The quantity of *Streptococcus mutan* colony in breastfeed and formula milk infant (Jumlah koloni *Streptococcus mutan* pada bayi peminum air susu ibu dan peminum susu formula)

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ABSTRAK

Kerusakan gigi pada bayi sangat dipengaruhi oleh jenis substrat yang dikonsumsi. Substrat tersebut merupakan sumber energi utama dalam proses kolonisasi bakteri dalam rongga mulut khususnya *S.mutan* sebagai bakteri utama penyebab kerusakan gigi. Bagi anak usia 1 tahun, umumnya sumber nutrisi berasal dari konsumsi susu baik air susu ibu (ASI) maupun susu formula. Penelitian ini dimaksudkan untuk mengetahui perbedaan jumlah bakteri *S.mutans* pada saliva antara anak usia 1 tahun yang mengkonsumsi ASI dengan susu formula. Studi observasi analitik dengan menggunakan desain cross-sectional study dilakukan pada 60 bayi yang memenuhi kriteria inklusi dengan metode simple random sampling, masing-masing 30 pada kelompok ASI dan pada kelompok susu formula di Posyandu Kelurahan Antang. Evaluasi *S.mutans* pada saliva dilakukan di Laboratorium Mikrobiologi Fakultas Kedokteran Universitas Hasanuddin. Orang tua responden mengisi kuesioner yang menanyakan hal-hal yang berhubungan dengan penelitian dan dilakukan microbial screening dengan mengambil sampel saliva. Hasilnya menunjukkan jumlah koloni *S.mutans* pada kelompok ASI rata-rata 19,7 CFU/ml sedangkan pada kelompok susu formula 37,97 CFU/ml yang berarti terdapat perbedaan jumlah koloni *S.mutans* yang signifikan antara konsumsi ASI dan susu formula pada saliva anak usia 1 tahun. Disimpulkan bahwa jumlah koloni *S.mutans* pada kelompok ASI lebih rendah dibandingkan susu formula.

Kata kunci: *Streptococcus mutan*, colony forming units, air susu ibu, susu formula, saliva, anak usia 1 tahun

ABSTRACT

Caries in infant was influenced by their substrat. Substrats are the primary source of energy in bacterial colonising process in oral cavity. *Streptococcus mutans* are especially the main cause of tooth decay. For 1-year-old infant the primary nutritions are generally from breast milk or formula milk. This study was aimed to determine the different numbers of bacterial colonizations of *S.mutan* found in breast-fed infants from those found in formula milk. This observational-analytics study used cross sectional study design was carried out to 60 infants who fulfil the inclusion criteria of simple random sampling method, 30 infants in each group from Integrated Health Centre in Kelurahan Antang, and the bacterium on the saliva was evaluated in Microbiology Laboratory of Medical Faculty Hasanuddin University. Each parent was given questionnaire which to asks the things related with this research and microbial screening was taken from saliva of 1-year old infant. The number of bacterial colonizations for *S.mutans* in breastfeeding group shows mean value 19.7 CFU/ml while the formula milk group was about 37.97 CFU/ml, so the consumptions of breast milk and formula milk in 1-year old infant show the significantly different number of bacterial colonizations of *S.mutans* in. It was concluded that colonization of *S.mutans* in breastfeeding group lower than formula milk group.

Key words: *Streptococcus mutans*, colony forming units, breast milk, formula milk, saliva, 1 year old infant

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PENDAHULUAN

The human body is composed of almost one trillion cells that can become a place to live bacteria, and one of the main entrance of bacteria after individual came out of the womb is the oral cavity. Theory of psychosexual development by Freud claimed that the process of learning and experience of our lives as human beings starts from the mouth. The mouth was explored by fingers, toys, food, and other things. Through the exploration process we unconsciously carry bacteria into the mouth.¹

Particularly in 1-year-old child, the exploratory process is done by providing food nutrition through breast milk or infant formula. Based on data from

the Food and Agriculture Organization (FAO) in the USA and WHO, carbohydrate content in breast milk as a source of primary energy in form of lactose is 7 g/100 mL while on infant formula ranged from 7-8.5 g/100 mL. Existing content of lactose in breast milk and formula milk increases amount of bacteria in the oral cavity by a process of splitting lactose into lactic acid in the form of acidic compounds. In addition, the entry of bacteria into the mouth can also be enhanced through media used to breastfeed, such as silicone or latex contained on bottles into bacterial transmission because direct contact with baby's oral cavity.²⁻⁴

Oral conditions are warm, moist, nutrient-rich and provide opportunities for the growth of bacteria.

The most abundant bacteria in the mouth is a kind of *Streptococcus*, and most cariogenic bacteria in this group is *S.mutans*. *S.mutans* colonizes on the surface of the teeth and soft tissues, and this is what will be the cause of the disease in the oral cavity.^{3,5}

Streptococcus mutan can synthesize extracellular polysaccharide insoluble 1-3 bond and through homo-fermentation process produces acidic compounds of the lactose-solving process that caused the mouth to acid pH. This is what can lead to caries in children, for example, the occurrence of caries that is indirectly caused by the feeding process either through breast milk and infant formula due to the increased number of *S.mutans*, and must be prevented early on to avoid severe damage to children's teeth.^{3,6}

This paper discusses the difference of number of *S.mutans* in infants 1-year old who are breastfed and infants of the same age but consume formula milk. It is based on the assumption that there are different levels of carbohydrate (lactose) conceived of breast milk and infant formula. Carbohydrates after process of glycolysis results in fermentation reactions and cause mouth becomes acidic pH. This has led to the development of bacteria, especially *S.mutans* most widely in the oral cavity.^{2,4}

Aside from the lower levels of lactose in breast milk, there are also anti-bacterial and anti-infection which is not found in formula milk such as Ig especially IgA, lactoferrin, lysozyme, and interferon which gives the possibility of assuming the amount of *S.mutans* in breastfeed group is fewer than those in formula feeding. According to American Academy of Pediatrics (AAP) kids can feel the full benefit of breastfeed after a total duration of breastfeed for the child reaches the age ≥ 1 year.^{2,7,8}

From the explanation above, it is necessary to explore more deeply, and the main problems of this phenomenon can be traced from the microbiological aspects of *S.mutans*. The results of the study of *S.mutans* are expected to provide an interpretation of the differences and the cause of the differences that may affect the amount of *S.mutans* in children 1-year of age who consume breast milk or infant formula. In this way caries in children can be prevented early.

MATERIALS AND METHODS

This observational analytic cross-sectional study design research was conducted in two places and sampling was conducted in the Village Antang IHC, while culturing and colony counting were conducted in Laboratory of Microbiology, Faculty of Medicine, Hasanuddin University. The study population was children aged 1 year who breastfeed or consumed milk formula. While the sample of this study was

S.mutans from saliva of the children who become research subjects based on the predetermined criteria.

The sampling method used was simple random sampling because the population had the same properties or homogeneous. In this study, samples were taken from one-year-old infants who consume breast milk or formula and meet the criteria as a research subject - free of caries, not having systemic diseases, and willing to be the subject of research. The total samples were 60 samples; it is based on the normal distribution which took 30 samples for of 1 year-old who consumed breast milk and infant formula, respectively.

The first step was to ask the children's parents to fill in the questionnaire that asked about things pertaining to this study, to know the different types of variations that exist in every subject of study.

Next, record the children that will be the subject of this research at the neighbourhood health centre as our research location. Clinical examination was conducted to the selected subject to determine number of deciduous teeth have been erupted, and ensure that the child was caries-free. The next phase was microbial screening of *S.mutans*, from sample of saliva in infant by using a sterile cotton swab. A sterile cotton swab was placed on the dorsum of the tongue until cotton swab looks wet. Cotton swab was placed in the pot and brought to the microbiology laboratory to perform colony counting.

In the laboratory process, NaOCl dilution with 4.5 mL 3 times dilution performed to prevent the exceeding number of overforming colonies number and ease in calculation. The first dilution was done in pots containing cotton swab samples taken from the saliva of children aged 1 year and was left for 5 minutes so that all the microorganisms attached to the swab cotton removed. Then, the second and third dilution with a solution of 4.5 mL NaOCl were done in a test tube.

Then, streaking was performed on the medium nutrient BHIA of the third test tubes (third dilution), and then incubated anaerobically at 37°C for 72 hours. After that colonies of the *S.mutans* can be calculated based on the method of colony forming units (CFU/mL) and the measurement was based on qualitative observation (Fig 1).⁹

Figure 1 Results of colony cultured of *S.mutan*

Data and bacteria calculation results obtained were presented in tables and graphs, and statistical analysis was performed using the unpaired t-test.

RESULT

The results of the study were displayed in the following tables.

Table 1 Distribution of subject characteristics

Characteristics	n	%	Mean $\pm$ SD
<i>Gender</i>			
Male	32	53.3	32.56 $\pm$ 32.63
Female	28	46.7	24.57 $\pm$ 22.92
<i>Breast Milk</i>			
Male	19	63.33	
Female	11	36.67	
<i>Formula Milk</i>			
Male	13	43.33	
Female	17	56.67	
Number of colony of <i>S.mutans</i>			28.83 $\pm$ 28.57
<i>Breast Milk Group</i>	30	50	
Number of colony			19.70 $\pm$ 22.19
<i>Formula Milk Group</i>	30	50	
Number of colony			37.97 $\pm$ 31.55

Table 1 show the study sample characteristic distribution of 60 infants; number of males (53.3%) more than females for from the overall saliva samples of 1-year-old infants who consume breast milk or formula milk. While the 30 breastfed sample consists of 19 males (63.33%) and 11 females (36.67%), the other 30 samples were formula milk group, 13 males (43.33%) and 17 females (56.67%). Also, this table shows the average number of colonies of *S.mutans* (CFU/ml) was 28.83. While the breastfed group the average number of colonies of *S.mutans* (CFU/ml) was 19.70 and in the formula milk group was 37.97. It shows that number of colony of *S.mutans* (CFU/mL) between the consumption of breast milk and infant formula, where the formula consumption showed greater number of colonies.

Table 2 Differences number of colonies of *S.mutans* and other bacteria between the consumption of breast milk and infant formula

No	Milk	<i>S.mutans</i> (CFU)	Other Bacteria (CFU)
		Mean $\pm$ SD	Mean $\pm$ SD
1	Breast Milk	19.7 $\pm$ 22.19	10.27 $\pm$ 19.36
2	Formula Milk	37.97 $\pm$ 31.55	14.03 $\pm$ 27.13

Independent sample t-test: $p > 0.05$; not significant

Table 2 show clearly the difference in the number of colonies of *S.mutans* (19.7 CFU/mL lower than 37.97 CFU/mL) and other bacteria (10.27 CFU/mL lower than 14.03 CFU/mL) between the consumption

of breastfed and infant formula. This shows that not only the number of colonies of *S.mutans* but also of other bacteria (non-specific) were lower in infants who consume breast milk than those who consume formula milk. The possible cause was the anti-bacterial effect (autoimmune) contained in breast milk so it does not only have the effect of systemic immunity in bacteria but also have an impact on the number of *S.mutans* in the oral cavity.

Table 3 The difference in number of colony of *S.mutans* between breast milk and formula milk consumption by gender

No	Milk	Gender	<i>S.mutans</i> (CFU)
1.	Breast Milk	Female	14
		Male	23
2.	Formula Milk	Female	31.41
		Male	46.54

Table 3 indicate that gender differences may also influence the amount of *S.mutans* in breast milk and infant formula consumption. The number of colonies of *S.mutans* (CFU/mL) in the breast milk intake for females was 14 which is lower than the number for males which was 23 colonies. Similarly, the number of colony of *S.mutans* (CFU/ml) on the consumption of infant formula shows the same difference where females have a lower number of colonies than males. Female infants of formula milk group had the number of colonies of *S.mutans* (CFU/mL) was 31.41, while the male infants was 46.54.

Table 4 Differences in sterile conditions (no bacteria) on the consumption of breast milk and infant formula

No.	Milk	Sterile (No bacteria)
1.	Breast Milk	17
2.	Formula Milk	13

Differences in sterile conditions were the conditions where no other bacteria found in the consumption of breast milk or formula milk shown in table 4. This shows that the effect on breastfeeding excellent immunity against the number of bacteria present in the mouth of 1-year-old infants because the results of the study of 30 samples contained 17 samples of breast milk consumed within the sterile conditions of other bacteria. As for the formula milk it indicates a lower amount, where from 30 samples only 13 of them were in the sterile conditions of other bacteria. These effects of auto immunity in breast milk was expected to not only influence other bacteria systemically but also affect the *S.mutans*.

The number of colonies of *S.mutans* on breast milk consumption can also increase due to several factors; one of them was frequency of breast feeding in a day. Based on the results in table 5, the number

Table 5 The increasing number of colonies of *S.mutans* on breast milk consumption based on the breastfeeding frequency

No	Frequency	<i>S.mutans</i> (CFU)
1	> 5 times a day	22
2	4 times a day	20
3	3 times a day	10.33

of colonies of *S.mutans* in 1-year-old infants who consumed more than 5 times per day was 22 (CFU/mL), while those who consumed 4 times a day was 20 (CFU/mL), and taking 3 times a day was 10.33 (CFU/mL). This also provides evidence that the frequency of breastfeeding affects the increasing number of bacteria.

Table 6 The increasing number of colonies of *S.mutans* on formula milk consumption based on the bottle feeding frequency

No	Frequency	<i>S.mutans</i> (CFU)
1	> 5 times a day	43.63
2	5 times a day	18.5
3	4 times a day	33.5
4	3 times a day	46

Table 6 show the fluctuating results against the number of colonies of *S.mutans* in the sample who consumed infant formula based on daily consumption frequency. There was no relation between frequency of drinking formula milk with the increasing number of *S.mutans*. This aspect was proven by the number of colonies of *S.mutans* which did not increase and was likely to change from > 5 times, 5 times, 4 times, and 3 times a day consumption of milk, at 43.63, 18.5, 33.5, and 46 (CFU/ml) respectively. This condition was possibly caused by other predisposing factors that can affect the number of *S.mutans* colonies from each sample.

Table 7 The increasing number of bacterial colonization according to the way of bottle cleaning in formula milk consumption

No	Bottle cleaning	<i>S.mutans</i> (CFU)	Other bacteria (CFU)
1	Soap + rinsed with warm water	43.1	21.36
2	Soap+rinsed+boiled	13	19.2

The increasing number of bacterial colonization on the consumption of infant formula can also be influenced by several factors; one of which out was the way of cleaning the bottle after use. Shown in table 7, the way of cleaning milk bottles have an impact on bacterial colonization in the oral cavity not only for *S.mutans* bacteria but also other bacteria considering that bottle is the consume media for consuming the milk and directly in contact with to oral cavity. Saliva samples from 1 year old infants

who consume formula milk with bottles were only cleaned with soap, while those rinsed with warm water show the number of colonies (CFU/ml) at 43.1 in *S.mutans* and at 21.36 in other bacteria (non-specific). While saliva samples from 1 year old infants who consume formula milk with bottles which were cleaned, rinsed with soap and boiled indicates the less number of colonies of *S.mutans* at 13(CFU/ml) than of the other bacteria at 19.2 (CFU/ml).

Table 8 Difference number of bacteria colonies between breast milk and formula milk consumption based on pacifier uses

Milk	Using Pacifier	<i>S.mutans</i> (CFU)	Other bacteria (CFU)
Breast Milk	Yes	20.36	11
	No	10.5	0
Formula Milk	Yes	40.53	20.6
	No	35.93	6.2

The results showed that pacifier use is in line with enhancing the number of colonies of *S.mutans* and other bacteria (non-specific). Shown in table 8, the number of *S.mutans* colony (CFU/ml) using a pacifier at 20.36 on breast milk group while those on formula milk group showed a higher rate 40.53 while the subjects who did not use a pacifier on breastfeeding group was 10.5 and on the formula milk group was 35.93. In addition to the increasing number of *S.mutans* colonies there also an increasing number in other bacteria (non-specific) which is in line with pacifier use on breast milk and infant formula, respectively at 11 and 20.6 (CFU/ml), while in those not using a pacifier no colony bacteria was found in breastfed group (0 CFU/ml) and the number was 6.2(CFU/ml) in formula milk group.

Table 9 Difference number of colonies of *S.mutans* according to frequency of complementary food intake in breast milk and formula milk group

No	Milk	Frequency	<i>S.mutans</i> (CFU)
		3 times	20.56
1	Breast Milk	2 timed	19.33
		Not eaten yet	7
		> 3 times	81
2	Formula Milk	3 times	48.25
		2 times	22

Complementary food has generally been given to 1-year-old infant. However, the higher the number of substrates that are inserted into the oral cavity the higher the growth of microorganisms, especially bacteria *S.mutans* as the major cause of tooth decay. It was consistent with the findings of this study (table 9) which show the frequency of complementary food feeding affects the amount of *S.mutans*. The more often consumption the higher the number of bacterial

colonies were found. In the breastfed group who consumed complementary food 3 times a day, 2 times a day, and those without complementary foods the number of *S.mutans* colonies was 20,56, 19.33, and 7(CFU/ml), while the samples of formula milk group with complementary foods of > 3times, 3 times, and 2 times a day, respectively at 81, 48.25, and 22(CFU/ml).

In addition to the above factors, tooth cleaning was the other consideration of the high number of colonies in 1 year old infant who consumed either breast milk or formula milk. Table 10 show that there was also differences in the number of colonies of *S.mutans* between the consumption of breast milk and infant formula influenced by whether or not dental cleaning was performed by 1-year-old infants. Breast milk consumption with dental cleaning shows number of *S.mutans* was 17 (CFU/mL) and the consumption without dental cleaning was at 18 (CFU/ml). While the number of colonies in those consuming infant formula with dental cleaning was at 26.06(CFU/ml) and without dental cleaning was at 60.45(CFU/ml).

Table 10 Difference number of bacteria colonies between breast milk and formula milk consumption based on dental cleaning

No	Milk	Dental cleaning	<i>S.mutans</i> CFU)
1	Breast	Yes	17
	Milk	No	18
2	Formula	Yes	26.06
	Milk	No	60.45

Table 11 Difference in number of bacteria colonies between breast milk and formula milk consumption

Milk	<i>S.mutans</i> (CFU) Mean $\pm$ SD	p-value
Breast Milk	19.7 $\pm$ 22.19	0.012*
Formula Milk	37.97 $\pm$ 31.55	

*Independent sample t-test: $p < 0.05$; significant

Table 11 shows the difference in the number of colonies of *S.mutans* between the consumption of breast milk and infant formula. The number of colonies of *S.mutans* in the breast milk group (19.7 CFU/mL) was less than those in formula milk group (37.97 CFU/mL).

Statistically there was a significant difference in the number of colonies of *S.mutans* in breast milk and infant formula consumption in the saliva of 1-year-old infants ($p=0.012$; $p<0.05$).

DISCUSSION

This study discussed the difference in the number of *S.mutans* colonization between consumption of breast milk and of infant formula in the saliva of 1-

year-old infants assuming that there are different levels of carbohydrate (lactose) conceived in breast milk and infant formula. Based on data from FAO in the USA, and WHO, carbohydrate content in breast milk as the main energy source in the form of lactose is 7 g/100 mL while the formula ranges 7-8.5 g/100 mL. Existing content of lactose in breast milk and formula milk increases number of bacteria in the oral cavity by the process of splitting lactose into lactic acid in the form of acidic compounds.²⁻⁴

Oral conditions were warm, moist, nutrient-rich and provide opportunities for the growth of bacteria. The most numerous bacteria in the mouth are the kind of Streptococcus, and the most cariogenic bacteria group was *S.mutans*. *S.mutans* colonize the surface of teeth and soft tissues, and will be the cause of disease in the oral cavity.^{3,5}

Carbohydrates-contained in breast or formula milk have aglycolysis process that will result in fermentation reactions and cause acid pH. This is what triggers the growth of bacteria, particularly *S.mutans* which were mostly found in the oral cavity.⁵

The measuring tool in this research was *colony form unit*, a method used to calculate the number of colonies of *S.mutans*. The validity and reliability of this measurement has been tested and has been frequently used in research settings. From the trials, this measurement shows its validity by the presence of different numbers of colonies of *S.mutans* among 1-year-old infants who consume breast milk and infant formula.

The research found there was difference in the number of *S.mutans* colonies between consumption of breast milk and infant formula in which the number of colonies of *S.mutans* in 1-year-old infants were lower in breastfed group than formula milk group. The number of colonies of *S.mutans* on 30 samples of breast milk group average was 19.7 CFU/mL, whereas the formula milk group from the same number of samples was an average of 37.97 which means the higher rate of colonies of *S.mutans* was in formula milk consumption.

There was a significant difference the number of colonies of *S.mutans* between the consumption of breast milk and infant formula in 1-year-old infants. It was also found that the number of colonies of *S.mutans* was significantly lower in breast milk than in formula milk consumption. This suggests that there was a significant difference in the number of colonies of *S.mutans* between consumption of breast milk and infant formula.

The difference in number of colonies of *S.mutans* can be traced in terms of the content of breast milk or formula. In addition to the lower levels of lactose,

breast milk also has anti-infection properties that employs auto immunity not owned by a formula milk. It has been known that breastfed infants, were not easily infected with the disease, because breast milk contains a variety of factor such as the body's defense, especially IgA, *lactoferrin*, *lysozymes*, *interferon* can destroy harmful bacteria and also is protective against a variety of viruses.^{2,7,8}

In addition to the above, the difference in the number of colonies of *S.mutans* between breast milk and infant formula consumption can also be caused by other predisposing factors such as frequency of consumption, use of pacifier, milk bottle cleaning, consumption of complementary foods, and cleaning teeth.

The increasing number of *S.mutans* colonies was in line with the frequency of breastfeeding in 1-year-old infants, as can be seen from Sharmaetal's study which found that the significantly increasing number of *S.mutans* colonies with the frequency of breastfeeding was due to carbohydrate in the breast milk itself, which is the main energy source for the bacteria in the oral cavity. This was unlike the case with consumption of infant formula. Although the frequency of drinking milk also affects the increase in the number of colonies of *S.mutans* many other factors can affect the number of colonies of bacteria. For example, the media used to drink milk in this case the child's milk bottle, and pacifier use that can not be controlled in this study. So further research is needed to know the effect of frequency of milk consumption to an increasing number of colonies of *S.mutans*.⁵

Another factor that could affect a high number of colonies of *S.mutans* on consumption of formula milk was the use of infant formula milk bottle as medium and direct contact with the oral cavity that needs to be kept clean. Based on questionnaire data from 30 samples of formula milk group, on average, provide answers that the mother of the child clean up a bottle of milk by means of the use of soap and then rinsed with warm water, others answered cleansing with soap, rinsed, then boiled bottle. And turns of microbiology laboratory examination result found that the number of colonies of *S.mutans* were higher in the first clean up ways without boiling the milk bottle. Not only the *S.mutans* but also found others bacteria whose numbers are also much more in the same way of cleaning.

In addition, results of the data collection from questionnaire showed that infant who consume formula milk has generally been oriented to the use of pacifier, coupled with the use of milk bottles also use the pacifier. Unlike the case with children who

consume breast milk, 30 samples of breast milk only two subject use the pacifier, so it can not be used as a comparison in the results. This suggests that children who are not breastfeed are generally oriented with pacifier use. The results showed pacifier use can also increasing number of *S.mutans* colonies and other non-specific bacteria. The use of pacifier generally made from latex and silicone facilitate attachment of bacteria and dirt on the surface of the pacifier and a transmission media of bacteria by direct contact with the child's mouth cavity.⁴

A 1-year-old child, generally has complementary foods in addition to breast milk or infant formula. The results of the questionnaires total of 60 samples generally at the age of 1 year old mother giving complementary foods such as porridge strain with the highest frequency that is 3 times a day. Based on the results of data processing show the frequency plays a role in increasing the number of colonies of *S.mutans* where the formula milk group still showed a significant difference compared to breast milk group. Increasing the number of colonies of *S.mutans* based on the frequency of complementary feeding occurs due to elevated levels of carbohydrates in the oral cavity derived from complementary foods become supply of nutrients for bacteria in the oral cavity, especially *S.mutans*. However, shortcomings of this study was uncontrolled types of given complementary foods, and there was no doubt that parents give other foods which unrecommended as complementary foods for 1 year old infant, so that more research is needed on this subject.³

However, the main predisposing factors that also need to be controlled was a factor of tooth cleaning. From the questionnaire given to total of 60 samples showed the effect of cleaning the teeth play important role in increasing the number of colonies of *S.mutans*. But for breastfeeding itself, indicate the numbers are not too significant, although it still shows that the teeth were not cleaned has a number of colonies greater than cleaned. As the formula milk group, showed an increase in the number of colonies of *S.mutans* highly significant among infants who do cleaning their teeth and not cleaned. However, shortcomings of this study because it was not control the way their parents clean the teeth, so we should do more research on this subject. Cleaning teeth is important to prevent early caries. *American Academy of Pediatric Dentistry* recommends to maintain oral hygiene from tooth eruption and teeth checked began at 1 year old as early prevention of caries, and introduce and familiarize the child to the dentist.¹

From the overall results of this study we concluded that the number of colonies of *S.mutans*

on breastfed group less than the formula milk group, both comparisons showed a significant difference with a considerable amount of difference between breast milk and formula milk groups.

Limitations of this study was the number of predisposing factors, such as the frequency of consumption, the consumption of complementary foods, and cleaning teeth can affect the results of the study, so the researchers was difficult to control these factors in order to avoid biased research. So, it can be offered that parents should give breast milk

to the child in order to obtain the benefits of breastfeeding, especially in terms of microbiology. However, should still consider child's oral hygiene. Parents should be taught the proper way to clean teeth for 1 year old infant, as well as providing information on how to cleaning the milk bottle for infant who consume formula milk. Further research needs to be conducted with a larger study sample on the issue. However, further research of each of the factors that affect an increasing number of colonies of *S. mutans* also required, and preferably with a larger sample.

REFERENCES

1. Wong, Donna L. Wong's essentials of pediatric nursing. 7th Ed. St. Louis: Mosby; 2004. p.117.
2. Kim JH, Froh EB. What nurses need to know regarding nutritional and immunobiological properties of human milk. CNEJ California 2011;41:122-37.
3. Law V. Cariogenic factors influencing oral colonization of mutans streptococci in young children. Aust Dent J 2007; 52(2): 93-100.
4. Kolenbrander, Andersen, Blehert, Egland, Foster, Palmer. Latex allergies & latex-safe protocol. Maryland 2007: 486-505.
5. Sharma R. Mutans streptococci colonization in relation to feeding practices, age, and the number of teeth in 6-30 months-old children: an in vivo study. Int J Clin Pediatr Dent [10.5005] 2012; 5(2):124-31. [internet] Available from: URL:<http://www.jaypeejournal.com/eJournals/ShowText.aspx>. Accessed December 7, 2012
6. Simon, Lisa. The role of *Streptococcus mutans* and oral ecology in the formation of dental caries. Lethbridge Undergraduate Res J 2010; 2(2)
7. Holmlund, Amoudruz, Johansson, Haileselassie, Ongoiba, Kayentao, et al. Maternal country of origin, breast milk characteristics and potential influences on immunity in offspring. Breast milk and neonatal immune responses. J Transl Immunol 2010;162:500-9
8. NDRI. Composition of breast milk and comparison with cows and standard formula milk. 2008. [internet] Available from URL: http://ndri.com/article/composition_of_breast_milk_and_comparison_with_cows_and_standard_formula_milk-425.htm. Accessed December 7, 2012
9. Brugger, Baumberger, Jost, Jenni, Brugger U, et al. Automated counting of bacterial colony forming units on agar plates. J PlouS One 2012; 7(3)