Assessment of Lactoferrin Levels For The Detection of Early Childhood Caries

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Abstract

Early Childhood Caries (ECC) is one of the most common diseases that occur in children throughout the world. Salivary proteins such as lactoferrin (LF) can play an important role in the etiology and prevalence of oral diseases and in the development of dental caries. LF has demonstrated a strong action against Streptococcus mutans.

The aim of this study was to analyze the level of LF as a caries prediction test that can be used as a biomarker in children with ECC.

Unstimulated saliva was collected from 28 children (14 caries-free and 14 with ECC; age range, 3–6 years) using a disposable plastic pipette and inserted into a tube separator sample. The saliva samples (minimum, 2 ml) were taken from under the tongues of the children. The levels of LF in the saliva were measured via enzyme-linked immunosorbent assay (ELISA). Significant differences in LF levels between the children with ECC and the caries-free children were ascertained using the Mann Whitney U test.

The LF levels were significantly higher in the children with ECC compared to those without caries ($p = 0.006$).

This study shows that lactoferrin can be used as a biomarker to detect ECC.

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Introduction

One of the most common chronic infectious diseases in preschoolers is dental caries. It is characterized by damage to the dental tissues due to a complex synergistic effect between the acids produced from the fermentation of carbohydrates derived from food by bacteria and host factors such as the teeth and saliva.[1] The American Academy of Pediatric Dentistry describes Early Childhood Caries (ECC) as a condition where, there are one or more carious lesions (with or without cavities), missing teeth (due to caries), or fillings on the surface of each primary tooth in children under 71 months of age.[2-4,5] ECC is one of the most common diseases that occur in children throughout the world. The prevalence of ECC in Indonesia is high; 90% of children aged 3–5 years have caries with a dmft of > 6.[6] ECC affects not only the oral health but also the general health of the children.[7,8] Although not life-threatening, the impact on individuals and society is considerable, resulting in pain, impaired functions, and adverse effects on the growth rate, weight, and development, thereby reducing the quality of life.[9] Thus, early prevention and diagnosis of ECC are important. Recent studies have assessed the oral defense system and the risk factors for the prevention of ECC.[1]

Saliva, one of the innate defense systems of the human body, consists of organic and inorganic elements that are useful for maintaining the health of the oral cavity; additionally, it can provide protection to the teeth via various mechanisms, such as repairing of the enamel by remineralization, increasing the pH of the plaque, removing food debris, sugar, and microorganisms, and acting against the bacteria in the oral cavity.[1] It is important to maintain the levels of proteins and polypeptides in the saliva in order to maintain oral health and homeostasis because quantitative and qualitative changes in salivary proteins are often associated with enhancements in the frequency and severity of

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the oral disease.[1,10] Therefore, the composition of salivary proteins has an important role in the etiology and prevalence of oral diseases, including the development of dental caries.

Protein molecules, such as lactoferrin (LF) use its antibacterial effects to directly control the microbial flora in the oral cavity.[1] LF is a cationic glycoprotein that binds to iron. It can bind and kill bacteria through direct interaction, and has a strong action against *Streptococcus mutans*, viruses, parasites, and fungi; therefore, LF considered is to play a major role in the occurrence of caries.[1,11,12] In an in vitro study, the removal of *S. mutans* was found to depend on the size of the LF dose.[13] The study showed that LF can provide protection from *S. mutans* and can, therefore, be used for the prevention of dental caries. Another study, which focused on children aged 36–71 months, reported that LF concentrations were lower in children with ECC than in those without caries. In addition, the authors stated that salivary LF could be used to predict ECC because indications of a relationship between ECC and low unstimulated LF salivary concentrations was observed in their study.[14] The high incidence of ECC and the important role of salivary proteins as an antibacterial agent led to the study on LF levels for the early detection of ECC. Hao et al. detected lower levels of LF in caries-free children when compared to those with high dmft and proposed that it may be related to the prevention of caries in primary teeth.[15]

The aim of the present study was to analyze the levels of LF as a caries predictor that can be used as a biomarker in children with ECC.

Materials and methods

Ethical clearance for the study was obtained from the Faculty of Dentistry, University of Indonesia Ethics Commission (protocol number 050190219).

Subjects

A total of 28 children (14 caries-free and 14 with ECC) aged between 3 to 6 years were included in the study. ECC assessment was done using the dmft index. Children with systemic disease, who routinely consumed drugs, refused to participate in the study, were uncooperative, and those undergoing radiation therapy were not included in the study.

Saliva Collection

Saliva was collected in the morning between 8.00 and 9.00 am, 1 h after breakfast. The child was required to sit in a chair, rinse their mouth with boiled water and wait for 1 min before the salivary samples were taken. Unstimulated saliva was collected using a disposable plastic pipette and inserted into the tube separator sample. A minimum of 2 ml of saliva was collected from under the tongue using a long plastic pipette. The samples were then directly placed into a Sample Separator Tube and stored in a cooler box. Subsequently, the samples were then taken to the Oral Biology Laboratory, at the University of Indonesia, and stored at -20°C.

LF Expression Analysis

LF levels were detected by ELISA using the Human LTF/LF ELISA Kit (Elabscience, USA) as described previously.

Statistical Analysis

Data analysis was performed using. Significant differences in LF levels between the two groups of children were evaluated using the Mann Whitney U test with a significance level set at <.05.

Results

The research samples were collected at the Makara Daycare, University of Indonesia and Pediatric Dentistry Clinic, University of Indonesia, from April–May 2019. The Shapiro Wilk normality test was first performed on the data obtained; subsequently, the non-parametric Mann Whitney U test was used because the data were abnormally distributed (*P*<.05).

Group	n	Median (Min-Max)	*P*
Free Caries	14	0.64750 (0.165-3.822)	.006*
ECC	14	2.70650 (0.239-15.116)	

Table 1. Differences in lactoferrin levels in the caries-free children and in those with early childhood caries (ECC).
n, number, * *P* < 0.05

The average amount of LF in the caries-free children was 0.64750 µg/mL with a min-max value of 0.165–3.822 µg/mL, whereas in the children with ECC, the average LF level was

2.70650 µg/mL with a min-max value of 0.239–15.116 µg/mL (Table 1). Thus, the children with ECC presented with higher levels of LF (P = 0.006).

Discussion

The aim of this study was to analyze the levels of salivary LF in children with ECC and to use it as a biomarker of caries development. This research focused on Indonesian children aged 3–6 years. The sampling was performed at the Makara Daycare in a time-efficient manner; furthermore, all children at the daycare had the same eating habits, performed the same activities, and were from the same family background.

LF is a non-heme iron-binding protein (iron that does not form part of the coordination complex with porphyrin molecules [iron-binding molecules]) that which belongs to the family of transferrin proteins along with serum transferrin, ovotransferrin, melanotransferrin, and carbonic anhydrase inhibitors, which serve to carry iron in the blood serum.[1,12] Some of the physiological functions of LF include regulation of iron absorption in the intestine, immune response, protection against microbial infections, and exertion of antioxidant, anti-cancer, and anti-inflammatory properties.

As shown in Table 1, the levels of LF in the saliva of the children with ECC were higher than those in the caries-free children. This finding is in line with several previous studies, which showed that the amount of salivary LF was higher in children with ECC.[1] An in vitro study conducted by Velusamy SK *et al* showed that LF provides protection against the colonization of *Streptococcus mutans* and, therefore, can be used for the prevention of dental caries. The removal of *S. mutans* by human LF is dose-dependent.[13] Furthermore, Hao *et al* detected higher concentrations of LF in children with high dmft compared to those without caries; this may be related to caries prevention in the primary dentition.[15] Based on previous studies, it is known that LF has antimicrobial activity.[12] The incidence of caries is high in children with ECC; therefore, the body will respond by increasing the amount of LF as a defense mechanism against the disease.

However, other studies have stated that the level of LF in children with ECC was lower than that in the caries-free children. Masoumeh Moslemi *et al* showed that the LF levels in saliva were significantly higher in the caries-free group compared to the ECC group, thus indicating that the amount of LF in saliva is not dependent on caries in the oral cavity. Alternatively, ECC may have a relationship with lower and unstimulated concentrations of LF and may function as a risk factor for dental caries in children.[14] Inconsistencies in research design and the methods of saliva collection and analysis may account for the differences in the results of the various studies, these are the limitation of this study. Therefore, further research is needed to clarify the role of LF in children with ECC.

Conclusions

In this study, LF levels were significantly higher in the children with ECC than in the caries-free children. Thus, we believe that LF can be used as a biomarker to detect ECC.

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Declaration of Interest

The authors report no conflict of interest.

References

1. Hemadi AS, Huang R, Zhou Y, Zou J. Salivary Proteins and Microbiota As Biomarkers For Early Childhood Caries Risk Assessment. Int J Oral Sci 2017;9(11):e1.
2. Alazmah A. Early Childhood Caries: A Review. J Contemp Dent Pract 2017;18(8):732-37.
3. Dye BA, Hsu KL, Afful J. Prevalence and Measurement of Dental Caries in Young Children. Pediatr Dent 2015;37(3):200-16.
4. Colak H, Dulgergil CT, Dalli M, Hamidi MM. Early Childhood Caries Update: A Review of Causes, Diagnoses, and Treatments. J Nat Sci Biol Med 2013;4(1):29-38.
5. Meltharyna M, Suharsini M, Sutadi H. Effect of Different Types of Milk Formula Against Streptococcus Mutans Biofilm Formation In Vitro. Asian J Pharm Clin Res 2017;10(5):34-37.
6. WHO Expert Consultation on Public Health Intervention Against Early Childhood Caries. Bangkok, Thailand; 2016.
7. Naidu R, Nunn J, Donnelly-Swift E. Oral Health Related Quality of Life and Early Childhood Caries Among Preschool Children In Trinidad. BMC Oral Health 2016;16(1):128.
8. Meyer F, Enax J. Early Childhood Caries: Epidemiology, Aetiology, and Prevention. Int J Dent 2018:1-7.
9. Zafar S, Harnekar SY, Siddiqi A. Early Childhood Caries: Etiology, Clinical Considerations, Consequences and Management. International Dentistry SA 2009;11(4):24-36.

10. Dawes C, Pedersen AM, Villa A, et al. The Functions of Human Saliva: A Review Sponsored by The World Workshop on Oral Medicine VI. Arch Oral Biol 2015;60(6):863-74.
11. Fine DH, Toruner GA, Velliyagounder K, et al. A Lactotransferrin Single Nucleotide Polymorphism Demonstrates Biological Activity That Can Reduce Susceptibility to Caries. Infect Immun 2013;81(5):1596-605.
12. Gonzalez-Chaves SA, Arévalo-Gallegos S, Rascón-Cruz Q. Lactoferrin: Structure Function and Applications. Int J Antimicrob Agents 2009;33(4):301.e1-8.
13. Velusamy SK, Markowitz K, Fine DH, Velliyagounder K. Human Lactoferrin Protects Against Streptococcus Mutans-Induced Caries in Mice. Oral Dis 2016;22(2):148-54.
14. 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 Prospects 2015;9(2):109-14.
15. Hao GF, Lin HC. Relationship of Concentration of Lactoferrin and Lysozyme in Saliva and Dental Caries in Primary Dentition. Zhonghua Kou Qiang Yi Xue Za Zhi 2009;44(2):82-4.