研究者：Mega Moeharyono Puteri（所属：Graduate School of Biomedical Sciences Applied Biomedicine, Department of Pediatric Dentistry, Hiroshima University）

研究題目：*Mutans Streptococci* Transmission Genotypic Diversity in Children

Objective

The purpose of the study is to investigate the *mutans streptococci* transmission genotypic diversity in children.This study is conducted in several stages ,as the first preparation was to detect incident of *Streptococcus mutans* and *Streptococcus sobrinus* in children in age ranged from 1 to 6 years old at a day nursery

Material and Methods

Subjects

Thirty seven children（18 boys and 19 girls）in age from 1 to 6 years old who at the day nursery in Hiroshima University were examined. Consent of participation in this study was collected from parents.

Plaque Sampling

Dental plaque was collected by brushing all of the erupted teeth with sterile toothbrush. Plaques which were adhered to the toothbrush were removed by washing several times in sterile distilled water. The plaque samples were immediately transported to the laboratory for DNA preparation.

Preparation of Genomic DNA

The chromosomal DNA was prepared according to standard mini prep method with some modification as followed. Plaque sample in distilled water were centrifuged 7000 rpm for 5 min until a pellet formed and the supernatant was discarded. Pellet was resuspended in 567 μl of TE buffer and all transferred to the new micro centrifuge tube added with glass beads 0,150 g. After 30 μl of 10% SDS, 3 μl of 20 mg/ml proteinase K was added and vortex for 3 min followed with incubation at 37°C for 1 hr. Then, 100 μl 5M NaCl was added and the solution was completely mixtures. Next, 80 μl CTAB was added and incubated at 65°C for 10 min. Furthermore, 0.7 vol of Chloroform/isoamyl alcohol（24：1）was added and the solution was mixed thoroughly. After centrifugation 13000 rpm for 5 min, supernatant was collected and equal volume of phenol/chloroform/isoamyl alcohol（25：24：1）was added. Again centrifugation at 13000 rpm for 5 min was performed. The supernatant was transferred to the new micro centrifuge, 0.6 vol isopropanol was added and stored at −20°C overnight. The following day, suspension was centrifuged 13000 rpm for 5 min. The precipitate was added 700

μl 70% ethanol followed with centrifugation at 13000 rpm for 5 min. The supernatant was discarded and pellets dried for 1hr 30 min at room temperature. The precipitate containing DNA was suspended in 30 μl TE buffer and stored at −30°C.

PCR

S. mutans 8148 and *S. sobrinus* were used as the control. PCR was performed with the primers as described by Igarashii et al (1996 ; 2000). Primer for *S. mutans* (SD1 and SD2) specifically amplified a 1272-bp fragment sequences were 5'-TAT GCT GCT ATT GGA GGT TC 3' (positions 973 to 992) and 5'-AAG GTT GAG CAA TTG AAT CG-3' (positions 2225 to 2244) respectively. Primer for *S. sobrinus* (SOF14 and SOR1623) amplified a 1610-bp fragment sequences were 5'-TGC TAT CTT TCC CTA GCA TG-3' (positions 134-153) and 5'-GGT ATT CGG TTT GAC TGC-3' (positions 1743-1726) respectively. Amplification of PCR was performed in 25 μl reaction mixture (Bioline Biotaq DNA polymerase) in Bio-rad iCycler. Positive and negative controls were used to minimize the impact of false positives. The Thermal cycle parameters were performed at 94°C for 2 min, 30 cycles of 94°C for 20 s, 50°C for 30 s and 72°C for 1 min 30 s. PCR reactions ended with final elongation step at 72°C for 5 min and 4°C for ∞. PCR products were separated on 1 % agarose gel electrophoresis, stained with ethidium bromide.

Results

To evaluate the incident of *S. mutans* and *S. sobrinus* in children, the bacterial expressions were detected. PCR showed that 67.6% were positive for *S. sobrinus* ; 8.1% were positive for *S. mutans* ; 18.9% were positive for both *S. mutans* and *S. sobrinus* and 5.4% were negative for both *S. mutans* and *S. sobrinus*. (figure.1)

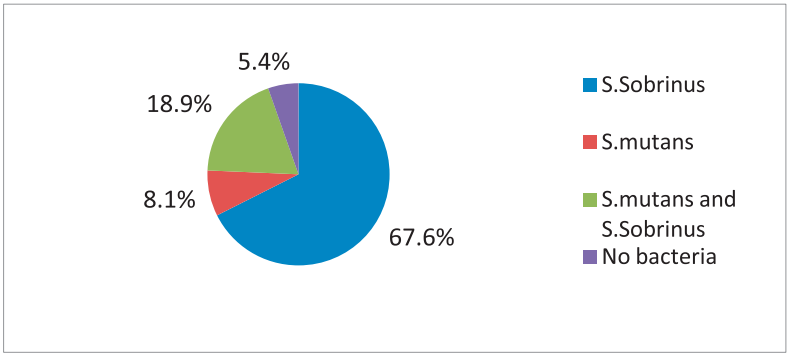

Figure 1 Organism present in all of the subjects

Distribution of *S. mutans* and *S. sobrinus* in different age of groups was shown in table 1. From the table 1 it was suggested that 75.0% children less than three years of age positive for *S. sobrinus* alone, no one from this group positive for *S. mutans* and 8.3% positive for both *S. sobrinus* and *S. mutans*. However, there were 16.7% children under three years of age negative for both *S. sobrinus* and *S. mutans*. On the other hand, children above three years of age in this study none of them negative for both S. sobrinus and *S. mutans*. Children aged 3 – 4 years old were detected positively for *S. sobrinus* alone (61.5%); 15.4% were positive for *S. mutans* and 23.1% were positive for both *S. sobrinus* and *S. mutans* respectively. Data showed those children aged 5 years old were positively detected for mutans streptococci ; 66.7% for *S. sobrinus* alone, 8.3% for *S. mutans* and 25.0% for both *S. sobrinus* and *S. mutans*.

Table.1. Distribution of mutans streptococci in different age groups

Organism	Number (%) of subject age							
	<3		3≤age<5		5≤		Total	
S. sobrinus	9	75.0	8	61.5	8	66.7	25	67.6
S. mutans	0	0	2	15.4	1	8.3	3	8.1
S. mutans and *S. sobrinus*	1	8.3	3	23.1	3	25	7	18.9
No bacteria	2	16.7	0	0	0	0	2	5.4
Total	12	12	13	13	12	12	37	100

Discussion

In the present study, data showed that most of the children positive for *S. Sobrinus* alone (67.6%) followed with (18.9%) positive detection for both *S. mutans* and *S. sobrinus*, and 8.1% for *S. mutans* alone. The prevalence of *S. mutans* and *S. sobrinus* in this study was 27.0% and 86.5% respectively. However the outcome of this present study quite different from the previous study (Okada et al, 2002) who reported the prevalence of *S. mutans* and *S. sobrinus* was 72.8% and 61.1% in aged 3 years to 5 years old of Japanese preschool children. These conditions probably occur as saliva role as protection against oral bacteria. The galactosides in saliva destroy the surface protein antigens of *S. mutans* that may prevent adherence of *S. mutans* (Law. V, 2007). Furthermore, this condition supported with study done by Tedjosasongko (2002) reported that *S. sobrinus* transmit easier than *S. mutans* and also speculated that bacteria with high ability to produce insoluble glucan may easily colonize on the tooth surface.

Mutans streptococci groups were found to be closely related to the incidence of dental caries in children. The initial acquisition of mutans streptococci were occurred at median age of 26 months, the period reported as “the window infectivity” (Tedjosasongko, 2002 ; Liu Y, 2007). Since, mutans streptococci are considered as the major cause of dental caries, it is important to detect as early as possible to make prevention from dental caries. From the

table1, it is shown that most of all the children 3 years old above, suggested for mutans streptococci. This study supported by previous study by Kozai et al (1999) reported the initial acquisition of mutans streptococci was observed at the average age of 26 months and at least within the first 5 years. Since that, some of the children may suggest from *S. sobrinus* alone, *S. mutans* alone or both *S. sobrinus* and *S. mutans*. The unsimiliar result occurred in this study to another study, could be also influenced by many factors such as bacterial properties, host factors, diets and environmental factors including habits and life style. In conclusion, in this study most of all the children harboring *S. Sobrinus* and *S. mutans* during "the window of infectivity".

In the future studies, DNA fingerprinting analysis will be perform to those subjects who harbor *Mutans Streptococci*. This is in order to evaluate the *mutans streptococci* genotypic diversity, transmission and bacterial properties that related in transmission.

Reference

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