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Relationship of the Number of Streptococcus Mutans Bacteria ATCC 25175 With DMF-T Index Based on Stunting Occurrence in Andalas Health Center Patients in Padang City

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Abstract

Caries is a multifactorial disease that is affected by saliva and teeth as hosts, microorganisms, substrate, and time. Stunting children experience salivary gland atrophy that causes a decrease in saliva flow rate, buffer function, and immune and antimicrobial components, so it can increase the risk of caries.

This study aims to find out the relationship of the number of bacteria with the DMF-T index based on the incidence of stunting. The method used is observational with a cross-sectional study design. The sample was selected by stratified random sampling, with as many as 30 stunting children and 30 normal children. Based on the results of the study, the number of Streptococcus mutans ATCC 25175 bacteria is higher in stunted children than in normal children, but in statistical tests, it does not mean $p = 0.659$ ($p>0.05$). The DMF-T index is higher in stunted children than in normal children. Statistical test analysis shows the result of $p = 0.004$ ($p<0.005$) that is there is a relationship between the DMF-T index and stunting occurrence.

In this study, there was no association of the number of Streptococcus mutans ATCC 25175 bacteria with the DMF-T index based on stunting events.

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Introduction

Caries is an infectious disease caused by demineralization of email and dentin which is closely related to the consumption of cariogenic foods. [1] In Indonesia, caries is still a major problem, almost 90% of the Indonesian population has problems with dental and oral health. Based on Riskesdas in 2013, Indonesia's DMF-T of 4.6 is a fairly high number according to the World Health Organization. The 2018 National Basic Health Research (Riskesdas) Results suggest that the number of dental and oral problems in Indonesia was 57.6%. With the highest percentage of cavities, which is 45.3%. [2] In West Sumatra, the percentage of the population experiencing problems in dental and oral health reached 58.8%. The prevalence of caries in the 3–4-year age group is 81.5% and in the 5-year-old age group is 90.2%. In the city of Padang in 2018 the prevalence of caries was 36.71%. [3] The occurrence of caries due to the role of caries-causing bacteria, namely streptococcus *mutans* .[4] Streptococcus *mutans* is the most common bacteria found in salivary and plaques are associated with the prevalence and incidence of caries. *Streptococcus mutans* is a gram-positive, nonmotile (immobile), and facultative anaerobic bacterium. [5] Streptococcus mutans is most prominent dental caries bacteria due their ability to attach to tooth surfaces connected by the pellicle and form biofilms known as plaque. [6,7] They produce enzyme *glucosyltransferase* to convert disaccharides into glucan and facilitating more bacterial accumulation .[8] These bacteria are aciduric (able to live) in an acidic environment and acidogenic (produce acid). [9] Streptococcus *mutans* has three *serotypes* namely *serotypes* c, e, and f. One type of *serotype* c is *Streptococcus mutans* ATCC 25175. Stunting is a condition in which children experience growth disorders so that the

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child's height based on age (H/A) is below WHO growth standards. [10] WHO has established stunting as a global priority and aims to reduce stunting by 40% by 2025. The number of stunting cases in Indonesia is in the 5th highest position in the world. [11] Based on a survey of the nutritional status of toddlers conducted by the Ministry of Health of the Republic of Indonesia in 2019, 1 in 4 Indonesian toddlers experienced stunting with a prevalence of 27.7%. West Sumatra is ranked the 20th highest stunting case in 34 provinces in Indonesia. The prevalence of stunting in children 0-59 months in the city of Padang is 22.45%. [12]

Materials and methods

This study is an observational study with a *cross-sectional study design,* to find out the relationship between *independent* variables and *dependent variables,* and *confounding variables* by taking measurements at times. The population in this study is elementary school students in the working area of the Andalas Health Center in Padang city consisting of normal children and stunting children with first molar teeth that have erupted perfectly. Samples were taken based on inclusion and exclusion criteria, namely elementary school students aged 6-12 years, their parents who filled *in informed consent* and did not have systemic diseases. *Stratified random sampling* technique was used. The research was conducted from September to October 2021. The tools used in the study included approval sheets for respondents, microtoise stature meters, and standard H/A tables for children based on WHO standards. Glass funnels and microtubes are used for salivation. For DMF-T index examination, *nierbeken* is needed to install diagnostic tools such as mouth glass, sonde, flashlight, *handscoon,* mask, and DMF-T index sheet. The ingredients used are 70% alcohol, cotton, and aquades for mouthwash before the study. INVITROGEN KIT for DNA isolation of *Streptococcus mutans* ATCC 25175 bacteria. *Real-Time* PCR Machine (Biorad CFX96 Touch Real-Time PCR).

The sample was asked not to eat before the study. First, a nutritional status check was measured using anthropometric measurements, based on height for age (H/A). Height is measured using a microtoise stature meter and the measurement results are converted into the form of standardized values (Z-score) and obtained child's nutritional status. Then sampled as many as 30 children with short nutritional status (stunting) and 30 children with normal nutritional status as a comparison. Stunting children are given the S code and normal children are given the N code. After that, saliva sampling is done by using glass funnels and microtubes. Samples are collected by using *the draining method technique.* The collected sample (2ml) is then put into a *coolbox* containing *dried ice* and stored at a temperature of -200C. Then the DMF-T index examination is carried out with the help of standard tools in the form of glass mouth and sonde, then calculated using the caries index and obtained caries levels are very low, low, medium, high, and very high. The collected data is then processed using the *Shapiro-Wilk* test and presented in table form. Saliva samples collected were carried out isolation and purification of the DNA of *Streptococcus mutans* ATCC 25175 bacteria and further clarified using *Real-Time* PCR.

Isolation and Amplification of DNA DNA Streptococcus mutans ATCC 25175

The collected saliva samples were isolated and purified by the DNA of *Streptococcus mutans* ATCC 25175 bacteria at the Biomedical Laboratory of the Faculty of Medicine, Andalas University. Pickpocketed 200µl saliva samples was used to isolate bacterial DNA by following the KIT Invitrogen procedure. DNA amplification was carried out using *Real-Time* PCR.

Respondent Group	Stunting Group (n=30)		Normal Group	
	F	%	F	%
Sex				
-Male	19	63,34	15	50
-Female	11	36,66	15	50
Elementary Schools				
-Elementary 23 Marapalam	10	33,33	28	93,33
-Elementary 14/15 JTT	7	23,33	2	6,6
-North Jati Elementary	7	23,33	-	-
-Sawahan Elementary	2	6,6	-	-
-Tan Malaka Elementary	2	6,6	-	-

Table 1. Characteristics of Respondents.

Based on table 2. The average DMF-T index is higher in stunting children than in normal children, which is 8.30± 3.21 versus 5.36± 3.44. Statistically, the difference is meaningful ($p<0.05$).

Respondent Group	DMF-T Index	
	Average ± Elementary School	P
Stunting	8,30 ± 3,21	0,001
Normal	5,36 ± 3,44	

Table 2. DMF-T Index Examination Results with Stunting Events.

Respondent Group	Number of Bacteria	
	Average ± Elementary School	P
Stunting	212.848.221 ± 281.192.086	0,199
Normal	152.600.955 ± 220.793.277	

Table 3. Relationship of *Streptococcus mutans* ATCC 25175 Bacteria Count with Stunting Events.

Based on table 3. The average number of *Streptococcus mutans* ATCC 25175 bacteria was higher in stunted children than in normal children at 212,848,221 ± 281,192,086 versus 152,600,955 ± 220,793,277. Statistically, the difference is meaningless (p>0.05).

Respondent Group	P
DMF-T Index	0,004
Number of Bacteria	0,659

Table 4. Relationship of Streptococcus Mutans ATCC 25175 Bacteria Count with DMFT Index Based on Stunting Occurrence.

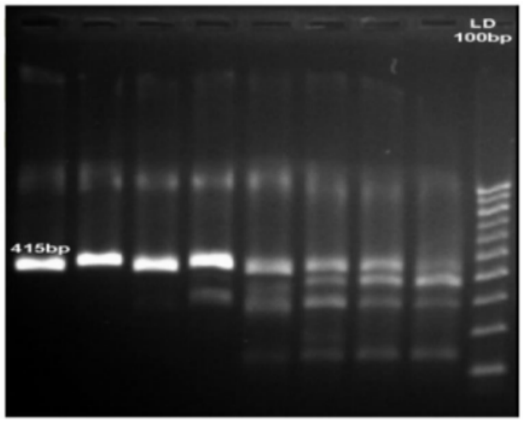

Figure 1. Electrophoresis of GTADIENT PCR results on DNA bands resulting from Real-Time PCR optimization.

The multivariate analysis shows that DMF-T index plays more role than the number of *streptococcus mutans* ATCC 25175 bacteria with a value of p = 0.004 (p<0.05) stunting occurrence. Based on Table 4, the number of *Streptococcus mutans* ATCC 25175 bacteria in stunting children is higher than the number of bacteria in normal children. But in statistical tests, it does not mean p = 0.659 (p>0.05). The results of the bacterial DNA electrophoresis Streptococcus mutans ATCC 25175 show that the sample was amplified on the fragment of 415 bp, as seen in Figure 1.

The amplification curve indicates on what cycle the *bacterial gene Streptococcus mutans* ATCC 25175 was detected. In this study streptococcus *mutans* at 25175 bacteria were certified in cycles 20-34 as seen in Figure 2.

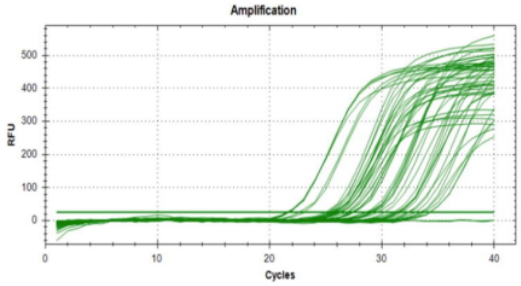

Figure 2. Amplification curve.

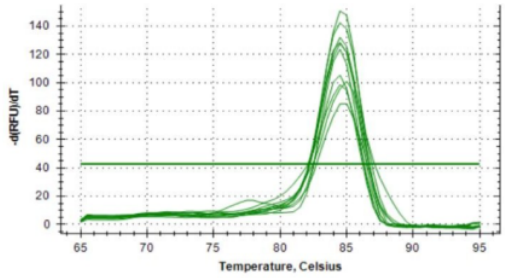

Figure 3. Melt Peak Curve.

The Melt Peak curve shows at what temperature the amf[pikasi] result is. A single peak shows specific amplification results, as seen in Figure 3.

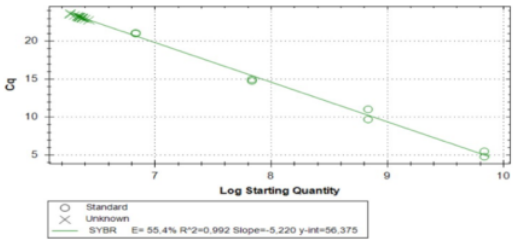

Figure 4. Standard Curve.

Absolute calculations were performed to calculate the number of Streptococcus mutans ATCC 25175 bacteria in the saliva of stunting children and normal children. To convert the results obtained from the Real-time PCR machine into sums in CFU units, standard curves are made as seen in Figure 4.

Discussion

Malnutrition is a state of malnutrition caused by inadequate consumption, low absorption, or loss of nutrition. Stunting is a manifestation of malnutrition that affects children the most in the world. Stunting children have a condition of height for age (H/A) below normal according to world health organization growth standards. [10,13] This study proves that stunting children have a higher average DMF-T index compared to normal children (table 2). This is related to chronic malnutrition that occurs in stunting children, both macronutrients and micronutrients. [14,15] Malnutrition, especially lack of protein and vitamin A results in atrophy of the salivary glands. [16] Stunting children experience salivary gland atrophy which causes a decrease in saliva flow rate, buffer function, and immune and antimicrobial components in saliva. [17,18] These conditions, combined with bad oral health attitude, increases the ability of caries-causing bacteria to develop in the oral cavity. [19] The microorganisms that cause caries produce acids that play a role in the initial process of caries, namely demineralization. The loss of minerals causes porosity so that the acid can diffuse deeper into the tooth structure. [1] If the *buffer* function fails to neutralize the acid, then the demineralization process will continue causing caries to expand and deepen. [20]

The results of this study are in line with Hooley's 2012 study which stated low weight and growth failure such as stunting are closely related to the incidence of caries. This is because stunting children experience macronutrient and micronutrient deficiencies causing impaired tooth growth (odontogenesis), late tooth eruptions, and salivary gland disorders. [21] Supported by research conducted by Rengg *et al.*, 2021 in Cambodia that examined the relationsh between dental caries and stunting incidence in children under 2 years of age, and proved that severe tooth decay is associated with stunting. [22]

This result is not in line with longitudinal research conducted by Dimaisip-Nabuap *et al* in 2019 in Indonesia which stated that children who have a higher DMF-T index at the age of 6-7 years have a chance of stunting at the age of 8-9 years. [23] In line with this research Folayan *et al.*, 2019 argue that children who experience caries at an early age are associated with low body weight and growth disorders such as stunting in the future. [24]

This study proved that the average number of *Streptococcus mutans* ATCC 25175 bacteria in stunted children's saliva was more than the average number of *Streptococcus mutans* ATCC 25175 bacteria in normal child saliva (Table 3). This is caused by malnutrition that causes dysbiosis in the environment in the oral cavity. The atrophy of the salivary glands and the hypofunction of the salivary glands make it easier for *Streptococcus mutans* ATCC 25175 bacteria to colonize in the oral cavity. These results are the same as research conducted by Vieira *et al* (2020) on 82 children aged 1-5 years with chronic malnutrition. [25] Salivary flow rates can also be influenced by several factors such as emotional conditions, habits, and degrees of hydration. [26]

The study used saliva as a sample to determine the number of *Streptococcus mutans ATCC 25175* bacteria. Stunting children have a poor immune system compared to normal children, coupled with decreased salivary function, especially *buffer* function can increase the risk of caries events. It was proven in this study that the DMF-T index is higher in stunted children than in normal children. Yanis and Agustin (2020) studied 28 saliva samples of healthy children aged 5 to 10 years and found that there was an association of *streptococcus mutans* bacteria with the severity of caries. [27]

It was proven in this study that the DMF-T index plays a more role than the number of bacteria when associated with stunting events (table 4). This is because *Streptococcus mutans* ATCC 25175 bacteria remain in the oral cavity, both in individuals with caries and in caries-free individuals. This proves that the number of *Streptococcus mutans* ATCC 25175 bacteria will be high in individuals with a high DMF-T index in both normal children and stunting children.

Conclusions

Based on the results of the study, it can be concluded that there is a relationship between the DMF-T index and stunting occurrence, but there is no relationship between the number of *Streptococcus mutans* ATCC 25175 bacteria and stunting events. In this study, the DMF-T index played a more role than the number of *Streptococcus mutans* ATCC 25175 bacteria with stunting events.

Suggestion

Further research focusing on dental parameters and oral health maintenance is needed as a comparison in the measurement of research samples.

Declaration of Interest

The authors report no conflict of interest.

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