Analysis of Salivary Lysozyme Levels for the Early Detection of Early Childhood Caries

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Abstract

Early Childhood Caries (ECC), one of most common chronic diseases affecting preschool children worldwide, affects 10.4% children in Indonesia. Saliva, which comprises lysozymes, protects the tooth structure, plays a role in the development of ECC, and could be a biomarker for ECC.

This study aimed to analyze salivary lysozyme levels in caries-free children and children with ECC and to determine its use as an indicator for early detection of ECC.

This cross-sectional study comprised 14 children with ECC and 14 caries-free children (aged 3–6 years). Unstimulated saliva (2 mL) was collected, and lysozyme levels were measured using ELISA. Mann–Whitney U test was used to evaluate statistical differences in salivary lysozyme levels between the ECC and caries-free groups. Spearman's correlation test was used to analyze the correlation between salivary lysozyme levels and dmft index.

The mean salivary lysozyme level in the ECC group (0.15 μg/mL) was significantly higher ($p = 0.04$) than that in the caries-free group (0.05 μg/mL). A significant moderately positive correlation ($r = 0.50$; $p = 0.01$) between dmft index and salivary lysozyme level was observed.

Salivary lysozyme levels in the ECC group were higher than those in the caries-free group.

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Introduction

Dental caries is a common, chronic multifactorial disease caused by specific bacteria, particularly *Streptococcus mutans*, and affects preschool children.[1,2] It is a major problem in many countries, including Indonesia.[3] According to the American Academy of Pediatric Dentistry, early childhood caries (ECC) is defined as the presence of one or more decayed, missing due to caries or filled tooth surface in a child aged 71 months or younger.[1] The prevalence of caries in Indonesia has increased over the years from 2007 to 2013;[3] it has reached 6.9% in children aged 1–4 and increased by 3.5% to 10.4%.[3] According to a survey conducted in 2000 by the Ministry of Health in Indonesia, the DMFT score continues to remain above 3, especially in Jakarta and Central Java.[4]

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Dental caries is a multifactorial disease due to the involvements of several factors such as microorganisms, the substrate, salivary proteins, and time.[5] However, the factor that plays a major role in the occurrence of dental caries is saliva.[4] Saliva has an important role in preventing dental caries due to its properties such as salivary flow, pH, buffering capacity, proteins, and defense mechanisms.[1] The saliva consists of molecules such as antimicrobial peptides (cathelicidin LL-37 peptide, statherin, alpha-defensin, beta-defensin, and histatin), major salivary glycoproteins (immunoglobulin, proline-rich protein [PRPs], and mucin), and minor salivary glycoproteins (lysozyme, lactoferrin, agglutinin, and cystatin) that are involved in the protection of the oral cavity.[6]

Lysozyme is a nonspecific antimicrobial salivary protein[7,8] that plays a role in protecting the teeth from bacterial and fungal attack in the oral cavity.[8] It is an antibacterial enzyme present in high concentrations in the serum, plasma, amniotic fluid, saliva, and tears; low concentrations of this enzyme have been detected in the urine.[1] Despite the low

concentrations of this enzyme, it is known to have significant biological activity.[3] Lysozymes play an important role in controlling bacterial overgrowth by reducing the bacterial count in the dental biofilm, inhibiting colonization, and modifying the metabolism of the bacteria.[7] Lysozymes kills bacteria via hydrolysis by breaking the β (1–4) glycosidic bond between *N*-acetylmuramic and *N*-acetylglucosamine on the bacterial cell wall (muramidase activity).[1,9]

Saliva can be used as a monitor of the local or systemic conditions in the human body.[10] A protein biomarker in the biological fluid can be used to provide information about the body's response to a condition or disease.[10] Biomarkers can be used as an early indicator of a disease, such as that in the oral cavity; for example, in a previous study, mucin-1, mucin-2, and acidic PRP-1 were found to be absent in individuals with high DMFT.[10] Lysozyme levels can be used as an important sign of the development of dental caries.[3] One study demonstrated that lysozyme levels were higher in the caries-free group than in the group with ECC.[3] Alternatively, another study demonstrated that increased levels of lysozyme were correlated with increased levels of *S. mutans* in oral cavity.[3] Owing to inconsistencies in the data on salivary lysozyme levels in children with ECC and in caries-free children, additional studies are required to explore this phenomenon.[11]

The current study was conducted to investigate the salivary lysozyme levels in caries-free children and use that as a measure for the early detection of ECC. We hypothesized that there is a significant difference in the levels between the groups of children.

Materials and methods

This study was approved by the ethics committee of the Faculty of Dentistry, Universitas Indonesia (protocol number 0501402019). Written informed consent was obtained from the parents. The study was conducted at the Taman Pengembangan Anak Makara and the Pediatric Dentistry Clinic at Universitas Indonesia from April to May 2019. In total, 28 children (14 with ECC and 14 caries free) aged 3–6 years were included in this cross-sectional study.

Children with dmft ≥ 1 were classified into the ECC group, and those with dmft = 0 were classified in the caries-free group. The exclusion criteria for the two groups were as follows: presence of systemic disease, use of medications, those who underwent radiotherapy, uncooperative children, and lack of consent by the parents of the child to participate in the study.

Before the collection of samples, children in both groups were instructed to rinse their mouths with water. The children were instructed to not swallow their saliva. Unstimulated saliva was collected in the morning, 1 hour after breakfast, from the sublingual region using a transfer pipette (3 mL; JetBiofil). A minimum of 2 mL of the collected saliva was poured into a centrifuge tube (15 mL; OneMed), stored in a cooler box, and immediately sent to the Oral Biology Laboratory at the Faculty of Dentistry, Universitas Indonesia. The samples were stored at −20°C until further analysis.

The samples were thawed at room temperature and centrifuged at 1500 × g for 15 min at 22°C. The saliva supernatant (25 μL) was collected and poured into a labeled microcentrifuge tube (1.5 mL, Biologix). All reagents were prepared, and salivary lysozyme levels were measured using Lysozyme ELISA kit (Elabscience, Human LZM [Lysozyme] ELISA kit, USA) protocol. The optical densities of the samples were measured at 450 nm using a microplate reader, and the lysozyme levels (μg/mL) were measured. The data collected from the ELISA experiment were analyzed using a statistical program (IBM SPSS Statistics). Shapiro–Wilk test was used to analyze the distribution of the data, and Mann–Whitney U test was used to measure the statistical differences in the salivary lysozyme levels between the two groups. A p value of <0.05 was considered statistically significant. Spearman's correlation analysis was used to analyze the relationship between the dmft index and the salivary lysozyme levels.

Results

The salivary lysozyme levels in the ECC and caries-free groups are demonstrated in Table 1. The mean dmft index in the ECC group was 7.57 (± 1.24). The Mann–Whitney U test

showed a significant difference (p = 0.04) in salivary lysozyme levels between the two groups (Table 2). The salivary lysozyme levels were higher in the ECC group than in the caries-free group. A moderately positive correlation (r = 0.50; p = 0.01) was observed between the dmft index and salivary lysozyme levels (Table 2). The sensitivity and specificity of the salivary lysozyme levels for ECC were 92.86% and 14.29%, respectively, when measured with the ELISA kit.

	ECC group	Caries-free group
Gender (% within gender)		
Male	7 (41.20)	10 (58.80)
Female	7 (63.60)	4 (36.40)
Age		
Mean ± SD (years)	4.14 (0.33)	3.57 (0.23)
dmft Index		
Mean ± SD	7.57 (1.24)	0

Table 1. Characteristics of the children in the early childhood caries (ECC) and caries-free groups based on gender, age, and dmft index.

SD, standard deviation.

Group	n	Median (Min-Max)	*P*
ECC	14	0.15 (0.05 – 1.02)	.04*
Caries-free	14	0.05 (0.01 – 0.37)	

Table 2. Differences in lysozyme levels (μg/ml) between the two groups of children.

ECC, early childhood caries; n, number. *, Mann–Whitney U test, $p < 0.05$.

Discussion

The aim of this study was to analyze the difference in salivary lysozyme levels between the ECC and caries-free groups. Furthermore, the use of salivary lysozyme levels as a biomarker of ECC was evaluated. The caries-free group included children aged 3–6 years without one or more decayed, missing due to caries, or filled tooth surfaces.[1] The selection of the children in the caries group was based on previous studies of higher prevalence of ECC in many countries, especially in Indonesia in 2007 and 2013 demonstrated increased number of caries prevalence.[3,4] The prevalence of caries in children aged 1–4 years was reported to have reached 6.9% and increased by 3.5% to 10.4%.[3] In another study, the prevalence of caries among children aged 3–5 years was reported to be 81.2% in Jakarta with a national DMFT index of 4.6 in 2013.[12]

According to the results of the ELISA assay, salivary lysozyme levels were higher in the ECC group compared to the caries-free group. According to a study by Hemadi (2017), the high levels of lysozyme in the ECC group were the result of a compensatory defense mechanism,[6,13] probably due to the presence of caries or due to high levels of *S. mutans*.[6] The protective mechanism in the oral cavity is stimulated through the secretion of lysozyme.[6] Lysozymes can activate autolysis of the bacteria and destroy the cell walls by aggregation and adherence.[14] Bai *et al* reported a possible correlation between increased lysozyme levels in unstimulated and stimulated whole saliva and ECC.[10]

Grychtol *et al* reported high levels of salivary lysozyme levels in children with ECC.[13] According to them, a high rate of caries is related to poor oral hygiene, which leads to increased levels of salivary lysozyme.[13] Similarly, Sharma *et al* demonstrated decreased amounts of the microorganisms in the oral cavity will lead to a decrease in the level of the salivary lysozymes.[2] Lysozyme is an antimicrobial enzyme that catalyzes the degradation of the negatively-charged peptidoglycan matrix in the microbial cell walls.[2]

Another factor related to ECC that contributes to increased levels of salivary lysozymes is poor oral hygiene resulting in gingivitis, especially in the interdental area.[13] The number of PMNs is increased in the gingival crevicular fluid due to gingival inflammation.[13] Lysozymes are found in azurophilic granules in PMNs and released into the saliva after stimulation or the lysis of the PMNs.[13]

Some studies have shown higher levels of salivary lysozymes in the caries-free group compared to the ECC group.[1,3] Bahhla *et al* demonstrated a decrease in the DMFT index as the levels of lysozyme increased.[1] Lysozymes exert protective and antimicrobial effects, thus playing an important role in caries prevention.[1] The levels of lysozyme in the current study were lower than those reported in another study.[3] This

discrepancy may be due to differences in the experimental conditions and methods.[11] In the present study, the sensitivity of salivary lysozyme for ECC was high, but the specificity was low. Additional studies are required to ascertain the use of salivary lysozymes as a biomarker for ECC.

Conclusions

Salivary lysozyme levels in children with ECC were higher than those in caries-free children. However, further studies on salivary lysozyme level and its relationship with ECC are required for salivary lysozyme level to be used as a biomarker for ECC.

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Declaration of Interest

The authors declare no conflict of interest.

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