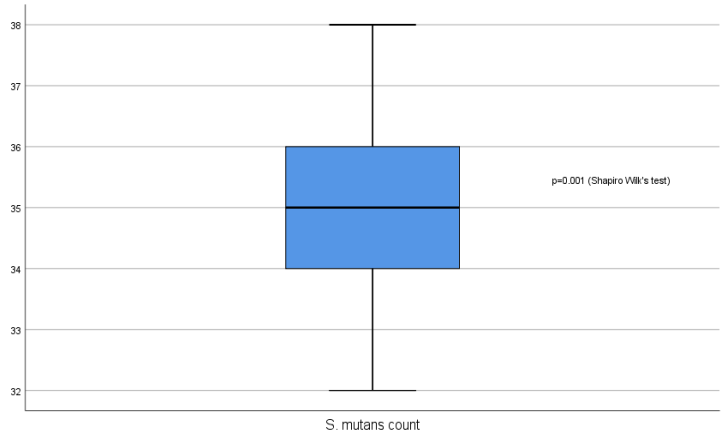

Figure 1: Descriptive statistics of *S. mutans* count (CFU).

	S. mutans count		
	Median (IQR)	**Mean rank**	**p-value**
Age in years			
≤25 years	35 (34-36)	59.36	
>25 years	35 (34-36)	61.31	0.757
Gender			
Male	35 (34-36)	62.57	
Female	35 (34-35.5)	50.15	0.137
Ethnicity			
Balochi	35 (34-36)	64.60	
Lasi	35 (34-36)	54.84	
Brahui	36.5 (35.5-37.5)	93.50	
Pashtoon	36 (34-37)	79.70	0.049*
Gutka			
No	35 (34-36)	64.75	
Yes	35 (34-36)	57.14	0.225
Smoking			
No	35 (34-36)	57.06	
Yes	35 (34-36)	67.65	0.110
Oral hygiene			
Brushing not at all	35 (34-36)	62.79	
Maswaak	35 (34-36)	56.56	
Brushing once a day	35 (34-36)	63.13	
Brushing twice a day	35 (34.5-36)	65.00	
Brushing once a week	35 (34-35.5)	54.07	
Brushing once a month	34 (33.5-37)	61.50	0.952
pH levels			
<7 (Acidic)	34.5 (34-36)	53.49	
7 (Neutral)	35 (34-36)	59.50	0.401
>7 (Alkaline)	35 (34-36)	62.84	

Table 2: Comparison of *S. mutans* count with socio-demographic factors

In the current study, saliva was used as the sampling medium for evaluating the microbiological results of dental caries and microbial content in the saliva was determined by using the dilution and spread plate method. *S. mutans* is a significant cause of dental caries, because these can adhere to the enamel salivary pellicle and to dental plaque microorganism (Forssten *et al.*, 2010). It is an acid producer, causing an acidic environment that raises the incidence of cavities. Dental caries

normally develops six to twenty-four months after a cavity has formed. *S. mutans* generates a small amount of acid, but it is produced locally and lowers the pH in the bacterium's microenvironment.

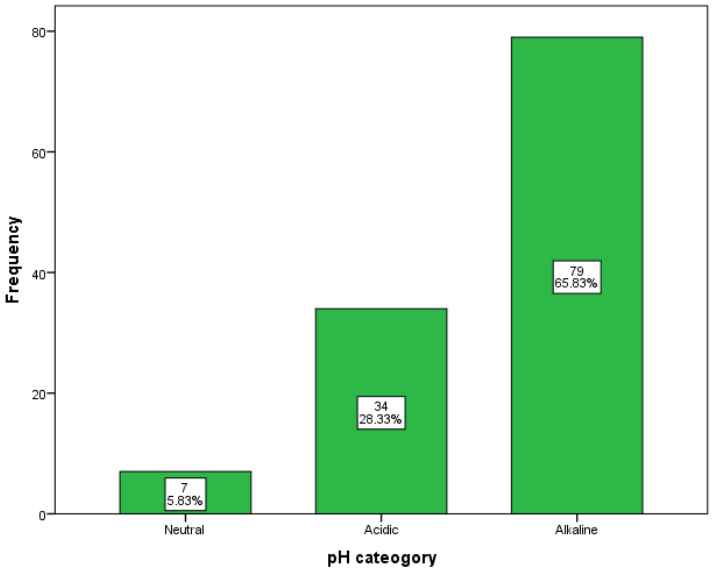

Figure 2: The pH level categories among patients with dental caries.

Because *S. mutans* may adhere to the hydroxyapatite matrix of the tooth, the pH of the tooth surface can quickly drop below the hydroxyapatite demineralization threshold (pH 5.5) (Forssten *et al.*, 2010). In the current study, the detection of *S. mutans* in saliva sample was 100%. In the previous study, *S. mutans* was present in all of the patients with dental caries (Sounah and Madfa, 2020). *S. mutans* was present in all saliva samples and that there was a statistically significant link between the number of *S. mutans* and the DMFT score (Kishi *et al.*, 2009). *S. mutans* was present in 87% of the adults with dental caries and here was a positive association between *S. mutans* and DMFT score (p=0.0001) (Babaeekhou *et al.*, 2020). Various researchers have also observed positive correlation between S. mutans and DMFT score in different age groups (Hebbal *et al.*, 2011; Pannu *et al.*, 2013; Oda *et al.*, 2015; Babaeekhou *et al.*, 2020). In Pakistan, the overall prevalence of dental caries is 57%, wherein KPK and Balochistan, the prevalence of caries is almost 51.2% (Siddiqui *et al.*, 2021). In our study, we included participants from Balochistan, where most of the participants belonged from Lasi ethnicity (58%). Further, we found mean S. mutans count were significantly higher in Brahui ethnicity ($p<0.05$) as compared to other ethnic groups. This might be due to the fact that different ethnic groups have different dietary habits and oral health status. The appropriate salivary pH level is necessary for microorganism development and proliferation in the oral cavity. The frequency of acidophilic microorganisms increased in saliva with a very low pH (acid environment), whereas the frequency of acidosensitive bacteria decreased (Jeong *et al.*, 2006; Singh *et al.*, 2015). In our study, we also found that majority of the participants with dental caries had low pH of less than 7 (66%). Furthermore, we observed a weak correlation between S. mutans count and salivary pH among individuals with dental caries. Hence, there are various factors which differ according to biological and physiological conditions and host environment that affect the S. mutans count in the oral cavity (Mittal *et al.*, 2021).

CONCLUSION: Frequency of *S. mutans* is 100% in individuals with dental caries. *S. mutan* counts have a significant association with ethnicity and weak correlation with PH in saliva.

CONFLICT OF INTEREST: None to declare.

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