Prevalence of Salivary *Streptococcus mutans* Serotype *k* in Children Undergoing Congenital Heart Surgery

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Objective: *The prevalence of Streptococcus mutans serotype k, which was speculated that might be associated with the development of cardiovascular diseases, has been reported in adult cardiovascular surgery patients. There is no information about presence of serotype k in children with cardiac disease. The aim of this study was to determine the salivary prevalence of S.mutans serotype k in children with congenital heart disease.* ***Study Design:*** *Salivary samples of 25 patients undergoing elective surgery for congenital heart defects with cardiopulmonary bypass and an age and gender matched control group of 25 healthy children were enrolled in the study. Species-specific 16SrRNA gene sequences were used for S. mutans and serotype-specific rgpF gene sequences were used for S.mutans serotype k determination in stimulated saliva samples.* ***Results:*** *S.mutans was detected in 19 (76%) of the study and 15 (60%) of the control children. The difference was not shown to be statistically significant. Serotype k was determined from 3 (12%) of the study group, while it was not determined from the samples of the control group.* ***Conclusions:*** *Our results indicate that those children with congenital heart disease may possess S.mutans serotype k in oral cavity at a higher frequency as similar with the adult cardiac surgery patients.*

Keywords: *Streptococcus mutans, serotype k, congenital heart surgery, saliva, children*

INTRODUCTION

Patients with congenital cardiac disease are at high risk of developing dental caries at a young age, thus adding a burden to their quality of life.[1-4] Difficulties with nutrition during their first years of life, chronic vomiting, high diet frequency, sucrose-containing medicine usage and negligence of oral hygiene as a result of a greater concern with cardiac disease are certain predisposing factors for poor oral hygiene.[4,5] Several guidelines have existed which have identified specific underlying cardiac conditions that placed children at increased risk of infective endocarditis (IE).[6-8]

The relationship between oral microorganisms and the development of IE is well known.[9,10] It was previously reported that, *Streptococcus mutans,* a major pathogen of dental caries, was the most frequently detected species among streptococci in cardiovascular specimens.[11] A close relationship of *S. mutans* with IE has been suggested, before.[12-15]

Based on the chemical composition of its cell surface serotype specific rhamnose–glucose polymers (RGPs), *S. mutans* have been classified into *c, e, f* and *k* serotypes.[16,17] It has been reported that approximately 70% – 80% of *S. mutans* strains in the oral cavity belong to serotype *c*.[18] Detection frequency of serotype *k,* a novel serotype, was estimated to range from 2% to 5%, while it was higher (10%) in cardiovascular surgery patients.[19] While it has been detected with a higher incidence in cardiovascular specimens,[17,20] it was speculated that *S. mutans* serotype *k* might be associated with the development of cardiovascular diseases, because it can resist to phagocytosis.[19]

S. mutans serotype *k* strains have also been detected in the oral cavities of healthy children with a prevalence of 2 – 2.9%,[20,21] but there is no information about oral presence of serotype *k* in children with congenital heart disease.

The aim of this study was to determine the salivary prevalence of *S. mutans* serotype *k* in children with congenital heart disease and compare with an age and gender matched control group of healthy children.

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Table 1. The distribution of the oral indices scores for the study and matched control groups.

	Study group (n = 25)			Control group (n = 25)			*P*
	Mean ± SD	Median	Min-max	mean ± SD	Median	Min-max	
dmft	5.47 ± 5.22	5	0 - 16	6.3 ± 2.98	6	1 - 11	0.275
dmfs	9.69 ± 9.72	7	0 - 30	11.6 ± 5.52	13	2 - 21	0.194
DMFT	2.23 ± 2.65	1	0 - 7	1.38 ± 1.50	1	0 - 4	0.613
DMFS	3.53 ± 4.72	1	0 - 13	2.23 ± 2.59	1	0 - 7	0.710
GI	0.08 ± 0.10	0.08	0.0 - 0.35	0.13 ± 0.17	0.11	0.0 - 0.77	0.466
PI	0.68 ± 0.29	0.68	0.19 - 1.51	0.76 ± 0.35	0.85	0.13 - 1.52	0.443

dmft= decayed, missed, filled deciduous teeth number; dmfs= decayed, missed, filled deciduous teeth surface; DMFT= decayed, missed, filled permanent teeth number; DMFS= decayed, missed, filled permanent teeth surface; GI= gingival index; PI= plaque index

MATERIALS AND METHOD

Patient selection and sample collection

Twenty-five patients at 3-12 years-old (mean age: 6) undergoing elective surgery for congenital heart defects with cardiopulmonary bypass under general anesthesia were enrolled in the study. An age and gender matched control group of healthy children who attended to the Pedodontics Clinic of Istanbul University Faculty of Dentistry, Istanbul, Turkey were also analyzed. All clinical procedures were approved by Local Ethics Committee of the Istanbul University Faculty of Medicine (2011/118-457) and informed consent was obtained from each parent prior to initiating the study.

All oral examinations were performed by one examiner (EB), following bio-security principles. The patients were examined on a medical examination table under good incident light. The examinations included: determination of the plaque index (PI) of Silness and Löe;[22] determination of the gingival index (GI) of Löe and Silness[23] and determination of caries experience (dmft and DMFT indices for deciduous and permanent teeth, respectively) following the World Health Organization (WHO) criteria.[24] Buccal, mesial, lingual and distal surfaces of each tooth were examined.

Paraffin-stimulated whole saliva samples were collected for 5 minutes. Swab samples from dental surfaces were taken using sterile swab sticks in smaller children, who were unable to chew. The swab samples were then placed in 2 ml sterile saline and homogenized by vortexing. The samples were separated equally for culture and PCR analysis.

Microbiological analysis

The levels of cariogenic microorganisms were detected using culturing techniques. The samples were immediately diluted 10-fold and 0.1 ml of appropriate dilutions were then plated onto Mitis Salivarius Agar (Acumedia Man Inc., Baltimore, Maryland) with bacitracin for mutans streptococci, onto Rogosa Agar (Merck KGaA, Dermstadt, Germany) for lactobacilli and onto Sabouraud Dextrose Agar (Merck) for yeasts. The typical colonies were counted and results were expressed as cfu/ml. The lower limit of detection was 10^3 cfu/ml for *mutans streptococci* and 10^2 cfu/ml for lactobacilli and 10 cfu/ml for yeasts.

PCR techniques were used for *S. mutans* presence and serotype *k* determination. For each sample, bacterial DNA was extracted and purified with the High Pure PCR Template Preparation Kit (Roche, Mannheim, Germany) according to the manufacturer's instructions.

Species-specific 16S rRNA gene sequences were used for *S.mutans* detection.[25] *S. mutans* positive samples were then analyzed for serotype *k* determination using serotype-specific *rgpF* gene sequences.[21] The sensitivity of all the PCR methods was evaluated using reference strains: *S. mutans* ATCC 25175 and *S. mutans* serotype *k* LJ23 (obtained from Dr K. Nakano, Department of Pediatric Dentistry, Osaka University Graduate School of Dentistry, Japan). The detection limit for simultaneous PCR was determined as 1000 cells by using the known numbers of bacterial cells diluted in sterile distilled water.

Statistical analysis

The data was tested for normality using the Shapiro Wilks test. Besides standard descriptive statistical calculations (mean and standard deviation), a Fisher's exact probability test, chi-square and Mann Whitney U tests were used for group comparisons. A *P* value of 0.05 was considered significant. Data were processed using the SPSS software Version 17.0.

RESULTS

Finally a total of 50 children were included in the study; 25 in the study group and 25 in the control group. The ages of the participants ranged from 3 to 12 for the study and the control group. The mean age of the children was 6.24 (± 2.93) in the study group and 6.24 (± 2.98) in the control group. There was no statistically significant difference in the mean age between the two groups.

The distribution of the dmfs/ dmft, DMFS/DMFT, PI and GI values are given in Table 1. There was no significant difference for the oral indices between the study and control children. A total of 12 in the study group and 12 in the control group had primary teeth. The dmft of these groups were 5.50 (± 5.63) and 6.75 (± 3.22) respectively. The difference was not shown to be statistically significant. A total of 13 in the study group and 13 in the control group were in mixed dentition, and so, had permanent teeth. The DMFT values in the Table 1 represent their diagnosis.

Table 2 shows the summary of the salivary concentrations of *mutans streptococci*, lactobacilli and yeast for the study and control group. There numbers of the salivary cariogenic microorganisms was not significantly different between the study and control children.

According to the PCR assay, *S. mutans* was detected in 19 (76%) of the study and 15 (60%) of the control children. The difference was not shown to be statistically significant (Table 3). Serotype *k* was determined in 3 (16%) of the 19 *S. mutans*-positive salivary samples. The determination rate for *S. mutans* serotype *k* among all the study group was 12%. Serotype *k* was not determined from the samples of the control group.

Table 2. Distribution of the numbers of salivary cariogenic microorganisms (log_{10} cfu/ml) for the study and matched control groups.

	Study group (n = 25)				Control group (n = 25)				*P*
	IF%	Mean ± SD	Median	Min-max	IF%	mean	Median	Min-max	
Mutans streptococci*	84	5.7 ± 0.5	6.0	0-7.1	80	5.6 ± 0.5	5.8	0-6.1	0.269
Lactobacilli**	68	4.7 ± 0.6	5.0	0-6.2	80	4.8 ± 0.4	5.0	0-5.0	0.517
Yeast***	56	3.7 ± 0.6	3.9	0-4.7	68	3.7 ± 0.4	3.8	0-4.0	0.769

IF%= isolation frequency; min= minimum; max= maximum.

*Detection limit=1000 cfu/ml; **Detection limit=100 cfu/ml; ***Detection limit=10 cfu/ml

DISCUSSION

Oral bacterial species such as *S. mutans* invade the bloodstream from the oral cavity, thus it is important to analyze the bacterial profiles of oral specimens from patients with high IE risk. *S. mutans* serotype *k* has been detected in higher frequencies among other serotypes.

This is the first report for oral serotype *k* prevalence in children with congenital heart disease. S. *mutans* serotype *k* was firstly isolated from the blood of IE subjects and differentiated by glucose side chain of the serotype-specific RGPs deficiency,[20] which leads less antigenic and less susceptible manor to phagocytosis by human polymorphonuclear leukocytes.[21] In this context, these properties increase the virulence of serotype *k* in blood and cause a greater duration of bacteremia and systemic inflammation,[26,27] while they cause to decrease the cariogenicity of these strains.[28-30] Serotype *k* also seems to be potentially associated with the pathogenesis of heart valve disease, due to *cnm* gene, which regards to their ability to attach to exposed endothelial collagen.[31]

In the present study, salivary prevalence of *S. mutans* serotype *k* in children with congenital heart disease was 12%, while it was not detected in the age and gender matched control group of healthy children. The isolation frequencies of *S. mutans* serotype *k* in the present study were closely similar to those reported by Nakano *et al* [19]: the detection frequency was 10.3% in 39 cardiovascular surgery patients with an age range of 49-80 years old. In the same study, serotype *k* was determined in 2.9% of the oral specimens of 69 healthy children with an age range of 2-12 years old, while it was not determined in their healthy mothers. In another study, *S. mutans* serotype *k* was detected at a much higher frequency in dental plaque specimens from subacute IE patients (75%) than in those from non-IE patients (20%).[32] So we speculate that the three children in our study with positive salivary serotype *k*, may be at high risk of IE.

Serotype *k* was originally designated for *S. mutans* strains isolated in Japan, and it is important to consider the geographical prevalence of this serotype. The reports regarding the detection of serotype *k* strains in worldwide are very limited. Serotype *k* has been found among strains isolated in Japan, Finland, Thailand and the UK,[17,33-35] whereas there is no information available on the serotype *k* prevalence in Turkish children. This is the first report for oral serotype *k* prevalence in Turkish population.

Stecksen-Blicks *et al* [4] have been observed a poor health of primary teeth in cardiac children and they argued that, this could indicate the difficult situations as these children face during their first years of life. Poor dental health also gives an increased risk of dental bacteremia that may lead to IE.[36] The mean dmft and DMFT values in our study group were 5.47 and 2.23, respectively. The mean DMFT values are higher than those for 12 years-old in Turkey, which has been reported as 1.9 according to WHO,[37] but there was no significant difference for the oral indices between the cardiac and healthy children in our study. In this case-control study, the same examiner has examined all the children in both groups to remove the effects of variation in caries diagnosis between different examiners. It is, however, unclear if there were any socioeconomic differences between the cases and the controls that may have influenced the results.

Since all chıldren in the control group had carıes experience, *S.mutans* was not detected in 40 % of them. This result may be controversial to much research which has suggested that *mutans streptococci* are the major pathogens of human dental caries. However, some recent studies indicate that the relationship between mutans streptococci and caries is not absolute: high proportions of mutans streptococci may persist on tooth surfaces without lesion development, and caries can develop in the absence of these species.[38-40] Recent molecular analyses have strengthened this concept by showing that the microbiota associated with white spot lesions is more diverse than hitherto appreciated and that novel phylotypes and species.[39,41,42] In addition, the samples of the children which *S. mutans* wasn't detected may harbor *S.mutans* under the detection limit of our PCR assay.

CONCLUSIONS

This is the first report to describe the presence of *S. mutans* strains serotype *k* in Turkish children and it adds the detection of serotype *k* in oral samples of cardiac children. Closer cooperation between pediatric cardiologists, pediatric dentists and oral microbiologists could help to improve dental care and systemic health for these children. Our results with those of others suggest that *S. mutans* serotype *k* presence in the oral cavity have to be taken into account for IE risk evaluation in cardiac children.

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Table 3. Distribution of salivary *S. mutans* serotype *k* in study and control groups

	Study group (n = 25)		Control group (n = 25)		*P*
	n	%	n	%	
S. mutans	19	76	15	60	0.365
Serotype *k*	3	12	0	0	0.235

*Detection limit is 1000 cells

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