Serotype c and e Streptococcus mutans from Dental Plaque of Child-Mother Pairs With Dental Caries

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Abstract

This study aimed to identify serotype c and e *Streptococcus mutans* (*S. mutans*) in child-mother pairs and determine the relationship between serotype S. mutans and dental caries in child-mother pairs. 119 pairs of 3- to 5-year-old child and mother participated in this study. Dental caries status were examined. Plaque samples were collected from 66 pairs subjects with dental caries. Polymerase chain reaction (PCR) using gtf B primer, Sc/Se primer was performed to detect *S. mutans* from dental plaque. Statistical analyzed used Pearson correlation, linear regression, one-way ANOVA, and Pearson chi-square.PCR confirmed *S. mutans* from 46 plaque samples pairs. There was a significant relationship between child-mother caries score ($p<0.05$). Child caries scores increased as mother caries score rose. Serotype c *S. mutans* distribution had more prevalent detected than serotype e. There was no significant relationship between serotype c/e *S. mutans* and child-mother caries score. There was also no significant relationship between serotype c and e *S. mutans* in child-mother.

We found thevarious composition of serotype *S. mutans* in child's mother's dental plaque that could be identified in dental plaque of child, mother, or both. The presence of serotypec and e *S. mutans* had no relationship with caries in child-mother.

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Introduction

Dental caries is still one of the dental health issues in children and adults in Indonesia. Study reported caries prevalence among 3- to 5-years-old preschool children in Jakarta was 81.2% whereas national report in 2013 said DMFT index was 4.6.[1,2]

Streptococcus mutans (*S. mutans*) are considered to be an important bacterial pathogen of dental caries. The presence and level of *S. mutans* in oral cavity could be a caries risk indicator due to its important role in caries initiation and progression. HYPERLINK \l "Zho13"[3] Children with early childhood caries have 30-50% *S. mutans* in plaque flora and 10% *S.mutans* in saliva flora, while children with low-level caries risk have less than 1% *S. mutans.*[3]

S. mutans colonies in children have been formed through *S. mutans* transmission from mothers, caregivers, siblings, and peers. However, the primary source of *S. mutans* is acquired from their mother, especially in early age children who depend on physically on their mother.[4] The mother's poor oral hygiene habit, dietary habit, and activity which increases the possibility of saliva contact between mother and child could lead *S.mutans* transmission risk.[5] A study reported that the number of *S. mutans*and preschool child's caries status had a correlation with the increasing number of *S. Mutans* and mother's caries status.[6]

S. mutans organisms have been classified into four serotypes (c, e, f, and k) based on the chemical composition of specific-

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serotype polysaccharide on its cell surface. Serotype c/e/f *S. mutans* are frequently found in human oral cavity samples, while serotype k is the latest serotype detected and usually found in a blood sample of people with cardiovascular disease.[7]

The specific-serotype is known to consist of rhamnose-glucose polysaccharide with a backbone of rhamnose and side chains of α- or β- linked glycosidic residues. The glucose side chains of specific-serotype have been assumed to play a major role in *S. mutans* colonization in the oral cavity.[8]

Due to the advanced technology, the polymerase chain reaction (PCR) method has been used in many studies to detect and identify *S. mutans* from plaque and saliva samples.[3,7]

This molecular approach technique is more rapid, sensitive, and specific than theconventional method.[7] PCR also can identify serotype *S. mutans* by using DNA extracted from samples and specific primers which arranged based on the difference of sequence of the gene located in argp A –rgp F operon.[8]

Serotype c *S. mutans* has been reported to be the most predominated in human dental plaque and saliva sample, its prevalence is 70-80%. Serotype e *S. mutans* is the next most common which is approximately 20%.[7]

Identification of serotype *S. mutans* can be advantageous to estimate *S. mutans* transmission from mother to child. There is a high-level similarity of serotype *S. mutans* between child and mother.[9,10] Furthermore, this study was aimed to identify serotype c/e *S. mutans* in child-mother pairs, to determine the correlation of caries status in child-mother pairs, and to analyze the relationship between serotype c/e *S. mutans* and child's mother's dental caries.

Materials and methods

This study was approved by Research Ethical Committee of the Faculty of Dentistry, Universitas Indonesia. Subjects were preschool children and their mothers in Central Jakarta and East Jakarta. All mothers who participated in this study had signed the written informed consent before the study. The inclusion criteria were children aged 3 – 5 years old, biological mother, a mother who is the primary caregiver to her child and frequently contact with her child at least 8 hours per day, both child and mother have dental caries. The exclusion criteria were both child and mother had been undergone any oral antibiotic therapy for the last three months and subjects with medically, physically, mentally disabilities.

119 child-mother pairs were examined to check the def-t score in child and DMF-T score in mother based on WHO.[11] All mothers filled in the questionnaire on the same day with the intraoral examination. After the intraoral and questionnaire screening, 66 child-mother pairs continued the study.

Dental plaque samples were collected by swabbing it from upper right posterior teeth using swab transport system (COPAN Diagnostics Inc., USA), both in child and mother, at 09.00 – 10.00 am. Child dental plaque was labeled as "A," while "M" was a label for mother dental plaque. Samples then were stored in 4^{o}C ice container and transported to the laboratory in less than 4 hours. Next, samples were cultivated in selective solid media trypticase soy-yeast-20 per cent sucrose-bacitracin (TYS20B) in Petri dishes. Each sample contained petri dish was placed in ananaerobic jar and incubated at 37^{o}C for 48 hours. Colonies of *S. mutans* would harvest on TYS20B media after 48 hours. Five colonies would be picked from each petri dish to recultivate in agar media TYS20B and trypticase soy broth (TSB). After recultivated, the next step was bacteria's DNA extraction using Wizard® Genomic DNA Purification Kit(Promega, USA). DNA concentrations were calculated by measuring each DNA extraction products using spectrophotometer at 280 nm wave lengths.

Species/ serotype	Primer	Sequence (5' – 3')	Product size (bp)
S. mutans	GTFB-F	ACTACACTTTCGGGTGGCTTGG	517
	GTFB-R	CAGTATAAGCGCCAGTTTCATC	
c	SC-F	CGGAGTGCTTTTTACAAGTGCTGG	727
	SC-R	AACCACGGCCAGCAAACCCTTTAT	
e	SE-F	CCTGCTTTTCAAGTACCTTTCGCC	517
	SE-R	CTGCTTGCCAAGCCCTACTAGAAA	

Table 1. Primer that used in PCR process[8,12]

PCR method using sequenced (5' – 3') primer was performed to identify *S. mutans* as presented in Table 1.[8,12] The composition of 1 tube for PCR reaction mixture was DreamTaq Green PCR Master Mix (Fermentas, USA) 10μl, Primer-1 2 μl, Primer-2 2 μl, water 2 μl, DNA 4 μl.

PCR detection of *S. mutans* was

performed with GTFB primer. The reaction mixture was denatured at 95°C for 1 min followed by a series of amplification for 35 cycles: denaturation at 94°C for 30 sec, annealing at 53°C for 1 min, and elongation at 70°C for 2 min. The final cycle comprised 72°C for 7 min for post elongation. Sc primer was used to identify serotype c *S. mutans* while Se primer was used to identify serotype e. *S. mutans*. The reaction mixture was denatured at 95°C for 1 min followed by 30 cycles of amplification: denaturation at 95°C for 30 sec, annealing at 59°C for 30 sec, elongation at 72°C for 30 sec. The final cycle comprised 72°C for 4 min for post elongation.

After amplification, the PCR products were analyzed and visualized by electrophoresis on an agarose gel 1.5%. 5 µl of PCR products were placed into well on an agarose gel. DNA ladder 100 bp was used to be DNA marker. Electrophoresis was performed at 400 mA, 80 volts, for 75 min. DNA visualization was performed by placing the gel on UV light gel-doc, then the resulting was documented on printed paper or saved file on the computer.

The result data were analyzed using bivariate analytical of the Pearson correlation test followed by a linear regression test of the caries score. One way ANOVA test was employed to determine the difference of serotype *S. mutans* and caries score. Pearson chi-square test for serotype *S. mutans* between child and mother. Statistical confidence was considered to be at p≤0.05.

Results

PCR confirmed the presence of *S. mutans* in dental plaque samples of 46 child-mother pairs. Seven child's plaque samples had *S. mutans,* but *S. mutans* undetected in their mother's samples, three mother's plaque samples had *S. mutans* but *S. mutans* undetected in their child's samples, then it made ten subjects pairs eliminated. Total subjects that would be analyzed were both child and mother who had *S. mutans* in their plaque samples (46 child-mother pairs).

The correlation between deft score and DMFT score are shownin Table 2 and Graphic 1. There was significant, positive correlation between deft score and DMFT score ($p < 0.05$). Moreover, deft score increased as DMFT rise by y= 5.71 + 0.44*x.

	Mean	r	p
Child's deft	8.50 ± 5.04	0.41	0.01*
Mother's DMFT	6.41 ± 4.70		
* signifancy: p < 0.05			

Table 2. Correlation between child's deft score and mother's DMFT score.

	Number of subjects that identified	%	Number of subjects that not identified
Child			
Serotype c*S. mutans*	29	63.04	17
Serotype e *S. mutans*	10	21.74	36
Mother			
Serotype c *S. mutans*	21	45.65	25
Serotype e *S. mutans*	9	19.57	37

Table 3. Distribution profile of serotype c and e S. mutans c in child and mother.

S. mutans	Mean of DMFT	p
serotype c	7.20 ± 4.39	
serotype e	3.33 ± 2.08	0.12
combination serotype c & e	3.83 ± 2.48	
*significancy: p<0.05		

Table 4. The difference between DMFT distribution and serotype c and S. mutans in mother.

Serotype c and e *S. mutans* in the present study were found in single and combination variation. Single variation is defined as one serotype has been detected in one subject, while multiple serotypes which have been detected in one subject refer to combination variation. For the next, serotype c and e *S. mutans* which have been detected in one subject will be defined as combination serotype c and e *S. mutans*. In this study, Table 3 shows serotype c/e *S. mutans* both in single and combination variation which has been successfully identified from both child's and mother's plaque samples. Prevalence of single variation of serotype c *S. mutans* in children and mothers was 2.17%. However, asingle variation of serotype e *S. Mutans* has not been identified in mothers subject, whereas it was 2.17% in children. Combination variation was 15.22% in children and 13.04% in mothers.

Most of the children and mothers subject have more several combination serotypes than single serotype.

S. mutans	Mean of deft	p
serotype c	7.86 ± 4.22	
serotype e	5.67 ± 3.21	0.10
combination serotype c & e	11.86 ± 6.52	
*significancy: p<0.05		

Table 5. The difference between deft distribution and serotype c and *S. mutans* in child.

		Serotype c *S. mutans* in child			
		(no)	(yes)	R	p
Serotype c *S. mutans* in mother	(no)	10 (21.74%)	15 (32.61%)	0.07	0.64
	(yes)	7 (15.22%)	14 (30.44%)		
		Serotype e *S. mutans* in child			
Serotype e *S. mutans* in mother		(no)	(yes)	R	P
	(no)	30 (65.22%)	7 (15.22%)	0.14	0.35
	(yes)	6 (13.04%)	3 (6.52%)		
		Combination of serotype c and e *S. mutans* in child			
Combination of serotype c and e *S. mutans* in mother		(no)	(yes)	r	P
	(no)	34 (73.91%)	6 (13.04%)	0.02	0.92
	(yes)	5 (10.87%)	1 (2.17%)		
*significancy: p< 0.05					

Table 6. The relationship of serotype c and e *S. mutans* of child-mother pairs.

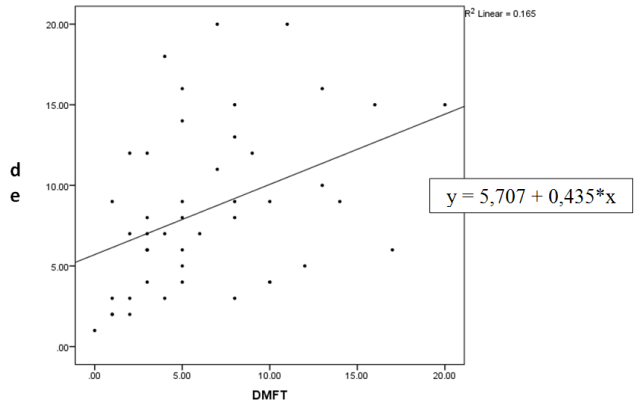

Figure 1. Correlation between child's deft score and mother's DMFT score.

The caries score and serotype c and e *S. mutans* are shown in Table 4 and Table 5. One way ANOVA test showed neither DMFT score nor deft score had statistical difference significantly on serotype c and e *S. mutans* (p>0.05).

There was avariation in the presence of serotype c *S. mutans*, serotype e *S. mutans*, acombination of serotype c/e *S. mutans* in child-mother pairs whichare shown in Table 6. The statistical test showed there were no statistically significant correlation serotype *S. mutans* between child and mother (p > 0.05).

Discussion

It has been known that many factors related to the child's and the mother's caries status.[3-6] The aim of the present study was for linking the relationship of child's and mother's caries status based on the bacterial etiology factor, according to the proportion of serotype c and e *S. mutans* in plaque samples.

In the present study, *S.mutans* were cultured from dental plaque because it is the primary habitat of *S. mutans* in the human oral cavity. According to previous studies, there is no significant difference between dental plaque and saliva samples for *S. mutans* organism identification.[3]

The result showed that the child's and the mother's caries score had a significant positive correlation, consequently child's caries score increased as mother's caries score expanded. In other words, child's caries score depend onto mother's caries score. This result relates to studies that have reported acorrelation between preschool child's and mother's caries status.The study indicated that mother's caries status would increase child's caries status to double.[13] Further, it has been reported that mother's caries score is one of the child's caries risk. A mother who has a higher DMFT score also has ahigh level of *S. mutans* so that it can increase the transmission risk of *S. mutans* from mother to child.[6]

The proportion of serotype e *S. mutans* in both child and mother was less than serotype c *S. mutans* and non-serotype c/e. Several studies revealed that serotype c *S. mutans* is the most predominated in oral cavity followed by serotype e *S. mutans*.[7,10,14] This is different than what we found in present study, but it relates to one previous study which subjects were preschool children in Jakarta. It reported that serotype e *S. mutans* was the least in preschool children's dental plaque.[15] The similar result is from Brazil which subjects were adults. It showed that serotype c *S. mutans* was the most common found but serotype e *S. mutans* was not identified from all samples.[16] Data of serotype *S. mutans* distribution in the present study might be used to

confirm the distribution of serotype *S. mutans* in child and adults in Indonesia, which is different from data from other countries. This difference may be due to caries prevalence. Caries mean score in the present study was high (deft/DMFT>5). Studies that reported serotype c *S. mutans* is predominated in samples and followed by serotype e *S. mutans,* are conducted in developed countries which have lower caries prevalence than Indonesia.

The highest caries mean score in the mother was a mother who had serotype c *S. mutans* while the least mean score was a mother who had serotype e *S. mutans*. The highest caries mean score in the child was a child who had multiple serotypes *S. mutans* where as the least mean score was a child who had serotype e *S. mutans*. What we found in child subject relate to previous studies that mixture colonies of multiple serotypes *S. mutans* are predicted to have a close relation with caries development.[14] However, mother subjects showed a different kind. This difference may be caused by the different of oral cavity condition between child and mother, level of oral hygiene status, level of frequency of refined intake carbohydrate, nutritional status, and immune system that involve bacterial interaction to initiate caries.[3,5,17,18]

Statistically, mother's caries was not related to serotype c and e *S. mutans* neither did child's caries. It is related to the previous study said that serotype *S. mutans* has not significant relation with caries prevalence.[10] *S. mutans* factor only is not adequate to develop dental caries because it has multifactorial etiology.[17]

In the present study, we did not found the presentof serotype c/e *S. mutans* simultaneously between child and mother. Contrary, previous study revealed there was a high matching rate between serotype *S. mutans* of child and mother.[10] The frequency of *S. mutans* similarity between child and mother may depend on culture and race factors, but it will decrease as child's age increase. Each strain of *S. mutans* may has difference virulence, colonization capacity, and level of infectivity.[3,18]

In our opinion, *S. mutans* might have adaptation and mutation caused there were no serotype *S. mutans* that simultaneously found in child and mother. In this study, the child age subject was 3 to 5 years old, while according to "window of infectivity" theory, early transmission of *S. mutans* is an occurrence in young age period before 3 years old.[3] During that span time, we assumed there might be adaptation and mutation. It has been reported that cell has ability to communicate with other cells in biofilm environment. Mechanisms of the signal system among cells (*quorum sensing*) can help the cell to adapt and survive in the environment. This communication system also presence in *S. mutans,* and it is believed to be involved in biofilm development. Adaptation and survival ability will continue after bacteria has been transmitted to a new environment, even there is mutation effect in latest studies.[19,20]

Conclusions

All subjects were from caries population and had caries score correlation between child and mother. We found various compositions of serotype c/e *S. mutans* in dental plaque child and mother. Serotype c and e could be identified in dental plaque child, mother, or both of child-mother. The presence of serotype c and e *S. mutans* in the child's and the mother's dental plaque samples were confirmed by PCR method. The presence of serotype c and *S. mutans* in mother have no relationship with the presence of serotype c and e *S. mutans* in a child.

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Declaration of Interest

The authors report no conflict of interest.

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