Comparison of the salivary bacterial colonies number and pH value in early and non-early childhood caries due to consuming infant formula using the nursing bottle

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ABSTRACT

Introduction: Saliva plays a vital role in the caries process. The critical function of saliva is to defence against caries. A decrease in the saliva secretion speed can be followed by an increase in the number of bacterial colonies. This study was aimed to determine the comparison of salivary bacteria colonies number and salivary pH on early and non-early childhood caries in children who consumed infant formula using the nursing bottle. **Methods:** This research was pure experimental research with cross-sectional approach. Fifteen children with ECC and fifteen children with non-ECC aged 2-4 years on Early Childhood Education Programs (PAUD) of Padang, West Sumatera, were included in this study. Thirty subjects were participated in this study and asked to collect saliva into a sterile container provided. Statistical analysis used was independent sample t-test. **Results:** There was a significant difference in the salivary pH and the number of salivary bacterial colonies on early childhood caries and non-early childhood caries children after consuming infant formula with the nursing bottle ($p < 0.05$). The number of salivary bacteria colony on the infant with early childhood caries was higher with an average of 407.2 x 10-4/ml, whereas in infant non-early childhood caries was lower with an average of 125.5 x 10-4/ml. The salivary pH on infants with early childhood caries was acid with an average of 6.2, and in infants with non-early childhood caries was normal with an average of 6.9. **Conclusion:** There is a difference in the number of salivary bacterial colonies in infants with early childhood caries and non-early caries after consuming infant formula using the nursing bottle.

Keywords: Early childhood caries, non-early childhood caries, salivary bacterial colonies, salivary pH

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INTRODUCTION

In Indonesia, oral health problems among the community are still commonly found, especially among children. The oral health profile showed that dental and oral disease ranked 10th of the most common illnesses suffered by Indonesians.[1] Dental and oral diseases, mainly dental caries

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are still one of the most problems often occurs in Indonesians, not only in adults but also in children.[2] Dental health in infants must be considered by parents to avoid dental problems and make teeth healthy. Not all children's teeth are healthy, especially during primary teeth phase. There are many preschool children suffer from caries at an early age.[3]

Factors causing caries consist of host factors or hosts, agents or microorganisms, substrates or diet and combined by time. The first bacteria inhabiting the salivary pellicle are mainly coccus-shaped, and the most widely is *Streptococcus*. Number of microorganisms is higher in the person with active caries than in caries-free person.[4] Early childhood caries (ECC) is often associated with poor dietary habits since infants, but in reality, the mechanism of the incidence of this disease is more complicated because not all children who drink bottled milk will suffer from ECC.[4] Many factors can worsen caries among infants. These factors are oral cavity conditions factors that are directly related to the technique of brushing teeth, drinking milk containing high carbohydrates using bottles, drinking time of milk bottles, adding sugar in milk, the number of frequencies of food and drinks that cause caries and the level of saliva acidity related to drinking milk time.[5]

The high frequency, amount, and time of the consumption of foods and beverages containing carbohydrates or sugars, mainly sucrose, can increase the possibility of caries occurrence. Based on some previous research discovered that there is a very strong relationship between the frequency of sugar consumption and the increase in the caries prevalence; however, the amount/quantity of sugar consumption does not have a strong relationship to the increased caries prevalence. Children with ECC usually prefer to consume sugar, such as fruit juices, and other sweet foods.[6]

Saliva plays an essential role in the caries process, a critical saliva function is as a defence against caries.[7] Saliva itself is a protective fluid in the oral cavity, when the secretion of saliva and buffer capacity is low, it can cause decrease in food scraping ability, acid neutralisation ability, and the ability to generate email demineralisation. A reduction in the speed of saliva secretion can be followed by an increase in the number of *Streptococcus mutans* and *Lactobacillus*. ECC is caused by the occurrence of the role of bacteria such as *Streptococcus mutans*, *Streptococcus sanguis*, *Streptococcus mitis*, *Streptococcus salivarius*, *Lactobacillus*, and some other strains.[8,9]

In general, some parents feeding their children using the nursing bottle, especially on busy par-ents. Sometimes formula feeding using a nursing bottle is habitual until the child falls asleep causing the formation of more acidic saliva which can damage primary teeth because the formula milk in the bottle contacted in the more extended period with the teeth. This feeding pattern can lead to progressive caries such as ECC. This study was aimed to determine the comparison of salivary bacteria colonies number and salivary pH on early and non-early childhood caries in children who consumed infant formula using the nursing bottle.

METHODS

The type of research used in this study was quasi-experimental with a cross-sectional approach. The research was conducted at the Early Childhood Education Programs throughout North Padang sub-district and at the Microbiology Laboratory of Baiturrahmah University Padang, West Sumatera, Indonesia, on December 2017. The subjects of this study were the students of Early Childhood Education Programs aged 2-4 years, consisted of 15 children with ECC and 15 children without ECC who consumed infant formula using a nursing bottle. Inclusion criteria were healthy children (without systemic disease), erupted primary teeth, consumed in-fant formula using the nursing bottle, aged 2-4 years. Exclusion criteria were children with systemic disease, primary teeth had not erupted, not consuming infant formula using a nursing bottle, aged more than 4 years old. Thirty subjects were asked to collect saliva into a sterile container provided.

Firstly, each of the collected saliva was measured for the pH value using a pH stick. Then, each of the collected saliva was diluted to 10-3 and inoculated to blood agar using a 10-1 calibration ose, then incubated at 37°C for 24 hours. After incubated for 24 hours, the number of grown bacterial colonies was counted manually.

The difference number of bacteria colonies between infants with ECC and without ECC after

consuming infant formula using a nursing bottle was examined using the t-test with the significance level of 0.05, and the difference of the salivary pH was analysed by counting the average of pH in each group. This research has been approved by the Research Ethics Committee of Andalas University, with the registration number 448/KEP/FK/2017 All participants in this study also had signed the informed consent under their parents consent.

RESULTS

Table 1 shows the average of salivary bacterial colonies number of infants with ECC and non-ECC.

Table 1. Comparison of the average number of the salivary bacterial colony in the infant with and without ECC after consuming infant formula with a nursing bottle

Number of bacterial colonies	
Early childhood caries (10^{-4}/ml)	Non early childhood caries (10^{-4}/ml)
992	80
352	186
278	164
390	170
335	13
305	229
126	108
315	54
626	134
355	136
335	136
454	109
440	95
362	143
443	144
407.2 x 10^{-4}/ml	**125.5 x 10^{-4}/ml**

The data presented in Table 1 concludes that the number of bacteria colonies which could be isolated from the saliva of infants with early childhood caries was higher than non-early childhood caries infants.

In early childhood caries group, as many as 11 children were having acid pH (pH < 6.8) and 4 children were having a normal pH (pH 6.8 - 7.2). In non-early childhood caries group, only 3 children had acid pH (pH < 6.8) and 12 children had a normal pH (pH 6.8 - 7.2). It can be concluded that the salivary pH of the infant with early childhood caries was more acidic than the salivary pH of the infant without early childhood caries. The research data obtained were then examined by the normality test.

Table 2. Salivary pH of infants with and without early childhood caries after consuming infant formula with a nursing bottle

Infant salivary pH		
No	Early childhood caries	Non early childhood caries
1	6	7.5
2	6.5	7
3	7	7
4	6	6
5	7	7
6	6	6
7	5.5	7.5
8	7	6.5
9	5.5	5.5
10	6	7
11	5.5	7.5
12	6	7
13	5.5	6.5
14	7	7
15	6	7
Average	**6.2**	**6.9**

Table 3 shows that the distribution of data on the number of bacterial colonies in early childhood caries was not normal because the p-value (p = 0.002, p < 0.05), thus to analyse if there were differences in the number of bacterial colonies in infant with and without early childhood caries, the Mann-Whitney test should be performed.

Table 3. Normality tests of the number of bacteria colony in the infant with and without ECC after consuming infant formula with a nursing bottle

Group	Statistic	df	Sig
Early childhood caries	0.781	15	0.002
Non early childhood caries	0.988	15	0.998

Result of Mann-Whitney test presented at Table 4 showed that there was a significant difference between the number of bacterial colonies in the infant with and without ECC after consuming infant formula using the nursing bottle, with the significance value of 0.001 (p < 0.05).

Table 4. Mann-Whitney test results

	Colony bacteria
Mann-whitney U	8000
Z	-4.335
Asymp. Sig. (2-tailed)	**0.000**

DISCUSSION

This study was aimed to determine the comparison of salivary bacteria colonies number and salivary pH on early and non-early childhood caries in children who consumed infant formula using the nursing bottle. This research was quasi-experimental with a cross-sectional approach. The research was conducted at the Early Childhood Education Programs throughout North Padang sub-district. Subjects consisted of 15 infants with early childhood caries and 15 infants without early childhood caries.

The results of the current study showed that the number of bacterial colonies in infants with early childhood caries was higher (407.2 x 10-4/ml) than infants without early childhood caries (125.5 x 10-4/ml). The pH measurement results of saliva showed that in infants with early childhood caries, as many as 11 children were having acid pH (pH < 6.8); in the group of non-early childhood caries, only 3 children had an acid pH (pH < 6.8) thus can be concluded that there was a difference between salivary pH in infants with and without early childhood caries after consuming infant formula using the nursing bottle. The salivary pH in the early childhood caries group was more acidic than the salivary pH in the non-early childhood group.

Each normal child has 6-9 million total of salivary bacterial colonies. Counting of the number of salivary bacterial colony indicated that there were 4 million bacterial colonies in the children with ECC. The calculation results of the salivary pH in this current study strongly influenced the existence of caries in the oral cavity. The lower the salivary pH, caries tend to be higher. According to research conducted by Soesilo[10], the optimal acidity (pH) of saliva to inhibit bacterial growth is between 6.5 - 7.5, and if the salivary pH is low, between 4.5 - 5.5, will facilitate the growth of acidophilic germs such as *Streptococcus mutans* and *Lactobacillus*. Also, a decrease in the pH value at the oral cavity can cause quick demineralisation of tooth elements.[10] Early childhood caries often occurs in infants at a very early age. At first, the affected area is four anterior maxillary teeth, then extending into the maxillary molar teeth, then the mandibular teeth, and the canines will be the last to be affected. This condition could occur due to the habit of drinking infant formula using the nursing bottles until the child fell asleep.[11]

The results of this study showed that the number of bacterial colonies was found to be higher in acidic pH of the early childhood caries infant than in the non-early childhood caries infant. This result is caused by sucking the nursing bottle during sleep which makes the front teeth flooded; thus, it will be faster to experience caries (perforated teeth). Liquids containing sugar such as milk, syrup, sweet tea, fruit juices with additional sugar can provide a good environment for microorganism to cause dental caries. Also, the decrease of salivary flow during sleep enhances the process of caries.[12] Prolonged use of the nursing bottle has a strong correlation to the onset of caries. Several studies reported that there had been a correlation between prolonged use of the nursing bottle (2 years or more) with the emergence of caries.[12] This condition will be severe if the use of the nursing bottle is done while sleeping (night feeding).[13] The use of milk bottles causes prolonged periods of milk to surround the teeth of infants and children, then the fluid is in contact with the dental enamel and merges with the oral bacteria such as *Streptococcus mutans*.[14] Research conducted on 150 children aged 18-36 months by Peressini[15] concluded that there was a significant correlation between the habit of drinking milk with a nursing bottle while sleeping with the emergence of caries and tooth decay.

Dental caries is a process of tooth damage that starts from enamel to dentin, caused by certain bacteria such as *Streptococcus* and *Lactobacillus* which able to ferment carbohydrates such as sucrose and glucose, forming acids, lowering the pH value to be < 5 and resulting in demineralisation of the susceptible tooth surface. Bacterial colonies that can be isolated in the infant with ECC were *Streptococcus sp.* and *Nieserria sp.*, while bacterial colonies from the infant without ECC were *Klebsiella sp.* and *Staphylococcus sp.* The bacteria shape that can

be identified microscopically were *Streptococcus sp.* with smectic blood shape, *Nieserria sp.* with slimy shape and transparent colonies, white and yellowish-coloured *Klebsiella sp.*, while *Staphylococcus sp.* was observed in a large colony, with a transparent and slimy shape. While the bacteria features observed under the microscope were as follows: *Streptococcus sp.* with round and chained feature, *Nieserria sp.* with coffee beans like feature, *Klebsiella sp.* with basil like capsule feature, and *Staphylococcus sp.* with coke or rounded like feature.

All bacteria above are commonly found oral bacteria. These bacteria are essentials in the formation of caries, especially *Streptococcus sp.* which are carcinogenic germs due to the capability of forming extracellular polysaccharides of sucrose form. This condition is what leads to the plaque attachment on the slippery tooth surface, and *Streptococcus* mutans in the dental biofilm are also able to make the acid from the carbohydrates. Furthermore, the acid produced by the bacterial activity will progressively destroy the inorganic part of the tooth so that dental caries eventually occur.[16] To prevent early childhood caries in children, parents are expected to give more attention and reduce the frequency of consuming infant formula using the nursing bottle, because it can increase the possibility of early childhood caries.

CONCLUSION

There is a difference between the number of salivary bacterial colonies in early childhood caries and non-early childhood caries infants after consuming infant formula using the nursing bottle, which is found to be higher in infants with early childhood caries than without early childhood caries. Also, more acidic pH is found in early childhood caries infant compared to non-early childhood caries.

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