The distribution of Streptococcus mutans and Streptococcus sobrinus in children with dental caries severity level

by Nur Dianawati

Submission date: 17-Sep-2020 01:04PM (UTC+0800)
Submission ID: 1389229060
File name: Nur_Dianawati_-_proofread_-109894.docx (388.13K)
Word count: 2347
Character count: 12444

The distribution of *Streptococcus mutans* and *Streptococcus sobrinus* in children with dental caries severity level

ABSTRACT

Background: *Prevalence of dental caries was still very high both in worldwide and Indonesia, especially in children. Cariogenic bacteria is the major couse of dental caries. Streptococcus mutans (S. mutans) is one of the bacteria often associated with caries due to its ability in producing acid and forming the bioflm for bacterial colonization on the surface of oral cavity. In addition to S. mutans, Streptococcus sobrinus (S. sobrinus) bacteria are also thought to play an important role in the process of caries.* ***Purpose:*** *This study aims to analyze the distribution of S. mutans and S. sobrinus in children with dental carries severity levels.* ***Methods:*** *This study was an observational analytical study. Bacterial isolation was conducted in carious lesions of 50 pediatric patients 6-12 years old with superficial dental caries. Samples of caries lesion were put directly in to a tube containing the brain heart infusion (BHI) Broth and incubated at 37^0 C for 24 hours. The samples, were sub-cultured on selective tryptone yeast cystine sucrose bacitracin (TYCSB-Himedia) agar, and then incubated for 2 days. Bacterial identification then was performed with PCR Multiplek method. Statistical analysis with Chi-square.* ***Results:*** *The total caries children were include in this study. Among these, showed that 94% positive for S. mutans, 30% positive for S. Sobrinus. The analysis of the prevalence of bacterial colonization (S. mutans and S. sobrinus) based on caries severity and OHI-S, there was no significant difference (p> 0.05).* ***Conclusion:*** *In this study showed that among 50 caries children, 94% were colonized S. mutans and 30 % S. sobrinus. There was not significant difference of the colonization of S. mutans and S. sobrinus, among children with severe and mild DEFT category, and also between bad and good OHI-S.*

Keywords: *caries severity; dental caries; OHI-S; Streptococcus mutans; Streptococcus sobrinus*

INTRODUCTION

Dental caries is still considered as a problem of oral health both in the world and in Indonesia. Based on the 2018 Basic Health Reasearch (RISKESDAS) data, the prevalence of caries in Indonesia was still very high and far below the World Health Organization (WHO) target.[1,2] According to the 2018 Basic Health Reasearch (RISKESDAS) data, at the age of 5-6 years the prevalence of caries reached 93%, while WHO and Federation Dentaire Internationale (FDI) had a target to make 50% of children free of dental caries. The decay missing filling teeth (dmf-t) index for primary teeth at the age of 5-6 years even was 8.43, indicating that the number of teeth suffering from caries in each individual was around 9 teeth categorized as severe early childhood caries.

Moreover, dental caries is mostly caused by cariogenic bacterial infection. *Streptococcus mutans* (*S. mutans)* is the main cariogenic bacterium in the pathogenesis process of caries.[3] Besides, *Streptococcus sobrinus* (*S. sobrinus*) is also thought to play a role in the process of

caries.[4] The pathogenesis process in the beginning of dental caries involving *S. mutans* is usually started with bacterial colonization. *S. mutans* biofilm then produces organic acids as a by-product of fermentable carbohydrate metabolism. This acid can cause the local pH to fall below the critical value resulting in demineralization of dental tissue. One of the main mechanisms of *S. mutans* producing high karyogenicity is its ability to adhere to the surface of a tooth. This attachment is successfully performed by extra-cellular polysaccaride (EPS) derived from sucrose. This process also involves the microbiological characteristics of the bacterial cell wall structure.[5]

In general, *S. mutans* is the main cause of caries.Nevertheless, the role of acidogenic and other asiduric bacteria, such as *S. sobrinus* is also assumed to be important. Based on several epidemiological and invitro studies *S. sobrinus* can be more cariogenic than *S. mutans*.[6] Virulence of the *S. mutans* group actually is related to its ability to colonize and develop on the tooth surface during acidic conditions. These properties include the production and regulation of adhesion proteins, glucosyltransferases (GTF), and extracellular polysaccharides, such as glucans which allow bacteria to attach firmly to the surface of teeth in biofilms. The two species (*S. sobrinus* and *S. mutans*), however, have different strategies in their attachment mechanism. *S. mutans* uses pellicles directly and specific surface antigens, while *S. sobrinus* uses glucans.[7]

Another previous research in Mongolia also shows that children at the age of 5-7 years old with both *S. mutans* and *S. sobrinus* in their saliva have significantly more dental caries than those who have only *S. mutans* or *S. sobrinus*.[8] In experimental animals, *S. sobrinus* even can produce more acids than other species of *S. mutans* group. The prevalence and level of *S. mutans* and *S. sobrinus* colonies, as a result, have been used as biological markers for caries prediction.[9] This study aims to analyze the distribution of *S. mutans* and *S. sobrinus* in children with dental caries severity levels.

MATERIALS AND METHODS

This study was approved by the ethics committee number: 328/HRECC.FODM/VI/2019 of the Faculty of Dental Medicine, Universitas Airlangga. This study was an observational analysis research. Samples were collected in October, 2nd-10th, 2019. The number of samples used was 50 pediatric patients at the Dental and Oral Hospital, Faculty of Dental Medicine, Universitas Airlangga, Surabaya. The age of the patients taken was 6-12 years. All parents of those pediatric patients also participated signing the informed consent. The teeth examined were deciduous molars. The type of caries examined was superficial caries or media caries with vital

teeth. Next, the severity of the caries was analyzed based on deft and OHI-S. *S. mutans* and *S. sobrinus* then were identified with multiplex PCR.

The caries lesion was taken from the first molar teeth using a sterile excavator, and put directly into the bottom of a tube containing the BHI Brain Heart Infusion Broth BHI-B (Merck, Darmstadt, Germany). Next, the tube was put into an incubator at 37ºC for 24 hours. On the second day, were sub-cultured on selective Tryptone Yeast Cystine Sucrose Bacitracin TYCSB (Himedia, Himedia Laboratories Pvt Ltd, India), and then incubated for 2 days. The growth of the colonies was indicated with the macroscopic characteristics of the colony of *S. mutans,* such as the hardened and sticky crystal form on the media, were then examine with PCR.

The PCR multiplex method was used to detect *S. mutans* and *S. sobrinus* bacteria. The results of the amplification then were visualized using electrophoresis method. DNA extraction was performed using boiling method in TE buffer. The suspected of 3-5colonies on TYCSB media were taken as and inoculated in ependorf tubes containing 100 µL of TE buffer, homogenezid by vortex mixer. The suspension was heated at a thermostat (Ependorf, North America) temperature of 95ºC for 10 minutes. After temperature reached the room temperature, the samples were centrifuged at 10,000 rpm for 10 minutes. The extracted DNA in supernatant was stored at -20ºC before use as DNA template for PCR.[10]

PCR was run 25 µL PRC mixture, as follow 12.5 µL of dNTPmix (Dream Taq Green, Thermo Scientific, USA), 0.5 µL (50pmol), 3.5 µL of bacterial DNA template, 1Ul tag pol, and then added 17 µLof distilled water. The primers sequence of *S. mutans* used GTF-B F :ACT ACA CTT TGC GGT GGC TTGG as forward and GTF-B R :CAG TAT AAG CGC CAG TTT CACT as reverse in 517 bp.[11] Primers sequence of *S. sobrinus* used GTF-I F : GAT AAC TAC CTG ACA GCT GAC T as forward and GTF-I R : AAG CTG CCT TAA GGT AAT CAC T as reverse in 712 bp.[12] DNA amplification then was performed using a thermal cycler PCR machine (Icyler, Biorad Thermal Cycler).[13]

The PCR was first run using a hot start temperature at 95ºC for 1 minute and amplified for 35 cycles with denaturation s at 94ºC for 30 seconds, annealing at 53ºC for 1 minute, elongation at 72ºC for 2 minutes, and end withat 72ºC for 7 minutes. PCR results were visualised using electrophoresis in 2% agarose gel (Spectronics Corporation, USA), with marker Ladder 100 bp. Elektrophoresis was run at 100 volts for 30 minutes. Next, the agarose gel was sained with Etidium bromid solution for 20 minutes. The amplicons were visualized using GelDoc (Digibox 7000, Mbiotech, Korea). Positive results were shown by the presenting of amplicon 517 bp for *S. mutans* and 712 bp for *S. sobrinus*.The results were analyzed using descriptive

analysis of the distribution of *S. mutans* and *S. sobrinus* with caries severity, and then statistically analyzed with Chi-square test.

RESULTS

After conducting research, bacterial isolates obtained in this study were identified by multiplex PCR to confirm *S. mutans* and *S. sobrinus* (Figure 1). Figure 1 Showed result of multiplex PCR positive *S. mutans* (GTFB) in the number 7038, 7039, 7040, 7041, 7042, 7043, 7044, 7045, 7046, 7048, 7049 and positive *S. sobrinus* (GTFI) in the number 7039, 7043, 7048, 7049. The results were then grouped based on deft severity and OHI-S then analyzed by frequency distribution and Chi-square.

The prevalence of the colonization of *S. mutans* was higher rather than *S. sobrinus,* 94% vs. 30% (Table 1). Based on severity level of caries using deft score, in severe score *S. mutans* was higher than mild score. Statistically there was no significant difference using Chi-square test p value >0.05. Same with *S. mutans, S. sobrinus* was higher in severe score than mild score of deft. And statistically there was no significant difference too between mild and severe score of deft. Based on OHI-S score, in good score *S. mutans* was higher than bad score. Statistic test using chi-square showed there was no significant difference p value >0.05. Same with *S. mutans, S. sobrinus* was higher in good score than mild score of deft. And statistically there was no significant difference too between good and bad score of OHI-S.

DISCUSSION

Several studies have shown that preventive efforts are effective to prevent early *S. mutans* colonization causing dental caries in children.[8] Hence, this study aims to reveal the incidence pattern of *S. mutans* and *S. sobrinus* in children based on the deft and OHIS. Next, the results of this study found that the highest incidence of bacteria causing dental caries was *S. mutans* (94%). Meanwhile, the incidence of *S. sobrinus* was 30%. These findings indicate the existence of *S. mutans* is considered not only as a microflora of the oral cavity, but also as a pathogenic bacterium causing caries. Both of *S. mutans* and *S. sobrinus* actually can proliferate in dental biofilm plaque. Their virulence is mainly due to their high adhesion ability, acidity, and asleepic properties.

Moreover, dental biofilm containing cariogenic bacteria (caries-related microorganisms) is one of the virulent factors associated with the development of tooth decay. Dental biofilms can be found on hard surfaces in the oral cavity, such as on surfaces, implants, orthodontic devices, or restorative materials. The development of biofilm processes involves several

progressive stages. The formation of initial biofilm accumulation also involves several specific processes. Variations in the biofilm coat in the oral cavity even have a significant impact on oral ecology and dental caries development.[14]

The higher colonization rate of *S. mutans* and *S. sobrinus* in this study, it would be roled by antigenI/II protein that strengthen the adherence to the tooh surface. It was also facilitated by glycoprotein receptor that present in saliva that is known as salivary agglutinin.[15] The other factors are cell-to-cell adherence and development of cohesive and pathogenic biofilms via the expression of GTFs. These enzymes (140 to 160 kDa) produce extracel-lular adhesive glucans that vary in chain length, content of α-1,3 and α-1,6 glucosyl linkages, and degree of branching and solubility. *S. mutans* expresses three genes for GTF: gtfB, responsible for in-soluble glucan synthesis; gtfC, for soluble and insoluble glucan synthesis; and gtfD, for soluble glucan synthesis. *S. sobrinus* expresses gtfI and gtfS, encoding enzymes that produce insoluble and soluble glucans, respec-tively.[15] The prevalence of *S. mutans* and *S. sobrinus* is widely associated with caries. In several epidemiological studies, there was a correlation between the existence of *S. sobrinus* and the high incidence of dental caries.[16]

The results of this study show that there was no statistically significant difference in the incidence of *S. mutans* and *S. sobrinus* between the higher and low caries severity level. This can be caused by several factors, such as host factors, bacterial virulence, diet, environment, and time. Besides, risk factors, such as sociodemographic factors, socio economic factors, knowledge level, as well as behavior also affect the incidence of caries.

Dental caries actually occurs because of an imbalance between demineralization and remineralization. When demineralization is higher than remineralization, caries can occur. Besides, oral hygiene also has a role in this balance.[17] Nevertheless, in this study there was no significant difference in the incidence pattern of *S. mutans*, *S. sobrinus* based on high or low deft and OHI-S.

Finally, this study interestingly reveals that there was no significant difference in the incidence pattern of *S. mutans* and *S. sobrinus* bacteria based on caries severity and OHI-S. This means that although these two bacteria are considered as the main factors causing dental caries, other factors may also have an equally important role in caries. Hence, further researches are expected to focus on more in-depth studies of *S. mutans* and *S. sobrinus* bacteria with other risk factors. Conclusion, this study showed that among carious tooth, 94% were colonized by *S. mutans* and 30% of cases co-colonization of *S. mutans* and *S. sobrinus*. There was not significantly differences of these bacterial colonization between various level of dental caries.

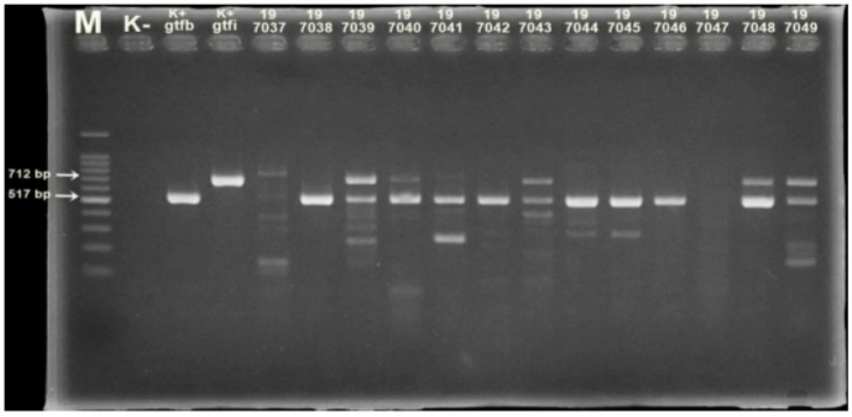

Figure 1. The results of multiplek PCR on *S. mutans* (GTFB) and *S. sobrinus* (GTFI).

Table 1. The isolation rate of *S. mutans* and *S. sobrinus* among children (n=50) with various of dental caries severity level from patients visiting Dental and Oral Hospital Universitas Airlangga

Severity Level		Children with dental caries (n=50)				
		S. mutans (n=47=94%)	p value	*S. sobrinus* (n=15=30%)	p value	Not identified *S. mutans* and *S. sobrinus* (n=3=6%)
DEFT	Mild	12 (24%)	0.124	3 (6%)	0.409	2
	Severe	35 (70%)		12 (24%)		1
OHI-S	Good	35 (70%)	0.315	9 (18%)	0.083	3
	Bad	12 (24%)		6 (12%)		0

The distribution of Streptococcus mutans and Streptococcus sobrinus in children with dental caries severity level

ORIGINALITY REPORT

16%	%	%	%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	QIONG ZHOU. "Genotypic diversity of Streptococcus mutans and Streptococcus sobrinus in 3-4-year-old children with severe caries or without caries : Genotypic diversity of two important cariogenic microbes", International Journal of Paediatric Dentistry, 11/2011 Publication	4%
2	Doron Steinberg, Shahar Eyal. "Early formation of Streptococcus sobrinus biofilm on various dental restorative materials", Journal of Dentistry, 2002 Publication	3%
3	www.tandfonline.com Internet Source	2%
4	EUN-JUNG CHOI. "Quantitative real-time polymerase chain reaction for *Streptococcus mutans* and *Streptococcus sobrinus* in dental plaque samples and its association with early childhood	2%

caries", International Journal of Paediatric Dentistry, 03/2009
Publication

5	www.bioline.org.br Internet Source	1%
6	Y. Li. "Differentiation of Streptococcus mutans and Streptococcus sobrinus via genotypic and phenotypic profiles from three different populations", Oral Microbiology and Immunology, 2/2001 Publication	1%
7	G. Hajishengallis, S. M. Michalek. "Current status of a mucosal vaccine against dental caries", Oral Microbiology and Immunology, 1999 Publication	1%
8	link.springer.com Internet Source	1%
9	worldwidescience.org Internet Source	1%
10	G. Hajishengallis. "Current status of a mucosal vaccine against dental caries", Oral Microbiology and Immunology, 2/1999 Publication	1%
11	Cyntia Rodrigues de Araújo Estrela, Fabiana Cristina Pimenta, Ana Helena Gonçalves de	1%

Alencar, Luis Fernando Naldi Ruiz et al. "Detection of selected bacterial species in intraoral sites of patients with chronic periodontitis using multiplex polymerase chain reaction", Journal of Applied Oral Science, 2010
Publication

---

12 academicjournals.org
Internet Source
<1%

---

13 Zhou Chen, Deepak Saxena, Page W. Caufield, Yao Ge, Minqi Wang, Yihong Li. " Development of species-specific primers for detection of in mixed bacterial samples ", FEMS Microbiology Letters, 2007
Publication
<1%

Exclude quotes	Off	Exclude matches	< 7 words
Exclude bibliography	On		

The distribution of Streptococcus mutans and Streptococcus sobrinus in children with dental caries severity level

GRADEMARK REPORT

FINAL GRADE

/0

GENERAL COMMENTS

Instructor

PAGE 1

PAGE 2

PAGE 3

PAGE 4

PAGE 5

PAGE 6