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Original Article

Lysozyme quantity and quality in relation with early childhood caries: A longitudinal study

[Cantidad y calidad de lisozima en relación con las caries de la primera infancia: Un estudio longitudinal]

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Abstract

Context: Several studies have reported the relationship between lysozyme quantity (concentration) and quality (activity) with early childhood caries (ECC), but the results remain controversial. These are controversial, probably due to the cross-sectional design used in previous studies.

Aims: To analyze the relation between lysozyme quantity and quality with ECC in two years old children.

Methods: An observational analytic with a cohort study for nine months, with a total sample of 68 caries-free children aged 9-24 months old, selected by purposive sampling from Integrated Healthcare Center in Medan, Indonesia. The subject must have at least two primary upper incisors that have erupted. Lysozyme examination was carried out three times, at the beginning of the month, the third and the ninth month. ECC examination was assessed using the American Association of Pediatric Dentistry criteria. Lysozyme concentration was measured using a competitive ELISA method with human lysozyme C (Fine Test). Then lysozyme reading was done at an absorbance of 450 nm using a microplate reader. Lysozyme activity was assessed using the Lysozyme Detection Kit (Sigma-Aldrich and ready by spectrophotometer. Data analysis was processed with an unpaired t-test, Mann-Whitney test, and longitudinal analysis using a generalized estimating equation (linear) test with a significant value, p<0.05.

Results: This showed that there was not any relationship found between lysozyme concentration and ECC (p>0.05). However, there was a relationship between lysozyme activity and ECC (p=o.oo8).

Conclusions: ECC children had higher lysozyme activity when compared to caries-free children.

Keywords: early childhood caries; longitudinal study; lysozyme activity; lysozyme concentration.

Resumen

Contexto: Varios estudios han informado la relación de la cantidad (concentración) y la calidad (actividad) de lisozima con las caries de la primera infancia (ECC), pero los resultados siguen siendo controvertidos. Estos son controvertidos probablemente debido al diseño transversal utilizado en estudios previos.

Objetivos: Analizar longitudinalmente la relación entre la cantidad y calidad de lisozima con la ECC en niños de dos años.

Métodos: Un análisis observacional con un estudio de cohorte durante nueve meses, con una muestra total de 68 niños sin caries de 9 a 24 meses de edad, seleccionados mediante un muestreo intencional del Centro de Salud Integrado en Medan, Indonesia. Los sujetos debieron tener al menos dos incisivos superiores primarios que hayan erupcionado. Examen de lisozima fue realizado tres veces, al mes inicial, al tercero y al noveno mes. El examen de ECC se evaluó utilizando los criterios de la Asociación Estadounidense de Odontología Pediátrica. La concentración de lisozima se midió utilizando un método ELISA competitivo con lisozima C humana (prueba fina). Luego se realizó la lectura de lisozima a una absorbancia de 450 nm utilizando un lector de microplacas. La actividad de la lisozima se evaluó utilizando el kit de detección de lisozima (Sigma-Aldrich y leido por espectrofotómetro. El análisis de datos se procesó con la prueba t no pareada, la prueba de Mann-Whitney y el análisis longitudinal utilizando una prueba de ecuación de estimación generalizada (lineal) con valor significativo, p<0.05.

Resultados: Esto mostró que no se encontró relación entre la concentración de lisozima y la ECC (p>0.05). Sin embargo, hubo una relación entre la actividad de la lisozima y la ECC (p=o.oo8).

Conclusiones: Los niños con ECC presentaron mayor actividad de lisozima en comparación con los niños sin caries.

Palabras Clave: actividad de lisozima; caries de la primera infancia; concentración de lisozima; estudio longitudinal.

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INTRODUCTION

Early childhood caries (ECC) is a carious lesion that occurs in infants, toddlers, and preschool children with a unique pattern. The definition of ECC is the existence of carious lesions on the surface of primary teeth (which can either be cavities or non-cavities), missing teeth due to carious lesions, or tooth filling in children under 72 months (AAPD, 2016). ECC is a major problem often found in developing countries, including Indonesia. According to the Ministry of Republic Indonesia, in 2018, its prevalence in children of three-four years was 81.1% with a mean deft 6.2, while in five years old children was 91.1% with a mean deft 8.1 (Ministry of Health Republic Indonesia, 2019).

ECC is caused by multifactorial interaction between the host (teeth and saliva), microflora, substrate or diet, and time. Saliva is one factor that had two roles, either in developing a carious lesion or preventing this event (Fejerskov and Kidd, 2008). Saliva's primary function is to protect the oral cavity from microbial growth and the overproduction of microbes that can cause dental caries. Saliva mechanism of action against microorganisms can be divided into two enzymatic and non-enzymatic systems. Enzymatic systems consist of lysozyme and lactoperoxidase. The non-enzymatic system consists of lactoferrin, aggregation factors, and immunoglobulins (Amerongen and Veerman, 2002).

Lysozyme is a small protein with a molecular weight of 14.6 kDa, cationic, and has an isoelectric point (pI) 10-11 (Vasilescu et al., 2016). Lysozyme can be found in fluids such as tears, breast milk, gastric fluid, nasal secretions, serum, saliva, urine, and cerebrospinal fluid. In the mouth, it is produced from salivary glands (major and minor), gingival sulcular fluid and salivary leukocytes (Moslemi et al., 2015; Vasilescu et al., 2016).

Lysozyme, also known as N-acetylmuramide glycanhydrolase, had a muramidase activity. This activity can hydrolyze β (1-4) bonds between N-acetylmuramic acid and N-acetylglucosamine in the peptidoglycan layer of bacterial cell walls. Hydrolysis results of the glycosidic bonds will induce the formation of small porous in bacterial cell walls so that it can kill bacteria. Lysozyme or N-acetylmuramide glycanhydrolase had a strong cationic effect, which can interfere with the aggregation of bacteria, prevent their attachment, and trigger autolysin by injuring their cell walls (Amerongen et al., 2004; Moslemi et al., 2015).

Salivary proteins and polypeptides concentration are key features to maintain oral health and homeostasis. The disease's increasing frequency and severity have always been associated with the quantitative and qualitative changes in the salivary proteome (Dawes et al., 2015; Vasilescu et al., 2016). The antibacterial effect of lysozyme is depended on their concentration and activity in the saliva. The activity level of lysozyme is more relevant in explaining the defensive role of these proteins rather than plainly describing the concentration (Jenzano et al., 1986). The relationship of lysozyme to ECC from previous studies is still controversial. Several studies found that salivary lysozyme concentrations in ECC children were higher than in caries-free children (Bai et al., 2007; Lertsirivorakul et al., 2015). Meanwhile, other studies reported that there was not any distinction in lysozyme concentration in caries-free and caries children (Hao and Lin, 2009; Felizardo et al., 2010). Lysozyme activity was found elevated in caries-free than in caries-prone children (Twetman et al., 1985). Others also reported no statistical difference in lysozyme activity between subjects with various caries statuses (Grahn et al., 1988).

These varying results might be due to the different designs or methods to collect and analyze the saliva. Numerous clinical papers have investigated in a cross-sectional way, and hence it is mandatory to examine this protein longitudinally (Moslemi et al., 2015). Based on this, this report aims to longitudinally examine the relation of lysozyme quantity (concentration) and quality (activity) with ECC in two years old child.

MATERIAL AND METHODS

This report is an observational analytic with a cohort study for nine months. This study had a total sample size of 68 caries-free children aged 9-24 months old, taken by a purposive sampling method from the Integrated Healthcare Center in Medan city (Indonesia). Inclusion criteria were healthy children with at least two erupted primary incisors who did not take any drugs that affected the saliva at least one month before the saliva was collected. Ethical clearance was acquired from the Research Ethics Commission, Faculty of Medicine, Universitas Sumatera Utara, number 59/TGL/KEPK FK USU-RSUP HAM/2018. All participants gave a consent form showing their willingness to participate in this study. The patients' families received detailed explanations regarding the study, consented to participate, and could withdraw from the study freely under any circumstances. The personal and medical information

of the patients was kept entirely confidential. Moreover, the study was conducted following the Declaration of Helsinki.

A dental examination was done by visually observing the teeth under sunlight, assisted with a mouth mirror and probe. At the beginning of the study, a dental examination was done three times in the third month (second observation), and ninth month (third observation). The ECC criteria are evaluated based on the American Association of Pediatric Dentistry (AAPD) criteria.

Saliva collection was taken in the morning, between 8-11 am. Children should not drink or eat one hour before saliva collection. Saliva was collected using an unstimulated saliva technique with a 2 mL disposable pipette. Saliva was put in a closed microtube and loaded into an icebox before being brought to the Integrated Laboratorium in the Faculty of Medicine, Universitas Sumatera Utara. Saliva samples were centrifuged with a centrifugal machine (Eppendorf, Eppendorf AG, Hamburg, Germany) for 1000 ×g for 20 minutes at 2-8°C and stored in the refrigerator at -80°C until analysis time. The saliva was collected three times by the time of the dental examination.

Lysozyme concentration was examined using a competitive ELISA technique with human lysozyme C (Fine Test, Wuhan Fine Biotech, Wuhan, China). Lysozyme concentration was assessed based on comparing the sample's optical density (OD) with a standard curve. Then the value was read at 450 nm using a microplate reader (Thermo Scientific Multiskan Go, Thermo Fisher Scientific, Vantaa, Finland). Lysozyme quantity was assessed three times.

Lysozyme activity was examined using Lysozyme Detection Kit (Sigma-Aldrich Co., Saint Louis, MO, USA). One unit (1 U) of lysozyme activity is described as 0.001 reductions in the absorption value at 450 nm/minute for the catalytic hydrolysis of *Micrococcus lysodeikticus* suspension as a substrate under pH 6.24 and temperature 25°C in 2.6 mL mixture of reaction (1 cm light path). The examination method was to insert 800 mL of *Micrococcus* cell suspension into three cuvettes, blank, control, and saliva samples. Then the cuvette is equilibrated at 25°C. Subsequently, 30 mL of each reaction buffer, lysozyme, and saliva was added to the blank, control, and sample cuvette. Furthermore, the examination was carried out using a spectrophotometer. By decreasing A_{450} lysozyme activity for 5 minutes, we can obtain a maximum linear mean (ΔA_{450}/minute) for test and blank samples.

The relationship between lysozyme concentration and activity with ECC in the second and third observations was evaluated using an unpaired t-test and Mann-Whitney test. The longitudinal analysis was assessed using a generalized estimating equation (linear) test. Data were coded and entered into the SPSS Statistics version 22 (IBM Corporation, Armonk, NY, USA). Significant values in this study were $p<0.05$.

RESULTS AND DISCUSSION

Of 68 caries-free children, 47.06% changed into ECC after three months and increased to 57.35% after nine months. The mean deft in the second observation was 1.53 and increased to 3.06 in the third observation. There was no correlation found between sex and ECC in the second or third observation, but there was a significant ($p<0.05$) relationship found between children's age and ECC (Table 1).

There was not any significant relationship between lysozyme concentration in caries-free and ECC children in second, third, and longitudinal observations ($p>0.05$). On the other hand, there was a significant relationship between lysozyme activity in caries-free and ECC children in longitudinal assessment ($p=0.008$) (Table 2).

There was no significant relationship between caries severity and lysozyme concentration, either in second, third, or longitudinal observation ($p>0.05$). On the other hand, there was a significant relationship between lysozyme activity and caries severity in the longitudinal observation ($p=0.015$) (Table 3).

Based on the longitudinal observation, of 68 children aged 9-24 months old who were initially free from caries, almost half had ECC with a prevalence of 47.06% after three months, and this value increased to 57.35% after nine months (Table 1). The prevalence of ECC in Medan (Indonesia) is similar to research conducted in Medan in 2012, which was 57.7% (Octiara and Tamba, 2012). These results are very concerning. With only less than a year, most children have suffered from ECC.

Besides that, the ECC prevalence and caries severity increase with age (Table 1). In the second observation, it was found that the deft score was 1.53, which then increased to 3.06 in the third observation (Table 1). This is similar to National Data in 2018, which reported that older children aged 3-4 years old have a prevalence of 81.5% with a mean deft 6.2, and the prevalence and deft score were increased in 5 years old group, respectively 90.2% and 8.1 (Ministry of Health Republic Indonesia, 2019).

Based on the same age group, ECC prevalence here was higher than in other Asian countries such as Japan, 3.2% (Nakayama and Mori, 2017) and Singapore, 17.8-42.9% (Hu et al., 2019). However, the prevalence of ECC in this study was lower than in Xinjiang, China, 64.5% (Li et al., 2017) and Thua Thien Hue, Vietnam, 72.4% (Nguyen et al., 2018). In Indonesia, the high prevalence of ECC is quite a challenge for dental health workers to achieve a 50% caries-free target for children aged 5-6 years old. Besides, to achieve Indonesia's target in 2030, which is that all 12 years old children can be caries-free, it is necessary to continuously prevent caries from an early age (Kebijakan Kesehatan Indonesia, 2019).

Table 1. Children categories based on sex, age, and deft.

Children categories	Total n (%)	Sex		p-value	Age (Month)	p-value	Index deft
		Male n (%)	Female n (%)		Mean (SD)		Mean (SD)
First observation							
Caries-free	68 (100)	36 (52.94)	32 (47.06)	-	15.34 (4.29)	-	0
Second observation							
Caries-free	36 (52.94)	17 (47.22)	19 (52.78)	0.45[1]	17.89 (4.07)	0.01*[2]	0
ECC	32 (47.06)	19 (59.38)	13 (40.62)		20.5 (4.3)		3.25 (1.5)
Total	68 (100)	36 (52.94)	32 (47.06)		19.12 (4.35)		1.53 (1.93)
Third observation							
Caries-free	29 (42.65)	13 44.83)	16 (55.17)	0.36[1]	23.14 (4.08)	0.04*[2]	0
ECC	39 (57.35)	23 (58.97)	16 (41.03)		25.49 (5.18)		5.33 (2.31)
Total	68 (100)	36 (52.94)	32 (47.06)		24.49 (4.85)		3.06 (3.18)

*Statistically significant (p<0.05); [1]Chi-Square test; [2]Independent t-test.

Table 2. Relationship between children category and lysozyme concentration and activity in first, second, third, and longitudinal observations.

Children categories	Lysozyme concentration (ng/mL) Mean ± SD/ Median (min-max)	p-value	Lysozyme activity (unit/mL) Mean ± SD/ Median (min-max)	p-value
First observation				
Caries-free (n = 68)	1270 (89.3-9280)		10 500 (667-157 000)	
Second observation				
Caries-free (n = 36)	696.95 ± 2.99	0.67[1]	17 955.60 ± 4.77	0.26[1]
ECC (n = 32)	788.13 ± 3.64		11 515.96 ± 5.41	
Third observation				
Caries-free (n = 29)	365.68 ± 3.45	0.19[1]	70 700 (5000 – 307 000)	0.19[2]
ECC (n = 39)	557.19 ± 3.76		86 700 (3330-550 000)	
Longitudinal I-II-III (9 months)				
Caries-free	324.631	0.105[3]	-30 394.961	0.008*[3]
ECC	Reference (Intercept = 1288.20)		Reference (Intercept = 73 826.239)	

*Statistically significant (p<0.05); [1]Independent t-test; [2] Mann-Whitney test; [3]General estimating equation (linear) test.

Table 3. Relationship between caries category and lysozyme concentration and activity in first, second, third, and longitudinal observation.

Caries category	n (%)	Lysozyme concentration (ng/mL) Mean ± SD/ Median (min-max)	p-value	Lysozyme activity (unit/mL) Mean ± SD/ Median (min-max)	p-value
First observation					
Caries-free	68 (100)	1270 (89.3-9280)	-	10 500 (667-157 000)	-
Second observation					
Caries-free (deft 0)	36 (52.94)	696.95 ± 2.99	0.95[1]	17 955.6 ± 4.77	0.5[1]
Low caries (deft 1-2)	13 (19.12)	834.26 ± 3.89		14 876.47 ± 6.66	
Medium caries (deft 3-4)	14 (20.58)	800.39 ± 3.88		8753.87 ± 4.58	
High caries (deft >5)	5 (7.36)	650.73 ± 3.23		12 752.64 ± 6.12	
Third observation					
Caries-free (deft 0)	29 (42.65)	365.68 ± 3.45	0.16[1]	76 700 (5000-307 000)	0.51[2]
Low caries (deft 1-2)	7 (10.29)	273.53 ± 3.63		113 000 (10 000-203 000)	
Medium caries (deft 3-4)	15 (22.06)	529.79 ± 3.27		86,700 (3330-207 000)	
High caries (deft >5)	17 (25)	780.73 ± 4.06		73 300 (20 000-550 000)	
Longitudinal (I-II-III)					
Caries-free (deft 0)	-	248.485	0.308[3]	-62 053.722	0,015*[3]
Low caries (deft 1-2)	-	11.360	0.987[3]	-39 311.700	0.159[3]
Medium caries (deft 3-4)	-	-194.259	0.517[3]	-50 397.862	0.053[3]
High caries (deft >5)	-	Reference (Intercept = 1364.345)		Reference (Intercept = 105 485)	

*Statistically significant (p<0.05); [1]One way Anova test; [2]Kruskal Wallis test; [3]General estimating equation (linear).

Relationship between lysozyme concentration with ECC and caries category

Based on Table 2, there was no relationship between lysozyme concentrations in caries-free and children with ECC (p>0.05), either in the second and third observation or longitudinally. This result is consistent with Hao and Lin (2009), who stated that there was no distinction in lysozyme concentrations between caries-free and caries groups.

This result differs from other studies that found a significantly higher lysozyme concentration in the caries-free group (p<0.05) (Moslemi et al., 2015). This result is also different from Bai et al. (2007) and Letsirivorakul et al. (2015) stated that lysozyme concentration was higher in ECC children than in the non-caries group. This can happen due to the compensatory mechanism in a carious lesion. In this event, the number of *S. mutans* is escalated, and lysozyme,

which serves as a defense component, will boost its secretion from this bacterial stimulation (Leone and Oppenheim, 2001). Letsirivorakul et al. (2015) stated that children with caries had significantly up-regulated lysozyme compared to caries-free children. This result is due to the lysozyme function as one of the innate immunity that acts as the body's first defense mechanism and is prominent in the oral cavity (Amerongen et al., 2004; de Andrade et al., 2014).

Table 3 also shows the same thing, which was not any relationship between caries severity and lysozyme concentration ($p>0.05$). This result is consistent with Felizardo et al. (2010) statement that lysozyme is not related to caries. However, this result was different from other studies where the DMF-T index decreased in line with the increase in lysozyme concentration ($p<0.05$) (Bhalla et al., 2010).

Relationship between lysozyme activity with ECC and caries category

In the second and third observations, there was not any relationship between the activity of lysozyme in ECC and caries-free children ($p>0.05$) (Table 3). However, longitudinal analysis for nine months (observations I-II-III) using a generalized estimating equation (linear) test found a significant association between this activity in ECC and caries-free children ($p=0.008$). Caries-free children had 30 394.961 units/mL lower lysozyme activity than children with ECC.

This result is consistent with the study that found that SECC children had higher lysozyme activity than caries-free children ($p=0.02$) (Bai et al., 2007). However, it is different from other studies that reported that children prone to caries had lower lysozyme activity than non-susceptible children (Twetman et al., 1985). It is also different from other studies that reported no difference in lysozyme activity between subjects with various caries statuses (Grahn et al., 1988).

A similar result is also seen in Table 3, based on the second and third observations, in which there was no relationship between caries severity and lysozyme activity ($p>0.05$). However, in longitudinal analysis, it can be seen that caries-free children had lower lysozyme activity 62 053.722 units/mL than children in the high caries group (deft>5) ($p=0.015$). Likewise, children with low caries had 39 311.700 units/mL lower lysozyme activity than the high caries group, and so did the moderate caries group with lower activity 50 397.862 units/mL than the high caries group. It was concluded that children with high caries (deft>5) had the highest lysozyme activity compared to other categories ($p=0.015$).

Protein concentration in each person's saliva varies with age, as the amount will increase with age. Alterations in salivary secretion with age are associated with a higher risk of carious lesion and caries incidence (Octiara et al., 2018). In general, this result can be seen in Table 2. At initial, lysozyme concentration was higher than in the second and third observations. This condition can make several studies with a cross-sectional method get different results. With aging, the likelihood of bacterial exposure to the oral cavity increases, which will impact lysozyme concentration. Besides, caries risk that occurred in the subjects of this study also caused variations in lysozyme concentrations.

Longitudinal observation can be a solution to analyzing salivary protein in children. These can be proven by lysozyme activity in cross-sectional observations (second and third) against ECC, which is not significant. When it is analyzed longitudinally, we can find the relationship between lysozyme activity and ECC. However, we could not find the association of lysozyme concentration with ECC, even though it has been analyzed longitudinally. This result is similar to other researchers who stated that lysozyme activity level is more relevant in explaining the defensive role of these proteins rather than plainly describing the concentration (Jenzano et al., 1986).

CONCLUSION

It is possible that lysozyme activity can be used as a biomarker candidate against ECC incidence in early childhood. Although lysozyme proteins have low concentrations in salivary glands, they have notable biological activity. In the future, it is necessary to consider using this salivary protein for caries prediction in early childhood. It was concluded that lysozyme activity had a relationship with ECC, with ECC children having higher lysozyme activity than children without caries.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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REFERENCES

AAPD - American Academy of Pediatric Dentistry (2016) Policy on early childhood caries (ECC): Classifications, consequences, and preventive strategies.

https://www.aapd.org/media/policies_guidelines/p_eccclassifications.pdf [Consulted January 10, 2020].

Amerongen AVN, Bolscher JGM, Veerman ECI (2004) Salivary protein: protective and diagnostic value in cariology? Caries Res 38: 247-253.

Amerongen AVN, Veerman ECI (2002) Saliva - the defender of the oral cavity. Oral Dis 8: 12-22.

Bai J, Zhou Q, Bao ZY, Li XX, Qin M (2007) Comparison of salivary proteins between children with early childhood caries and children without caries. Zhonghua Kouqiang Yixue Zazhi 42: 21-23.

Bhalla S, Tandon S, Satyamoorthy K (2010) Salivary proteins and early childhood caries: A gel electrophoretic analysis. Contemp Clin Dent 1: 17-22.

Dawes C, Pedersen AM, Villa A, Ekström J, Proctor GB, Vissink A, Aframian D, McGowan R, Aliko A, Narayana N, Sia YW, Joshi RK, Jensen SB, Kerr AR, Wolff A (2015) The functions of human saliva: A review sponsored by the World Workshop on Oral Medicine VI. Arch Oral Biol 60(6): 863-874.

de Andrade FB, de Oliveira JC, Yoshie MT, Guimarães BM, Gonçalves RB, Schwarcz WD (2014) Antimicrobial activity and synergism of lactoferrin and lysozyme against cariogenic microorganisms. Braz Dent J 25: 165-169.

Fejerskov O, Kidd E (2008) Dental caries: the disease and its clinical management, 2nd Edn. Oxford: Blackwell Munksgraard Ltd.

Felizardo KR, Goncalves RB, Schwarcz WD, Frederico RCP, Maciel SM, de Andrade FB (2010) An evaluation of the expression profiles of salivary proteins lactoferrin and lysozyme and their association with caries experience and activity. Rev Odonto Ciênc 25(4): 344-349.

Grahn E, Tenovuo J, Lehtonen OP, Eerola E, Vija P (1988) Antimicrobial system of human whole saliva in relation to dental caries, cariogenic bacteria and gingival inflammation in young adults. Acta Odontol Scan 46: 67-74.

Hao GF, Lin HC (2009) Relashionship of concentration of lactoferrin and lysozyme in saliva and dental caries in primary dentition. Zhonghua Kouqiang Yixue Zazhi 44: 82-84.

Hu S, Sim Y, Toh JY, Saw SM, Goodfrey KM, Chong YS, Yap F, Lee YS, Shek LPC, Tan KH, Chong MFF, Hsu CYS (2019) Infant dietary patterns and early childhood caries in a multi-ethnic Asian cohort. Sci Rep 9(1): 852.

Jenzano JW, Hogan SL, Lundblad RL (1986) Factors influencing measurement of human salivary lysozyme in lysoplate and turbidimetric assays. J Clin Microbiol 24(6): 963-967.

Kebijakan Kesehatan Indonesia (2019) Indonesia diharapkan bebas karies pada 2030. https://kebijakankesehatanindonesia.net/25-berita/berita/2500-indonesia-diharapkan-bebas-karies-pada [Consulted August 8, 2020].

Leone CW, Oppenheim FG (2001) Physical and chemical aspects of saliva as indicators of risk for dental caries in humans. J Dent Educ 65(10): 1054-1062.

Lertsirivorakul J, Petsongkram B, Chaiyarit P, Klaynongsruang S, Pitiphat W (2015) Salivary lysozyme in relation to dental caries among Thai preschoolers. Pediatr Dent 39(4): 343-347.

Li Y, Wulaerhan J, Liu Y, Abudureyimu A, Zhao J (2017) Prevalence of severe early childhood caries and associated socioeconomic and behavioral factors in Xinjiang, China: a cross-sectional study. BMC Oral Health 17: 144.

Ministry of Health Republic Indonesia (2019) Basic Health Survey, Jakarta- Ministry of Health Republic Indonesia. http://www.litbang.kemkes.go.id/laporan-riset-kesehatan-dasar-riskesdas/ [Consulted January 5, 2020].

Moslemi M, Sattari M, Kooshki F, Fotuhi F, Modarresi N, Sadrabad ZK, Shadkar MS (2015) Relationship of salivary lactoferrin and lysozyme concentrations with early childhood caries. J Dent Res Dent Clin Dent Prospects 9(2): 109-114.

Nakayama Y, Mori M (2017) Risk factors associated with early childhood caries in 18 to 23-month-old children in a Japanese city. J Natl Inst Public Health 66(5): 545-552.

Nguyen YHT, Ueno M, Zaitsu T, Nguyen T, Kawaguchi Y (2018) Early childhood caries and risk factors in Vietnam. J Clin Pediatr Dent 42(3): 173-181.

Octiara E, Sutadi H, Siregar Y, Primasari A (2018) sIgA and lysozyme as biomarker of early childhood caries. Adv Health Sci Educ Theory Pract 8: 96-101.

Octiara E, Tamba EA (2012) Relationship of family economic and mother education with early childhood caries (ECC) among children 12-36 months aged in district Medan Denai. Dentika Dent J 17(1): 78-82.

Twetman S, Lindner A, Modeer T (1985) Lysozyme and salivary immunoglobulin A in caries-free and caries-susceptible preschool children. Swed Dent J 5: 9-14.

Vasilescu A, Wang Q, Li M, Boukherroub R, Szunerits S (2016) Aptamer-based electrochemical sensing of lysozyme. Chemosensors 4(10): 10.

AUTHOR CONTRIBUTION:

Contribution	Octiara E	Heriandi S	Yahwardiah S	Ameta P
Concepts or ideas	x	x	x	
Design	x	x	x	
Definition of intellectual content	x	x	x	x
Literature search	x			
Clinical trial	x		x	
Experimental studies	x		x	
Data acquisition	x	x	x	
Data analysis	x	x		
Statistical analysis	x	x		
Manuscript preparation	x			
Manuscript editing	x	x	x	x
Manuscript review	x	x	x	x

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