The use of Lysozyme Toothpaste to Prevent Early Childhood Caries (Ecc) in 2 Years Old Children

Essie Octiara[1]*, Heriandi Sutadi[2], Yahwardiah Siregar[3], Ameta Primasari[4]

1. Department of Pediatric Dentistry, Faculty of Dentistry, Universitas Sumatera Utara.
2. Department of Pediatric Dentistry, Faculty of Dentistry, Universitas Indonesia.
3. Department of Biochemistry, Faculty of Medicine, Universitas Sumatera Utara.
4. Department of Oral Biology, Faculty of Dentistry, Universitas Sumatera Utara.

Abstract

The aim was to analyze the effect of using non-fluoride lysozyme toothpaste for five weeks against ECC prevention, which was evaluated from deft and defs records, S.mutans colonies, sIgA, and lysozyme activity in 2-years-old children.

This investigation is a clinical trial with 68 children aged 14-35 months, including 29 children without caries and 39 ECC. Dental examination and saliva were taken before and after using toothpaste. Saliva was reviewed to discover the amount of S. mutans, sIgA, and lysozyme activity. sIgA was evaluated by ELISA, and lysozyme activity by spectrophotometry. Data interpretation is determined using paired and unpaired t-test. The amount of S.mutans colonies in saliva following lysozyme toothpaste in the ECC group decreased significantly. After using lysozyme toothpaste, there was a higher decrease in sIgA in ECC than the control group. After using lysozyme toothpaste, the highest increase in lysozyme activity was found in ECC children.

Lysozyme toothpaste can reduce the increase in defs index by 50% in ECC compared to the control group. Lysozyme toothpaste can prevent ECC, marked by the reduction in defs index, the amount of S. mutans colonies, and its effect in the sIgA mechanism of action and lysozyme activity.

Clinical article (J Int Dent Med Res 2022; 15(2): 623-629)

Keywords: ECC, Lysozyme toothpaste, S.mutans, sIgA, Lysozyme activity.

Received date: 18 February 2022 **Accept date:** 10 May 2022

Introduction

One of the major oral problems in infants and preschoolers worldwide is Early Childhood Caries (ECC). It is manifested as the appearance of carious lesions in deciduous teeth surface, can either be cavity or non-cavity, missing teeth on account of caries, or tooth filling in children under 6 years old.[1] Its prevalence in children aged 3 years and under is relatively high in various countries. In Japan, ECC prevalence was 3.2%, Nigeria 6.1%, Chile 20.3%, Jerusalem 33%, India 36.4%, and increases in ECC were found in China by 64.5% and Vietnam by 72.4%.[2-8] In Indonesia, the prevalence of ECC was very high at 81.5%; Wajo Regency, South Sulawesi (Indonesia) at 74.1%, and Medan City (Indonesia) at 57.7%.[9-11]

Corresponding author:
Essie Octiara
Department of Pediatric Dentistry
Jl. Bakau No.14 Medan, Indonesia
ZIP Code: 20113
E-mail: eoctiara@gmail.com

The high prevalence of ECC in early childhood requires an effective prevention method. Effective management strategies to prevent ECC are based on their etiology. ECC had multifactorial etiology consisting of microorganisms, carbohydrate diet, susceptible tooth surface, and time.[12]

One of the effective caries prevention methods based on ECC etiology is regular tooth brushing using toothpaste. Children who clean their teeth once a day have 3.4 times the risk of suffering ECC compared to those who regularly clean their teeth twice per day.[13] Based on the research in Indonesia, it was found that only 1.1% of children aged 3-4 years old that brushed their teeth at proper time.[9]

The use of fluoridated toothpaste as a caries prevention agent has been widely reported in several studies.[14,15] However, for 2-years-old children and under, the use of fluoride-containing toothpaste must proceed with caution. If they have average or high caries risks, a smear layer of toothpaste is given to them, while low caries risk can use a non-fluoride toothpaste. Tooth brushing in one-year-old can be done using a soft toothbrush only, without toothpaste, or using a non-fluoride one.[16]

To increase the effectiveness of teeth brushing in early childhood, we can use toothpaste without fluoride but contains the active substance lysozyme that can act as an antibacterial. Lysozyme had antimicrobial activity by hydrolyzing the glycosidic bonds connecting N-acetylmuramic-acid and N-acetylglucosamine in the bacterial cell wall's peptidoglycan layer. This condition will induce small orifices in the bacterial cell wall so it will die.[17,18] As a powerful cation protein, lysozyme can interfere with bacterial aggregation and inhibit bacterial adhesion.[19] The lysozyme activity will last when the enzyme is incorporated into the pellicle.[20] Therefore, it is hoped that the addition of the lysozyme to non-fluoride toothpaste can have an antibacterial effect against *S.mutans* so it can prevent the incidence of new caries.

This study aimed to analyze the effect of using non-fluoride lysozyme toothpaste for five weeks against ECC prevention, which was evaluated from deft and defs records, *S.mutans* colonies, sIgA, and lysozyme activity in 2 years old children.

Materials and methods

Study Samples

This study means a clinical trial with a sample size of 68 aged 14-35 months children made up of 29 children without caries and 39 children with ECC. These children are obtained from Integrated Healthcare in Medan city, Indonesia. Samples' inclusion criteria in this study were healthy children without using a medicine that can alter saliva at a minimum of 30 days prior to the study. The guidelines for deciding on children with ECC are established by the American Association of Pediatric Dentistry (AAPD) criteria.[1] The study started after obtaining approval from the Health Research Ethics Commission, Faculty of Medicine, Universitas Sumatera Utara (no 059). 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 in the study, and could withdraw from the study freely under any circumstances.

Caries Examination and Saliva Sample Collection

Caries examination was assessed visually under sunlight with the help of a mouth mirror and dental probe. Data were recorded based on the deft and defs index according to ECC's AAPD criteria. Saliva collection was taken between 09.00 to 11.00 am. Drinking and eating were prohibited one hour before saliva collection. Saliva was taken using a disposable pipette for 2 ml with an unstimulated saliva method. Then, samples were taken to the Integrated Laboratorium and Microbiology Laboratorium in the Faculty of Medicine, Universitas Sumatera Utara to be analyzed.

Microbial Examination

S.mutans culture was done using trypticase soy-sucrose-bacitracin (TYS20B). The media then stored inside the anaerobic container (anaerobic condition, 10% H_2, 10% CO_2, dan 80% N_2) then kept in the incubator at 37^{o}C for 2x24 hours. Thus, the quantity of Colony Forming Units (CFU/ml) of *S.mutans* is counted after 2x24 hours.

Saliva Chemical Analysis

The chemical analysis was done to determine sIgA concentration and lysozyme activity. The sIgA concentration was assessed using the reagent human sIgA ELISA Kit (Fine Test, Wuhan Fine Biotech, Wuhan, China) with the sandwich technique by analyzing the optical density (OD) of the specimen with a standard curve. Specimen measurement was done using a micro reader with a 450 nm wave.

The lysozyme activity was examined using the Lysozyme Detection Kit (Sigma-Aldrich Co., Saint Louis, MO, USA). The examination method was 800 ml of *Micrococcus* cell suspensions were inserted into 3 cuvettes (1 for blank, 1 for control, and 1 for saliva specimen). Then the cuvette is equilibrated at 25^{o}C. Subsequently, in the blank, control, and specimen cuvette were added respectively with 30 ml Reaction Buffer, lysozyme solution, and saliva specimen. The cuvette was examined using a spectrophotometer (Thermo Scientific Multiskan Go, Thermo Fisher Scientific, Vantaa, Finland). The decreasing activity of ∆450 lysozyme for 5 minutes and obtained the maximum linear mean (∆A450 / minute) for the test and blank samples. One-unit (1 U) lysozyme activity was meant as a reduction of 0.001 in absorption value at 450 nm per minute for the catalytic hydrolysis of Micrococcus lysodeikticus suspension as a substrate, below pH 6.24 and 25°C in 2.6 ml reaction mixture (1 cm light path).

Tooth Brushing with Toothpaste

Children who had their teeth examined and saliva is taken, then they were given either lysozyme or control toothpaste. The basic ingredients for control toothpaste are calcium carbonate, sodium carboxymethyl cellulose, glycerol, sorbitol, tragacanth, sodium benzoate, flavorings, and distilled water. Lysozyme toothpaste is control toothpaste added with hen egg-white lysozyme or HEWL (Sigma-Aldrich Co., Saint Louis, O, USA). We used a 0.1% concentration based on the previous lab experimental test.[21] Acetic acid 0.4% then added as a stabilization agent. Lysozyme and control toothpaste were given randomly to the subjects. The 0.1% lysozyme toothpaste was given 22 ECC and 17 caries-free children, while control toothpaste was given to 17 ECC and 12 caries-free children. After 5 weeks of teeth brushing, their teeth were checked over to record the deft and defs index. Afterward, saliva was recollected to assess *S.mutans* colonies, sIgA concentration, and lysozyme activity. The obtained data were analyzed with the baseline one.

To define the distinction in the mean value of sIgA concentration, lysozyme activity, and *S.mutans* among children without caries and children with ECC, we used an unpaired t-test or Mann-Whitney test if the data weren't normally distributed. A paired t-test was used to learn the differences in sIgA concentration, lysozyme activity, S.mutans colonies prior to and after teeth brushing, or Wilcoxon test if not distributed normally. The significance value used in this study was $p<0.05$.

Results

The initial characteristics of subjects in this study consisted of 42.65% caries-free children with mean age 21.84 months and 57.35% ECC children with mean age 24.34 months (Table 1).

Following tooth brushing using lysozyme toothpaste, the number of *S.mutans* in ECC children had decreased significantly (p=0.03). Meanwhile, there wasn't any decrease in ECC children using control toothpaste. The sIgA concentration in both groups was significantly different before and after using lysozyme toothpaste (p=0.001). After using lysozyme toothpaste, ECC children experienced a significant increase in lysozyme activity (p=0.001) (Table 2).

There was a significant distinction in the sIgA difference among children without caries and with ECC following the use of toothpaste containing lysozyme (p=0.02), whereas, in children using control one, no notable distinction was found (p=0.63). Meanwhile, lysozyme activity also *S.mutans* colonies examination found no distinction obviously (p> 0.05) (Table 3).

There was a higher increase in the defs index in children who were given control toothpaste than in children who were given lysozyme toothpaste, although it wasn't remarkable from a statistic view (p=0.09) (Table 4).

Discussion

Effect of lysozyme toothpaste against *S.mutans* colony count

At the beginning of the study, it was found that ECC children had a higher number of *S.mutans* colonies than caries-free children (Table 2). The outcomes of our research were similar to Thakur et al and Setiawan et.al, who stated the same thing.[22,23] The amount of *S.mutans* within the saliva is a microbial parametric to determine individual caries' risk.

The use of lysozyme toothpaste affected the amount of *S.mutans* after 5 weeks of teeth brushing (Table 2). In ECC children, the quantity of *S.mutans* colonies lowered significantly by 3.25 times (p=0.03) compared to baseline. On the other hand, there wasn't any reduction in *S.mutans* population in the control group. There was an increase in the *S.mutans* population, but the result was insignificant.

Toothpaste containing lysozyme can help reduce numbers of *S.mutans* in children with ECC. This result is consistent with Gudipaneni et al., that used fluoridated toothpaste contains lysozyme for 7-days in children suffering from SECC and found a notable decrease in the amount of *S.mutans* and *L. acidophilus*.[24]

Similar results were found in the study by Pinheiro et al., who reported a drop of value in *S.mutans* colonies in teeth with dentin cavities coated with lysozyme and hydroxyapatite after one month of observation.[25] The antimicrobial activity of lysozyme was effectively used as a sealing cavity. Lysozyme had bactericidal activity and can reduce *S.mutans* adhesion. Lysozyme arrival will affect the number of glucans produced by glycosyltransferase B, but it does not affect its glucan structure.[26] Lysozymes also cause a massive loss of potassium in bacteria, which can lead to a noticeable reduction in the potential membrane because K+ is the major monovalent cation in bacterial cells which reduce energy-dependent membrane transport function, disabling potassium-dependent cellular enzymes, reduce turgor pressure, terminate growth, and cell death.[27]

When compared among children without and with ECC has given lysozyme toothpaste, it can be seen that distinction in the decrease of *S. mutans* in ECC children was relatively high. Meanwhile, in caries-free children, there was no decrease in *S.mutans* (Table 3). The effect of lysozyme toothpaste on the amount of *S.mutans* colonies in children without caries is not very visible due to the small value of *S.mutans* contained in caries-free children's saliva that is far below ECC children. Specific (sIgA) and non-specific (lysozyme, lactoferrin, etc) antibacterial abilities in children's saliva have been able to overcome the *S.mutans* infection. This is consistent with Sood et al., which found a correlation between the number of mutans streptococci and the concentration of sIgA, in which the lower the number of *S.mutans* in a person's saliva, the higher the sIgA concentration ($r = -0.46$, $p < 0.05$).[28]

Effect of lysozyme toothpaste against sIgA concentration

In the caries-free group, following the use of lysozyme and control toothpaste, there was an almost two-fold increase in sIgA concentrations compared to before tooth brushing ($p = 0.001$) (Table 2). Here, it can be seen that sIgA in caries-free children's saliva overcame *S.mutans* bacterial infection in the child's oral cavity. This was consistent with Omar, who stated that the sIgA response in *S.mutans* would be increased in children who have low caries risk activity because it is a form of the body's sIgA protective mechanism against caries attack.[29]

Besides that, the activity of brushing teeth without toothpaste has also been able to reduce the value of *S.mutans* colonies in children's oral cavity. Seow reported that after regular daily brushing of 107 children under two years old, there was a reduction in the number of children infected with *S.mutans* ($p < 0.01$).[30]

In contrast, there was a significant decrease in sIgA values in ECC children following the use of lysozyme and control toothpaste than before ($p=0.001$) (Table 2). However, the difference in decreasing sIgA concentration in ECC children after using lysozyme toothpaste was higher than control toothpaste.

Lysozyme toothpaste also helps reduce the amount of *S.mutans* colonies in ECC children. sIgA was formed due to microorganism antigens in the oral cavity,31 in relation to reduced S.mutans colonies on the teeth. The production of sIgA in ECC children was not as high as before the teeth were brushed with lysozyme toothpaste. Lysozyme toothpaste was effective for children with ECC to help handle *S.mutans* bacteria. This can be proven from Table 3 that notable differentiation in sIgA concentration was found following the use of lysozyme toothpaste among children without caries and with ECC ($p=0.02$), whereas, there wasn't any differentiation in the control group ($p=0,63$).

Lysozyme toothpaste effect against lysozyme activity

The antibacterial effect of lysozyme depends on the concentration and activity of saliva lysozyme. Lysozyme activity level is more suitable to explain the defensive responsibility of this protein. Studies that differentiate between active and inactive lysozyme in saliva will give better results than just describing the concentration.[32] Therefore, in this study, lysozyme activity evaluation was carried out to see the difference between using lysozyme and control toothpaste.

In Table 2, it can be seen the increase in lysozyme activity in ECC children given lysozyme toothpaste was the highest among other groups of children, which was 13 times higher than before tooth brushing, while the increased in lysozyme activity in caries-free children given lysozyme toothpaste and caries-free children assigned control toothpaste was 2.5 and 5 times respectively. Here, we can see the role of lysozyme toothpaste as antibacterial in ECC children, in which lysozyme activity in ECC children given lysozyme toothpaste was higher than those given control toothpaste (Table 2).

An insignificant difference was found

between the increase in lysozyme activity among children without caries and with ECC who were given lysozyme toothpaste (p=0.06). The same results were also found among children without caries and with ECC who were given control toothpaste (p=0.79) (Table 3). However, in substance, the difference in the increase in lysozyme activity in children who were given lysozyme toothpaste was higher than the control group. From these results, it can be concluded that lysozyme toothpaste was useful to give to children who experience ECC.

Effect of lysozyme toothpaste against defs index

In ECC children who used lysozyme toothpaste, there was an increase in the defs index of 2, while the ECC children using control toothpaste was 4, however, this was insignificant statistically (p = 0.09) (Table 4). In substance, lysozyme toothpaste can reduce the increase in the defs index by 50% in ECC children, compared to the control toothpaste group.

Conclusions

It was concluded that lysozyme toothpaste could reduce the increase in surface caries lesions (defs) in ECC children by acting as an additional antibacterial agent in saliva. The amount of S.mutans colonies was reduced will affect sIgA action and lysozyme activity. Lysozyme toothpaste can be used as a substitute to prevent ECC in 2-years-old children and under.

Acknowledgments

Affirmations are written to the Minister of Research, Technology, and Higher Education of Indonesia, at the expense of this research was received from DRPM Directorate General of Strengthening Risbang.

Declaration of Interest

The authors report no conflict of interest.

Children Category	n (%)	Gender		Mean Age (months)	Mean		Mean	
		♂ (%)	♀ (%)		Baseline deft	Final deft	Baseline defs	Final defs
Caries-free	29 (42.65)	13 (44.83)	16 (55.17)	21.84+3.91	0	0	0	0
ECC	39 (57.35)	23 (58.97)	16 (41.03)	24.34+5.16	5.34+2.32	5.34+2.32	5.83+3.04	6.65+3.31
Total	68 (100)	36 (52.94)	32 (47.06)	23.27+4.79				

Table 1. Subjects Characteristics.

Toothpaste Category	Saliva Examination	Caries-free children (n=29)			ECC children (n=39)		
		Mean/ Median	SD/Min-max	p-value	Mean/ Median	SD/Min-max	p-value
S.mutans colonies count (x10^3 CFU/ml) before and after using toothpaste							
Lysozyme toothpaste	*S.mutans* colonies before brushing	5	2-270	0.28[a]	117	3-4,400	0.03*[a]
	S.mutans colonies after brushing	14	4-132		36	6-1,144	
Control toothpaste	*S.mutans* colonies before brushing	8.5	3-57	0.27[a]	22	4-2,200	0.55[a]
	S.mutans colonies after brushing	11.5	6-47		82	3-2,592	
sIgA concentration (ng/ml) before and after using toothpaste							
Lysozyme toothpaste	sIgA before brushing	279.49	31.96	0.001*[b]	401.25	69.16	0.001*[b]
	sIgA after brushing	515.12	3.62		316.75	1.42	
Control toothpaste	sIgA before brushing	365.12	93.13	0.001*[b]	385.36	50.89	0.001*[b]
	sIgA after brushing	616.18	1.77		355.89	1.72	
Lysozyme activity (ng/ml) before and after using toothpaste							
Lysozyme toothpaste	Lysozyme activity before brushing	25,153.59	4.51	0.001*[b]	7,170	333-217,000	0.001*[a]
	Lysozyme activity after brushing	64,159.79	19.25		95,000	10,000-550,000	
Control toothpaste	Lysozyme activity before brushing	17,500	1,330-180,000	0.03*[a]	18,500	167-287,000	0.09[b]
	Lysozyme activity after brushing	86,650	36,700-833,300		68,300	3,330-203,000	

Table 2. The difference in the Amount of S.mutans, sIgA Concentration, and Lysozyme Activity Before and After Using Lysozyme and Control Toothpaste in Caries-free and ECC Children. *Significant; aWilcoxon test; bpaired t-test

Children Category	n	Lysozyme toothpaste		p-value	Control toothpaste		p-value
		Mean/ Median	SD/ Min-max		Mean/ Median	SD/ Min-max	
Difference in *S.mutans* Colonies Before and After Using Toothpaste (Δ *S.mutans* colonies x10³ CFU/ml)							
Caries-free	29	-5	-91-138	0.09ᵃ	-2,5	-143-141	0.39ᵃ
ECC	39	102.5	-409-3,256		4	-1,492-1,998	
Difference in sIgA Concentration Before and After Using Toothpaste (Δ sIgA Concentration (ng/ml))							
Caries-free	29	-328	-8,748-500	0.02*ᵃ	-258.78	402.32	0.63ᵇ
ECC	39	39.5	-1,320-660		27.47	396	
Difference in Lysozyme Activity Before and After Using Toothpaste (Δ Lysozyme activity (unit/ml))							
Caries-free	29	-10,800	-306,167-189,700	0.06ᵃ	-43,734.17	59,936.78	0.79ᵇ
ECC	39	-83,748.5	-500,000-127,000		-34,942.53	99,174.11	

Table 3. Comparability in the Distinction of S.mutans Colonies, sIgA Concentration, and Lysozyme Activity among Children without Caries and with ECC Based on the Toothpaste Given to Subjects. *Significant; a Mann-Whitney test: bunpaired t test.

Toothpaste Category	defs difference in all subject before and after using toothpaste (n=68)			defs difference in ECC children before and after using toothpaste (n=39)		
	Median	Min-max	p	Median	Min-max	p
Lysozyme toothpaste	-1	-6 - 0	0.47 ᵃ	-2	-6 - 0	0.09ᵃ
Control toothpaste	-2	-8 - 0		-4	-8 -0	

Tabel 4. Comparison in defs Difference Before and After Using Lysozyme and Control Toothpaste.
ᵃMann-Whitney test.

References

1. American Academy of Pediatric Dentistry (AAPD). Policy on early childhood caries (ECC): Classifications, consequences, and preventive strategies. Refer Man 2014; 37 (6): 50-2.
2. Nakayama Y, Mori M. Risk factors associated with early childhood caries in 18 to 23-month-old children in a Japanese city. J Natl Inst Public Health 2017; 66 (5): 545-52.
3. Folayen M, Kolawole KA, Oziegbe EO, et.al. Prevalence and early childhood caries risk indicators in preschool children in suburban Nigeria. BMC Oral Health 2015; 15: 72.
4. Hooffmeister L, Moya P, Vidal C, Benardof D. Factors associated with early childhood caries in Chile. Gac Sanit 2016; 30(1): 59-62.
5. **Livny** A, **Assali R**, **Cohen** HDS. Early childhood caries among a Bedouin community residing in the eastern outskirts of Jerusalem. *BMC Public Health 2007***:** 167.
6. Sharma K, Gupta KK, Gaur A, Sharma AK, Pathania V, Thakur V. A cross-sectional study to assess the prevalence of early childhood caries and associated risk factors in preschool children in district Mandi, Himachal Pradesh. J Indian Soc Ped and Prev Den 2019; 37(4): 339-44.
7. Li Y, Wulaerhan J, Liu Y, Abudureyimu A, Zhao J. Prevalence of severe early childhood caries and associated socioeconomic and behavioral factors in Xinjiang, China: a cross-sectional study. BMC Oral Health 2017; 17:144.
8. Nguyen YHT, Ueno M, Zaitsu T, Nguyen T, Kawaguchi Y. Early childhood caries and risk factors in Vietnam. J Clin Pediatr Dent 2018; 42(3):173-81.
9. Ministry of Health Republic Indonesia. Basic Health Survey. Jakarta: Ministry of Health Republic Indonesia (2019). Available online at: http://www.depkes. go.id/resources/download/info-terkini/hasil-riskesdas-2018.pdf (accessed Januari, 2020)
10. Achmad H, Samad R, Handayani H, et al. JIDMR 2018; 11(1): 107-15.
11. Octiara E, Tamba EA. 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 2012; 17(1): 78-82.
12. Jayashree K, Ghan B. Early childhood caries: Epidemiology, aetiology, clinical features, prevention and management. EC Dent Scienc 2019;18(7): 1569-603.
13. Sathyaprasad S, Dhanya KB, Krishnamoorthy SH, George J. Evaluation of risk factor of early childhood caries in preschool children. Int J Curr Researc 2017; 9 (7):1-4.
14. Huong DM, Hang LTT, Ngoc VTN, et.al. Prevalence of early childhood caries and its relatedrisk factors in preschoolers: Result from a crosssectional study in Vietnam. Pediatr Dent J 2017; 30: 1-6.
15. Nobile CGA, Fortunato L, Bianco A, Pileggi C, Pavia M. Pattern and severity of early childhood caries in Southern Italy: A preschool-based cross-sectional study. BMC *Public Health* 2014;14: 206.
16. American Academy of Pediatric Dentistry. Guideline on fluoride therapy. Refer Man 2014; 37 (6): 15,16.
17. Moslemi M, Sattari M, Kooshki F, et.al. Relationship of salivary lactoferrin and lysozyme concentrations with early childhood

caries. J Dent Res Dent Clin Dent Prospect 2015; 9(2):109-14.
18. Maulitasari WA, Sutadi H, Fauziah E. Analysis of salivary lysozyme levels for the early detection of early childhood caries. JIDMR 2020; 13(4): 1523-1524.
19. Zalewska A, Knaś M, Szulimowska J, Waszkiewicz N, Wołosik K, Waszkiel D. Nonspecific immune factors in the whole unstimulated salivaof human infants, children and adolescents. Dent Med Probl 2013; 50(3): 291–7.
20. Svendsen IE, Lindh L, Arnebrant T. Adsorption behaviour and surfactant elution of cationic salivary proteins at solid/liquid interfaces, studied by *in situ* ellipsometry. Colloid and Surf B: Biointerf 2006, 53: 157–66.
21. Octiara E, Sutadi H, Siregar Yahwardiah, Primasari A. *Streptococcus mutans* antibacterial activity of 0.1% lysozyme tooth paste as an alternative for children below 3 years old in preventing Early Childhood Caries (ECC) (Experimental laboratory study). Dentika Dent J 2021; 2(24): 28-34.
22. Thakur AS, Acharya S, Singhal D, Rewal N, Mahajan N, Kotwal B. A comparative study of Mutans Streptococci and Lactobacilli in mothers and children with early childhood caries (ECC), severe early childhood caries (S-ECC) and caries free group in a low income population. OHDM 2014; 13(4): 1091-5.
23. Setiawan AS, Darwita RR, Susilawati S, Maharani DA, Djais AA. Biological factors in 2-3 years old children in determining risk factors of early childhood caries: Pilot study. JIDMR 2019; 12(2): 666-71.
24. Gudipaneni RV, Vijay KV, Jesudass G, Peddengatagari S, Duddu Y. Short term comparative evaluation of antimicrobial efficacy of tooth paste containing lactoferrin, lysozyme, lactoperoxidase in children with severe early childhood caries: A clinical study. J Clin and Diagn Resear 2014; 8(4): 18-20.
25. Pinheiro SL, da Rocha NN, Peres MHM. Capacity of hydroxyapatite-lysozyme combination againts *Streptococcus mutans* for the treatment of dentinal caries. J Conserv Dent 2016; 19: 465-8.
26. Kho HS, Vacca-Smith AM, Koo H, Scott-Anne K, Bowen WH. Interactions of *Streptococcus mutans* glucosyltransferase B with lysozyme in solution and on the surface of hydroxyapatite. Caries Res 2005; 39:411-6.
27. Wang YB, Germaine GR. Effect of lysozyme on glucose fermentation, cytoplasmic pH, and intracellular potassium concentrations in Streptococcus mutans 10449. Infect and Immun 1991; 59(2): 638-44.
28. Sood LI, Al-Ezzy MYH, Diajil AR. Correlation between *Streptococci mutans* and salivary IgA in relation to some oral parameters in saliva of children. J Bagh College Dentistry 2014; 26(1): 71-9.
29. Omar OM, Khattab NMA, Rashe LA. Glucosyiltransferase B, mmunoglobulin A, and caries experience among a group of Egyptian preschool children. J Dent for Child 2012; 79 (2):63-8.
30. Seow WK. Biological mechanisms of early childhood caries. Com Dent and Oral Epidemiol 1998; 26 (Supplement): 8–27.
31. Cvetkovic A, Ivanovic M. The role of *Streptococcus mutans* group and salivary immunoglobulins in etiology of early childhood caries. J Serbian Dent 2006; 53: 113-23.
32. Jenzano JW, Hogan SL, Lundblad RL. Factors influencing measurement of human salivary lysozyme in lysoplate and turbidimetric assays. J Clin Microbiol 1986; 24(6): 963-7.