Relationship between CD14 and IgA Levels with the Early Childhood Caries Event Children Age 3–5 Years

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Abstract

BACKGROUND: Early childhood caries (ECC) is a problem in the field of dental health that occurs in infants and toddlers around the world.

AIM: This study aims to relationship between CD14 and IgA levels with the ECC event children age 3–5 years.

MATERIALS AND METHODS: This type of research is an observational cross-sectional comparative design between children with caries and non-caries. Samples are children aged 3–5 years with a history of consuming bottle milk. The number of samples was 28 people for each group (caries and non-caries) so that the total sample was 56 people. The CD14 and IgA levels measured using the ELISA method. The statistical test used is an independent t-test. p < 0.05 was said to be statistically significant.

RESULTS: The results showed that the mean of CD14 levels was higher in the group caries than in the non-caries, while IgA levels were higher in the group non-caries than in the caries group.

CONCLUSION: This study concluded that early caries childhood caries event children age 3–5 years increased the levels of IgA and decreased CD14 levels.

Introduction

Early childhood caries (ECC) is a problem in the field of dental health that occurs in infants and toddlers around the world [1]. The negative impact of caries on children can be functional changes such as chewing and speaking disorders, psychological disorders such as irritability, disruption of learning processes, and disruption of children's social interactions due to restraint when smiling and talking [2]. This also disrupts the child's growth and development because the incidence of this disease is also increasing from year to year and continues to develop into a pandemic throughout the world [1, 3, 4].

The prevalence of ECC in developing countries such as in the Khartoum City of Sudan in children aged 3–5 years is 52.4% [5]. The prevalence of ECC in children aged 3–5 years in the city of Bengaluru, India, is 24.39% [6]. Developed countries such as England, Hong Kong, Qatar, and Germany have an ECC prevalence of 27.9%, 55%, 89.2%, and 10% [7, 8].

Risksedas in 2013 reported 10.4% of children aged 1–4 years experienced dental and mouth problems, but only 25.8% received treatment [9]. The prevalence of ECC in children under 3 years old in DKI Jakarta was 52.7% with a deft score (decay extraction filling – teeth) average 2.85 [10]. The prevalence of caries in children aged 2–5 years in the city of Yogyakarta, Indonesia, reaches 76.5% [11]. Dental caries is an infectious disease caused by interactions between bacteria, such as Streptococcus mutans, Actinomyces sp., and Lactobacillus sp. with food in tooth enamel [1]. S. mutans is the main bacterium found in ECC children and plays an important role in caries development [12].

The American Academy of Pediatric Dentistry in 2014 stated that the frequency of consuming liquids containing fermented carbohydrates such as juice, milk, and soda can increase the risk of caries [13]. Bottled milk consumption at night is the most powerful ECC risk factor. The use of bottle milk at night increases the risk of caries because the rate of salivary flow decreases during sleep, which causes the cleaning of the oral cavity by saliva also is low [14].

Saliva has a variety of biomolecules, especially proteins that affect the survival of oral microorganisms through innate defensive mechanisms that modulate oral microflora [15]. More than 1400 salivary proteins have been identified, some of which are immunoglobulins, acidic proline-rich proteins, mucins 1 e 2 (mucous glycoproteins) agglutinin, lactoferrin, and lysozyme, cystatin S, and statherin, defensin,
CD14, glucosyltransferase, and amylase. The protein along with the glycoprotein contained in saliva protects oral tissue. Changes in salivary protein can result in changes in flora in the oral cavity that can lead to caries formation [16]. The absence of CD14 in saliva can be a potential biomarker of caries [17].

Salivary protein which also acts as the immune system in the oral cavity is immunoglobulin [15]. There are three subclasses of immunoglobulins found in saliva, namely, IgA, IgG, and IgM. IgA is the most common immunoglobulin in saliva (about 60%) [18]. The salivary secretory immunoglobulin A (sIgA) can bind specifically to the epitope of S. mutans section, so bacteria cannot bind to the salivary pellicle and S. mutans will not colonize the salivary tooth surface [15]. There is a significant relationship between the amount of IgA and the incidence of caries in children [18]. The high concentration of sIgA in children with ECC is associated with increased antigen exposure, thereby triggering high antibody production [15].

Based on this background, this study focused on one of the main factors in the caries process, namely, the host factor by analyzing the levels of sCD14 and sIgA saliva in the population of children with ECC as an effort to develop indicators of prediction of caries in children aged 3–5 years who have the habit of drinking bottled milk.

Materials and Method

Samples

This type of research is an observational cross-sectional comparative design between children with caries and non-caries. Samples are children aged 3–5 years with a history of consuming bottle milk. The number of samples was 28 people for each group (caries and non-caries) so that the total sample was 56 people. Samples were taken from saliva conservatively carried out in the morning around 8.00–10.00 with the aim as time to queue. At that time, it is expected that the state of the mouth is clean because the child has eaten breakfast and has not had lunch.

Saliva sampling

Samples were taken from saliva and plaque conservatively conducted in the morning around 8.00–10.00 with the aim as an intermediate time. At that time, it is expected that the state of the mouth is clean because the child has eaten breakfast and has not had lunch. Plaque collection is done using a sterile Copan swab, in the form of a long cotton bud, to avoid the child’s fear of plaque collection. Besides Copan was chosen because it is guaranteed sterilization as long as the Copen stalk is not touched and its application is very easy. The assessment of caries determination is based on the WHO deft criteria.

Measurement of CD14 by ELISA

All reagents were prepared and samples were placed at room temperature before use. Then, 50 µL CD14 is added to the standard well. After that, the samples were put into 40 wells for each well and added 10 µL of anti-ER antibodies and 50 µL of streptavidin-HRP into the sample wells and standard wells. Then, the well is covered with seal and incubated for 60 min at 37°C. After that, wash the well 5 times using wash buffer. Then, 50 µL of substrate A solution was added to each well and incubated again for 10 min at 37°C in a dark room. Next, 50 µL stop solution is added to all the wells and measure the absorbance value using a wavelength of 450 nm (product by Bioassay Technology Laboratory, Shanghai, China).

Measurement of sIgA by ELISA

All reagents were prepared and samples were placed at room temperature before use. Then, the saliva sample is diluted at a ratio of 1:5 in the saliva thinner. After that, transfer the sample, control and standard into a 1.5 mL sterile tube and add 4 mL of sIgA diluent into each tube. Next, add 50 µL of the enzyme conjugate antibody into each tube. Then, incubate for 90 min at room temperature. Subsequently, samples and controls and standards were transferred into the well and incubated again for 90 min while in the incubator at 400 rpm. After that, all wells are washed 6 times using wash buffer. Subsequently, 50 µL TMB substrate was added to each well. Leave 5 min on the shaker incubator and incubate for 40 min in a dark room. Next, add 50 µL stop solution and stir for 3 min. Then, the absorbance is measured with a wavelength of 450 nm (product by Salimetrics, LLC, USA).

Statistical analysis

Data are presented in mean and elementary forms. The statistical analysis used is SPSS 16.0. The statistical test used is an independent t-test. p < 0.05 was said to be statistically significant.

Results

Table 1 shows that the difference between caries and non-caries from CD14 (Figure 1).

Table 1: Differences in mean levels of CD14 by group

| Group   | CD14 levels (ng/ml) (Mean ± SD) |
|---------|---------------------------------|
| Caries  | 0.60 ± 0.03                     |
| Non-caries | 0.59 ± 0.24                   |
Table 2 shows the difference between caries and non-caries from IgA (Figure 2).

| Group   | IgA levels (ng/ml) (Mean ± SD) |
|---------|-------------------------------|
| Caries  | 1.04 ± 47.35                  |
| Non-caries | 80.62 ± 22.08               |

Discussion

The pathogenesis of caries involves the role of dental plaque that begins with adhesion of salivary proteins to the tooth surface and is followed by bacterial colonization. These bacteria convert sugar into energy which causes an acidic environment in the oral cavity resulting in demineralization of tooth enamel and cause dental caries. Saliva plays a role in the pathogenesis of caries, namely, in the process of formation of S. mutans colonies. Saliva plays a role as a defense system against bacterial infections and maintains a balance between demineralization and remineralization [19].

Dental caries is a term that describes a signs, symptoms, and side effects due to chemical imbalance from the tooth surface (enamel and dentin) caused by dental plaque and mediated by saliva [20]. The etiology of ECC is not much different from caries in permanent teeth, but the intake foods or drinks that are high in sugar are one of the strong factors against ECC [21].

Saliva plays a role in maintaining oral health and homeostasis because it has immunological and non-immunological content [16], [17]. Every change in salivary protein composition plays an important role in the etiology of oral disease and development of dental caries [17]. The immune system in children develops with age and becomes more sensitive to various microbiota and different antigens [16].

sCD14 is a glycoprotein that functions in lipopolysaccharides (LPS)/cell wall product signaling by controlling the level of the immune system response [17]. Low levels of sCD14 because specific immune responses are still not fully developed in children aged 3–5 years, an increase in sCD14 levels in saliva may be a response of the immune system which are reparative to inadequate immunoglobulins such as IgA and IgG [22].

Differences in sCD14 levels in the ECC and caries-free groups indicate the role of the innate immune system in the oral cavity. sCD14 plays an important role in the initiation of the immune response by recognizing several microbial products, such as LPS, endotoxins, and peptidoglycan, which are the main components of Gram-positive and Gram-negative bacteria [16]. SCD14 levels can be used as caries biomarkers and help in monitoring or evaluating effectiveness of karyostatic treatment [23].

CD14 is present on the surface of monocytes, macrophages, and neutrophils, through the glycosylphosphatidylinositol anchor, and is present in the soluble form plasma, sCD14. The main salivary gland secretes sCD14 into saliva. sCD14 acts as an important anti-cariogenic factor. This allows binding between epithelial cells and bacteria and activates the production of cytokines for phagocyte recruitment. This protein mediates activation of endothelial and epithelial cells, which are CD14-negative cells [24].

sIgA is the protein most commonly found in saliva. Antibodies in saliva are the first line in the immune system to fight antigens [25]. Salivary IgA secretions are believed to be factors that protect dental caries by controlling the growth of cariogenic oral microflora, preventing pathogenic adhesion, and activation of enzymes and toxins from bacteria [26]. Increased levels of sIgA in caries patients are an immune system response as a protective mechanism against the process of dental caries and S. mutans are active in the oral cavity [18].

Neutrophils in saliva are the first defense cells against pathogenic microbes. Neutrophils can...
recognize surface bound or free molecules secreted by bacteria including S. mutans such as peptidoglycan, lipoprotein, lipeiteichoic acid, LPS, CpG-containing DNA, and flagellin [23]. Neutrophils in the area of infection bind and swallow microbes through a process which is called phagocytosis. Interleukin-8 is a type of cytokine that acts as a mediator in the migration of neutrophils to sites of inflammation and infection [27].

Two main classes of antibodies present in saliva, namely, IgA (sIgA) and IgG, are produced as IgA dimmers by local plasma cells in the salivary gland stroma. Then, these antibodies are carried by the epithelium and are secreted by the polymeric immunoglobulin receptor which is also called a secretory membrane component. IgA polymers are produced by plasma cells in the lamina propria of the oral mucosa. IgA polymers bind to immunoglobulin receptors on the surface of epithelial cells, then by endocytosis, the complex IgA receptor passes through the cellular compartment before being released to the surface of epithelial cells. Then, there is proteolysis of the receptor, and the dimmer IgA molecule and free secretory components spread throughout the mucosa including the saliva [28], [29].

Conclusion

This study concluded that early caries childhood caries event children age 3–5 years increased the levels of IgA and decreased CD14 levels.

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