The colony number of *Streptococcus mutans* and *Lactobacillus* in saliva of dental caries and free caries children

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ABSTRACT

Streptococcus mutans (S. mutans) are regarded as the main initiator microorganism of caries, with Lactobacilli participating on caries progression, due to its carcinogenic capacity. The purpose of this study was to examine the number of S. mutans and Lactobacillus sp in children with in children's saliva with dental caries and free caries. Twenty children attending the Paediatric Dental Clinic in Airlangga University participated in our study. Their age ranged from 1–14 years old. Subject was divided into two groups, which were study group consisting of 10 children with 3–5 dmft/DMFT and control group with 10 caries free children. Subjects were examined and their caries number was recorded using WHO index. Stimulated saliva was collected from each subject for bacterial assessment. Colony counting of S. mutans and Lactobacillus sp count in each saliva sample group were done. The study showed that subject with 3–5 dmft/DMFT had higher number of S. mutans and Lactobacillus sp than caries free.

Key words*: StreptococcuS. mutans, Lactobacillus sp, saliva, primary teeth*

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INTRODUCTION

In the last decade, oral and dental health have remarkably increased, however, the prevalence of dental caries in children as clinical problem remains significantly high. Dental caries is one of pathological conditions mostly suffered by Indonesian children. In Indonesia the prevalence of dental caries in children of 10 years old is 80%. Dental caries is an infectious disease initiated by progressive demineralization in hard tissue on the crown surface and the tooth root. Several interrelated factors such as: the tooth and saliva (host), microorganism, substrate and time are involved in the process of dental caries.[1]

Streptococcus mutans (S. mutans) and *Lactobacillus sp* are the active microorganisms contributing dental caries. The latest study suggests that the essential role of *S. mutans* is in the initial process of caries while *Lactobacillus sp* is correlated with the active caries episode.[2] *S. mutans* is the main bacteria contributing dental caries.[3] In free caries patients, low number of *S. mutans* is found as normal flora in oral cavity, on the contrary it is dominant in plaque of patients with active multiple caries lesions. The critical period of *S. mutans* colonization occurs at the age of 19–31 months in which it is considered the window of infectivity.[4] However, the evidence showed that *S. mutans* could be found soon after tooth eruption, even in babies of 6 months old who do not have any teeth yet.[5,6]

The study conducted on children either with high caries or free caries revealed that children with good oral condition have low number of *Lactobacillus* and children with active caries indicate the presence of bacteria with higher adhesion capacity and agglutination process.[7] The objective of this study was to examine the number of *S. mutans* and *Lactobacillus sp* in saliva of caries children and free caries children.

MATERIAL AND METHOD

Twenty children (boys and girls) attending the Paediatric Dental Clinic in Airlangga University Surabaya participated in our study. Subject was divided into two groups, which were study group consisting of 10 children with 3-5 dmft/DMFT and control group with 10 caries free children. Both groups were classified into four groups consisted of 5 samples. The criteria for inclusion of the samples in this study were as follows: aged 1–14 years with primary dentition, mixed dentition, or permanent dentition, 3–5 dmft/DMFT for study group and free caries for control group. The criteria for exclusion were if the children are taking antibiotic, syrup form medicine or suspension with high carbohydrate content one month before the saliva was taken.

Sample of saliva was taken through stimulating salivary gland by chewing parafilm, and collected in a 15 ml tube. The samples was suspended in vial containing 1 ml saline, and incubated at 37° C for two hours. The saliva sample was shaken for 30 seconds using vortex mix, and then aliquots of 25 µml plaque sample was inoculated in both agar media. Mitis Saliva Agar media was used to culture *S. mutans* and

Rogosa Media to culture *Lactobacillus sp*. After the agar media were incubated in anaerobe condition at 37° C for 48 hours, colony counting was done.[8]

The number of *S. mutans* and *Lactobacillus sp* were statistically analyzed using Kolmogorov Smirnov test and the variable was shown by the mean value. Independent t-Test was applied to analyze the different number of *S. mutans* and *Lactobacillus sp* in saliva.

RESULT

The mean and deviation standard of number of *S. mutans* and *Lactobacillus sp* in both groups are shown in table 1. It showed that the mean number of *S. mutans* is significantly higher than *Lactobacillus sp*. In free caries group, the number of *S. mutans* is lower compared to 3–5 dmft/DMFT groups. The number of *Lactobacillus sp* is also higher compared to free caries groups.

Table 1. The mean colony number of *S. mutans* and *Lactobacillus sp*

Microorganism	Type of caries	n	$\overline{X}$	SD
S. mutants	Free caries	5	246	34.2
	3–5 dmft/DMFT	5	575.8	40.1
Lactobacillus sp	Free caries	5	55	4.47
	3–5 dmft/DMFT	5	117.2	25.44

Statistical analysis using Kolmogorov-Smirnov Test was done followed by test on the different number of *S. mutans* and *Lactobacillus sp* in free caries groups and 3–5 dmft/DMFT group. The result of Kolmogorov Smirnov Test is shown in Table 2.

Table 2. The result of Kolmogorov-Smirnov Test on number of *S. mutans* and *Lactobacillus sp*

Microorganism	Type of caries	Kolmogrov Smirnov Test
S. mutants	Free caries	p = 0.987
	3–5 dmft/DMFT	p = 0.917
Lactobacillus sp	Free caries	p = 1
	3–5 dmft/DMFT	p = 1

The result of Kolmogorov-Smirnov test in all groups $p > 0.05$ is shown in table 2 indicating all groups have normal data distribution.

Statistical analysis using Independent t Test was applied to determine the different number of *S. mutans* between free caries and 3–5 dmft/DMFT group, $p = 0.001$ ($p > 0.05$) indicating significant difference in the number of *S. mutans* between those two groups.

Statistical analysis using Independent t Test was also applied to determine the different number of *Lactobacillus sp* between free caries and 3–5 dmft/DMFT group, $p = 0.001$ ($p > 0.05$) indicating significant difference in the number of *Lactobacillus sp* between those two groups.

Statistical analysis using Independent t Test was applied to determine the different number of *S. mutans* and *Lactobacillus sp* in 3–5 dmft/DMFT group $p = 0.001$ ($p < 0.05$) indicating significant difference in *S. mutans* number and *Lactobacillus sp* group of 3–5 dmft/DMFT.

DISCUSSION

S. mutans frequently found in dental plaque are regarded as the main initiator microorganism of dental caries. However, micro flora found in saliva similar to those in any surface of oral cavity, thus the method of colony count using saliva sample could represent bacterial in dental plaque.[9]

Transmission and *S. mutans* colonization in oral cavity are important factors to prevent dental caries considering *S. mutans* has a potential role in caries initial formation but in further *Lactobacillus sp* would play role.[3] In this study, the number of *S. mutans* was significantly higher than the number of *Lactobacillus sp* found in twenty children aged 1–14 years either with 3–5 dmft/DMFT or caries free. The number of *S. mutans* and *Lactobacillus sp* in 3–5 dmft/DMFT children showed significant correlation ($p < 0.001$). This study suggested children with high number of *S. mutans* and *Lactobacillus sp* would also indicate to have high number of caries. The lower the number of bacteria, the lower the caries number.

The result of this study support the previous study on preschool children which found positive correlation between the number of *S. mutans* and dental caries.[10–13] Previous researcher reported[14] similar result, patients with active caries having higher number of *S. mutans* and *Lactobacillus sp* compared to caries free patients.

Some researchers also reported the positive correlation between the number of *Lactobacillus sp* and the severity of caries.[15–16] Brambilla *et al.*[17] stated that *Lactobacillus sp* is predisposition factor for caries due to the capability of bacteria to interact with microorganism during colonization. Brambilla *et al.*[17] also reported that there are positive correlation between the number of *Lactobacillus sp* in 3–5 dmft/DMFT in 21% children aged 9–13 years old. Van Houte[3] suggested high number of *Lactobacillus sp* in dental caries but not in caries free, showed that *Lactobacillus sp* play role more on caries process than initial process. Number of *S. mutans* in oral cavity closely related with the caries severity and the prediction of caries risk in the future.[18,19]

S. mutans virulence in dental caries depends on the host, the quality and the duration of bacterial adhesion in the plaque. *S. mutans* adhesion could be accelerated by purified salivary agglutinin which interacts with agglutinin receptor (high-molecular-weight surface protein) and presence of *S. sanguis* in oral cavity. Suspensions is obtained from micro bacterial interaction producing bacterial aggregation

to destroy the enamel.[20] In which agglutinin also involved in initial process adhesion of *S. mutans* in salivary pellicle. Purified agglutinin could bind hydroxyapatite to support *S. mutans* adhesion.[21] The latest data shows agglutinin could bind bacteria in dental surface simultaneously to support *S. mutans* adhesion.

In this study, saliva was used as the sample. *Lactobacillus* is bacteria with salivary component receptor while *S. mutans* receptor mostly found in dental plaque. However, all groups indicating to have higher number *S. mutans* compared with *Lactobacillus* is due to the capability of *S. mutans* more rapidly to multiply and to produce acid compared with *Lactobacillus* (aciduric and acidogenic).[22]

The study showed that *S. mutans* and *Lactobacillus sp* were significantly higher in 3–5 dmft/DMFT compared with free caries group. Detection of *S. mutans* and *Lactobacillus sp* number is important to be done in children in order to be able to evaluate and to prevent dental caries.

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