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GENDER-WISE PREVALENCE OF STREPTOCOCCUS MUTANS SEROTYPES E, F AND K IN SOUTH INDIAN SCHOOL CHILDREN

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ABSTRACT

Dental caries is widely prevalent disease affecting children worldwide. Streptococcus mutans (SM) is the single most common microorganism implicated in the initiation and progress of dental caries. The specific serotypes of SM are c, e, f and k. Serotype c is reported to be most commonly found in the human oral cavity followed by serotypes e, f and k. Currently no data is available on gender-wise distribution of SM serotypes in children. The present study highlights the prevalence of lesser known SM serotypes e, f and k in 6 to 12 years south Indian school going caries active (CA) and caries free (CF) boys and girls. Thirty five boys and 25 girls in the CA (DMFT/dmft≥5) group and 28 boys and 12 girls in the CF (DMFT/dmft=0) group participated in the present study. The prevalence of SM was identified in unstimulated saliva samples by Polymerase Chain Reaction (PCR) amplification method using primers that anneal and amplify a segment of SM-specific GTFB gene. SM specific bands were identified by gel analysis confirmed by direct sequencing. Samples positive for SM were analysed for presence of serotypes e, f and k using primers and PCR techniques. The prevalence of serotype k was found to be the highest in CA and CF boys (31.4% and 18.51%) and girls (36% and 33.3%) followed by serotypes e and f in CA boys and girls and f and e in CF boys and girls. Different combinations of serotypes e, f and k were found in boys and girls of CA and CF groups. The differences between the prevalence of SM serotypes between boys and girls in both CA and CF groups were statistically non-significant.

INTRODUCTION

Dental caries poses a significant public health issue in children worldwide [1,2]. Children's dental health is critically important to their overall health and successful development into high-functioning adults.[3]. Children with untreated ECC might have significantly poorer oral health-related quality of life than children without ECC [4,5]. A variety of factors, including microbial, genetic, immunological, behavioral and environmental, interact to contribute to the onset and development of dental caries [1]. Members of the genus Streptococcus are dominant in dental plaque biofilms, ten times more prevalent than the next most abundant genera [6]. The preferential colonization site of mutans streptococci (MS) is the tooth; [7]. they are highly localized on the surfaces of the teeth, and their abundance in the plaque is highest over initial lesions; [8]. their level of colonization within the plaque is increased by sucrose consumption; they synthesize molecules from sucrose that foster their attachment to the teeth; [9-11]. they are rapid producers of acid from simple carbohydrates and are tolerant to low pH; and they are recovered on cultivation of initial and established carious

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lesion sites. Their virulence expression is strongly associated with consumption of carbohydrates, especially sucrose [12].

Serotype refers to a group of closely related microorganisms distinguished by a characteristic set of antigens [13]. Using immunodiffusion, Bratthall [14]. demonstrated five serological groups of mutans streptococci. Serotyping by immunodiffusion, immunofluorescence or immunoelectrophoresis has been widely applied for typing of MS [15,16]. Recently Polymerase chain reaction (PCR) techniques have been used to evaluate Streptococcus mutans (SM) serotypes in epidemiological studies [17-21]. Strains of MS are classified into nine distinct serotypes a, b, c, d, e, f, g, h and k based on the chemical composition of cell surface rhamnose-glucose polymers (RGPs) as follows: *Streptococcus cricetus* (serotype a), *Streptococcus rattus* (serotype b), *Streptococcus mutans* (serotypes c, e, f and k) and *Streptococcus sobrinus* (serotypes d and g) [22,23].

Serotype c is the most found serotype in the human oral cavity (70% - 80%), followed by serotype e (approximately 20%), while serotypes f and k are more rare (less than 5%) [24]. Interestingly, geographical differences in the distribution of the MS in oral plaque samples have been observed [25,26]. Recent studies have indicated an association between cariogenic SM serotypes and infective endocarditis (IE), an inflammatory condition of endothelial cells of heart. SM serotype k has been found in the heart valves and blood samples of patients with IE [19,20,23]. These findings highlight the clinical significance of elucidating the prevalence of MS, more specifically SM serotypes in a population.

In the literature, studies on serotypes of SM are scarce. Kirtanya et al [27]. in their study had found SM matching serotype c in Indian population. A study was conducted to evaluate the prevalence of serotypes e, f and k in South Indian population of school going children utilizing PCR techniques and reported a higher prevalence of serotype k and e, followed by serotype f in caries active group [28]. In the present article, the prevalence of SM serotypes e, f and k among boys and girls are presented.

MATERIALS AND METHODOLOGY

Permission was obtained from concerned authorities to perform the study. Through random sampling children aged between 6 and 12 years studying in schools of a south Indian town, Chidambaram were selected. World Health Organization (WHO) Index was used to assess the dentition status and screened for dental caries [29]. Children with parental consent and individual assent were included in the study. Children consuming antibiotics for long periods were excluded from the study. The children were classified as caries active (DMFT/dmft $\geq$ 5) and caries free (DMFT/dmft = 0) [30,31]. Single examiner performed oral screening in natural day light. The findings were recorded by recording clerk.(Figure-1) Twenty five children were screened twice at an interval of two weeks and intra examiner reliability was found to be good (kappa>0.91). One hundred children including 60 caries active (CA) and 40 caries free (CF) boys and girls participated in the study.

Unstimulated saliva samples were gathered from both groups of children.(Figure-2) The prevalence of SM was identified by strain specific PCR amplification method using primers that anneal and amplify a segment of GTFB gene specifically from SM [17,18,28,32]. SM specific bands were identified by gel analysis of the PCR amplified samples. Direct sequencing confirmed the specificity of amplification of SM GTFB gene. (Figure-3) The samples from boys and girls which tested positive for SM in both caries active and caries free groups were analysed for presence of serotypes e, f and k using primers and PCR techniques [28]. The amplified products were run in agarose gel. Serotype-specific bands appeared on analysis with DNA/RNA GeneQuant Spectrophotometer. (Figure-4) The results obtained were statistically analyzed.

RESULTS

The study group comprised of 60 CA subjects including 35 boys and 25 girls. 40 subjects in CF group included 28 boys and 12 girls. The difference in the genders was statistically not significant. The DMFT/dmft scores in CA boys were 6.97 and 6.20 in CA girls. The difference was not statistically significant. Among the saliva samples, utilizing PCR techniques and gel electrophoresis, the samples with SM expression was found to be comparable in boys and girls (80%:80% and 44.4%: 41.6% in CA and CF groups respectively), though the CA group had more SM expression (Table-1).

Single serotype expression in the samples tested positive for SM was found to have no statistically significant difference among boys and girls (60%:68% and 46.4%:50% in CA and CF groups respectively). Multiple serotype expression in a given sample did not show statistical difference among CA and CF boys and girls (5.7%:20% and 14.29%:8.33%) (Table 1).

The prevalence of serotype k was found to be the highest in CA and CF boys (31.4% and 18.51%) and girls (36% and 33.3%) followed by serotypes e and f in CA boys and girls and followed by serotypes f and e in CF boys and girls. Combinations of serotypes e, f and k, and e and k were found in CA boys and serotypes e and k, and f and k was found in CA girls. Combinations of serotypes e, f and k, f and k, and e and k were found in CF boys and only serotypes e, f and k was found in CF girls. (Tables 2,3).

Table 1. Gender-wise distribution of age, DMFT/dmft, expression of SM and SM serotypes

Group	Gender	Age			DMFT/dmft			Expression of SM		Expression of Single Serotype		Expression of Multiple serotypes	
		Mean	S. D.	Signifi cance	Mean	S. D.	Signifi cance	Exp of SM	Signifi cance	Single serotype	Signifi cance	Multiple serotypes	Signific ance
Caries Active (n=60)	Boys (n=35)	8.63 Yrs	2.13	t = 1.45 p= 0.15	6.97	2.23	t = 1.49 p= 0.14	28 (80%)	chi-square = 0.11 p =0.74	21 (60%)	chi-square = 0.57 p =0.45	2 (5.7%)	F E Test p =0.11
	Girls (n=25)	9.56 Yrs	1.29		6.20	1.53		20 (80%)		17 (68%)		5 (20%)	
Caries Free (n=40)	Boys (n=28)	10.79 Yrs	1.81	t = 0.33 p= 0.74	Nil	Nil	Nil	12 (44.4%)	chi-square = 0.00 p=1.00	13 (46.4%)	chi-square = 0.04 p=0.84	4 (14.29%)	F E Test p=1.00
	Girls (n=12)	10.58 Yrs	1.78		Nil	Nil	Nil	5 (41.6%)		6 (50%)		1 (8.33%)	

Table 2. Gender-wise distribution of SM serotypes in the caries active subjects

	Serotype	Boys(n= 35)	Girls (n= 25)	Significance
Caries Active Group (n=60)	e	7 (20%)	5 (20%)	F E Test, p = 1.00
	f	3 (8.6%)	3 (12%)	F E Test, p = 0.68
	k	11 (31.4%)	9 (36%)	chi-square = 0.26 P = 0.61
	e & f	0	0	
	e & k	1 (2.85%)	3 (12%)	F E Test, p = 0.30
	f & k	0	2 (8%)	F E Test, p = 0.16
	e, f & k	1 (2.85%)	0	F E Test, p = 1.00

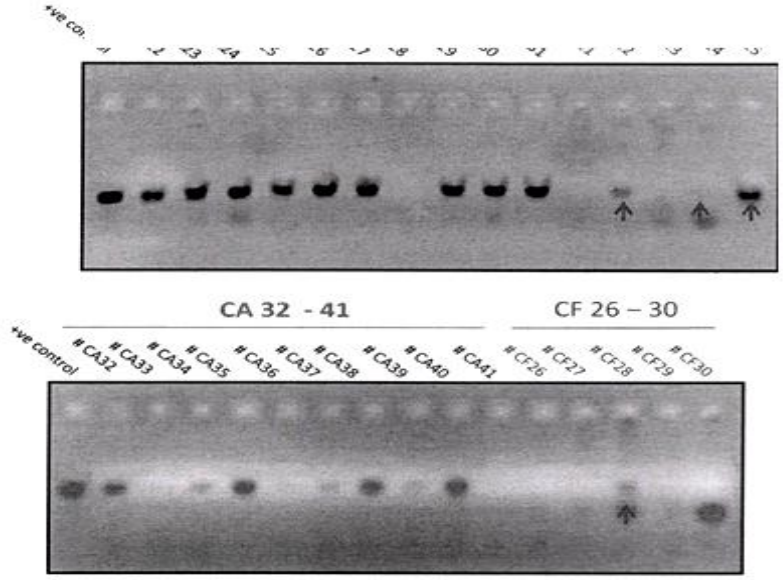
Table 3. Gender-wise distribution of SM serotypes in the caries free subjects

	Serotype	Boys (n= 28)	Girls (n= 12)	Significance
Caries Free Group (n=40)	e	3(11.11%)	1(8.33%)	F E Test, p=1.00
	f	5(18.51%)	1(8.33%)	F E Test, p = 0.65
	k	5(18.51%)	4(33.33%)	F E Test, p=0.41
	e & f	0	0	
	e & k	1 (3.7%)	0	F E Test, p=1.00
	f & k	2 (7.4%)	0	F E Test, p=1.00
	e, f & k	1 (3.7%)	1(8.33%)	F E Test, p=0.52

Figure 1. School children being screened

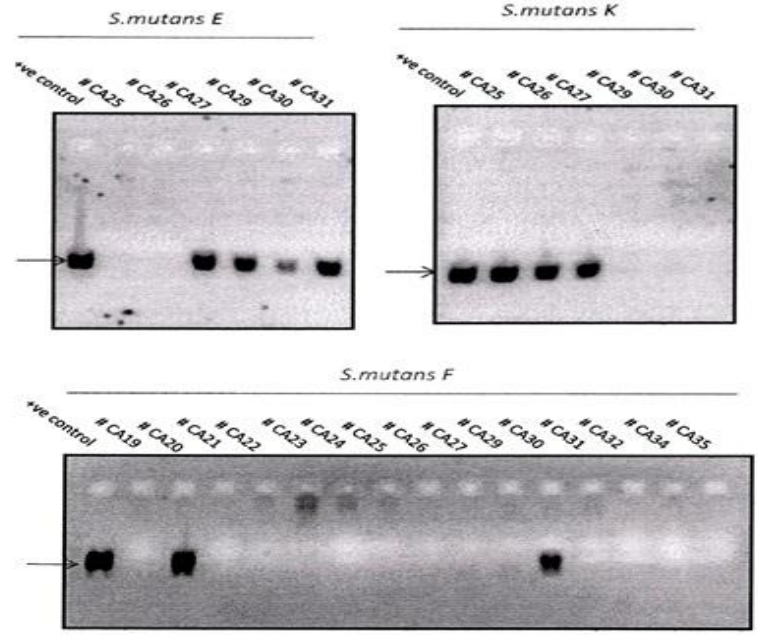
Figure 2. Saliva collection in plastic tubes

Figure 3. PCR amplification of all samples for prevalence of SM



The bacterial genomic DNA extracted from saliva samples were PCR amplified with S. mutans specific primers to identify their prevalence. Representative samples of CA and CF groups are shown in figure-3. Arrow marks represent bands indicating the presence of S. mutans in CF group.

Figure 4. PCR amplification of SM positive samples with serotype specific primers



The bacterial genomic DNA that tested positive with S. mutans specific primers were subjected to PCR amplification with serotype e, f & k specific primers. Representative samples of CA group are shown in figure-4. Serotype specific primers for e. f & k showing bands in few samples can be noted (Arrow marks)

DISCUSSION AND CONCLUSION

Initial acquisition of MS has been reported to occur in children at the median age of 26 months during a discrete period designated as the "window of infectivity". It was suggested that the children who escape MS acquisition during the initial window period will remain MS free until at least six years of age, when first molars emerge. Another window of infectivity for MS would be present when the permanent teeth begin emerging between 6 and 12 years of age [33,34]. According to the WHO, the importance given to this age group is due to the fact that it is this age that children leave primary school [29]. In many countries, it is the age at which reliable sample and data can be easily obtained through a school system. The age of 12 was determined as the age of global monitoring of caries for international comparisons and monitoring of disease trends [29,35,36]. The present study included school going children of Chidambaram in the age group of 6 to 12 years.

In the present study, DMFT/dmft index as per WHO criteria was used to categorize 6 to 12 years old school going children as CA and CF [29]. For the mixed dentition, the sum of the DMFT (Permanent teeth) and dmft (Primary teeth) scores was considered [37,38]. In the present study the caries activity is denoted as 'DMFT/dmft'. Published reports on the caries activity on

south Indian population have considered DMFT scores more than 5 as the criteria for CA group [30,31]. In the present study the mean DMFT/dmft scores were 6.97 and 6.20 in CA boys and girls respectively.

In an earlier study on 5 to 10 year old children studying in the primary schools in south Indian town of Chidambaram, the mean dmft and DMFT scores were 3.00 and 0.42 respectively. Although the mean dmft scores were not statistically significant different for the two sexes, the mean DMFT score was found to be higher among girls than among boys ($P < 0.02$) [39]. The DMFT/dmft scores in the present study belong to the CA study population selected with the DMFT/dmft scores ≥ 5, which might explain the increased score. In the present study, the DMFT/dmft score was marginally higher in boys than girls though statistically non-significant.

Reports on the gender predilection of dental caries are varied. Although, intuitively, the earlier exposure of girls' teeth to the oral cavity should provide explanation for the higher incidence of caries in females, contradictory information has been found to support this idea in children [40]. A study conducted on 771 children who were 2 years old in Zurich identified the number of initial or cavitated lesions in children and reported male gender as a risk factor for caries [41]. However, another study on of prevalence of dental health problems in 12–15 years old 1068 children of Kerala reported boys and girls being almost equally affected by caries, or with females slightly more affected (49% male versus 51% female) [42]. In a cross sectional examination of 10 and 11 years children in southern Italy, the mean DFT for boys was 3.20 versus a mean DFT of 1.96 in girls. This difference was statistically significant, in that it was more common for the male children to have caries than the female children [43]. The results of another study conducted in south Indian population reported higher prevalence of dental caries in 5 year old boys and 12 year old girls than their opposite gender subjects suggesting that there is a time between 5 and 12 years of age when higher caries prevalence in children switches from male to female [44]. The mean age of boys and girls of the caries active group in the present study were 8.63 years and 9.56 years.

SM is considered to be the primary initiator of dental caries. The presence of SM could greatly increase the risk of caries if the host's defense mechanisms did not override the bacteria. CA boys and girls in the present study exhibited greater expression of SM (80%:80%) than their CF counterparts (44.4%:41.6%), with no statistically significant difference between the genders. A study of twins (both monozygotic and dizygotic) tested the amount of SM in participants using the Stripmutans test and found no statistically significant gender differences in the amount of SM. Those results included those from 28 pairs of dizygotic opposite gender twins [45]. Loyola-Rodriguez et al [46]. found no statistically significant differences in SM between genders after inoculating saliva of CF and CA children.

PCR techniques and gel electrophoresis were utilized in the present study to elucidate the prevalence of SM serotypes. The present study identified a novel trend of hierarchy of SM serotype prevalence. Serotype k was highest in prevalence in all groups followed by other serotypes. In CA group, the serotype k was followed by e and f in boys and girls. In CF group, serotype k was followed by f and e in boys and girls in that the scores of serotypes f and e were equal in girls. The gender-wise differences were statistically non significant. Presently no other similar data are available for comparison. The findings of the present study are significant given the cardiac association of SM serotype k and its higher prevalence in the present study population.

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