sIgA and Lisozim as Biomarker of Early Childhood Caries Risk

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Abstract—Caries in children under 6 years old (Early Childhood Caries) is still a major oral health problem in many countries. The high prevalence of ECC should be prevented immediately based on the cause of caries. Saliva is one of the factors that play role in dental caries process, also play a role as caries prevention. This article describes the relationship between sIgA and salivary lysozyme to the incidence of ECC and the development of that protein as one of the caries biomarkers. Several studies reported that sIgA in caries-free children had higher concentrations than in ECC children. Lysozyme concentrations in unstimulated saliva are also significantly higher in the caries-free group compared with the ECC group. Determination of lysozyme and sIgA level in saliva may be helpful in diagnosing caries diseases and infections. It is concluded that the development of sIgA and salivary lysozyme can be used as one of the biomarkers for dental caries detection, risk assessment, diagnosis, prognosis and disease monitoring, and evaluation in ECC.

Keyword – ECC, sIgA, salivary lysozyme, biomarker

I. INTRODUCTION

Dental caries is still a major oral health problem in many countries including Indonesia. The unique caries lesions in infants, toddlers and preschoolers children are called Early Childhood Caries (ECC). The presence of carious lesions on the surface of deciduous teeth (cavity or not cavity), teeth lost by caries or restored teeth in children under 6 years old [1].

ECC epidemiological data from various countries shows varying numbers. In America, ECC incidence in 1 years old children has high prevalence which is 21% and increases in preschoolers age 5 years old by 75% [1,2]. Study in India found that the prevalence of ECC varied by 27.5% [3] to 56.6% [4], while in Northern Thailand, it has higher prevalence which is 68.5% [5].

National data in 2007 and 2013 found that there was an increased in the caries prevalence in Indonesian children, it was reported that caries prevalence in children age 1-4 years old was 6.9% [6], and increased by 3.5% to 10.4% [7]. Prevalence of ECC in children 3 years old and under in Surabaya and Medan was higher than the national data in Surabaya that reported 30.8% [8], while in Medan it was found higher by 57.7% [9].

The high prevalence of ECC in various countries must be prevent immediately based on the caries etiology. Caries occurs not because of one event but due to a series of processes over several periods, therefore caries is expressed as a multifactorial disease. There are three main factors that play role in caries disease which are host factors (teeth and saliva), agents (microorganisms) and substrates or diet and added with time factor. Caries will occur when each factor is mutually supportive [10].

Saliva is one of the factor that play role in dental caries process, also play a role as caries prevention [11]. Therefore, it is possible that saliva is used as a biomarker to assess a person’s caries risk. Biomarkers serve to detect, risk assessment, diagnosis, prognosis and disease monitoring and health evaluation [12,13]. Biomarkers can be antibodies, microbes, DNA, RNA, lipids, metabolites and proteins. The change in concentration, structure, function, or action in analyte can be attributed to the onset, developmental regression of a particular disorder or the outcome of how the body responds [14]. Biomarkers are assessed through quantitative tests to determine specificity, sensitivity and reproducibility [13].

Increased use of saliva as a non-invasive diagnostic tool has been developed recently. Non-invasive methods will avoid patient’s discomfort or fear in detecting the disease, because in sampling does not cause any pain. Besides economical, saliva is easily collected, delivered, and stored so that there is a decrease in overall costs for patients and healthcare providers [15].

Saliva proteins that can be used as dental caries biomarkers which serve as immune and nonimmune antimicrobial factors [16]. The main salivary immune factor is secretory IgA (sIgA) [17], while non-immune factors in saliva include lysozyme, lactoferrine, salivary peroxidase, aglutinin, histatine, cystatine, secretary
leucocyte protease inhibitor (SLPI), defensine-2, chromogranine A, cathelicidine, mucine, and prolin-rich glycoproteins [18]. Lysozyme is one of innate immune saliva and body's first defense and prominent in the oral cavity [18,19].

Salivary immunoglobulin is formed due to antigen microorganisms in the oral cavity. The sIgA anti-microorganism interacts with antibody bonds on specific antigens in the bacterial cell wall resulting in bacterial-leukocyte agglutination [20]. Research in children age 4-8 years found that the caries-free group had the highest total sIgA compared to control group with caries experience 1-5 teeth and 6-10 tooth [21].

The literature report shows that the determination of lysozyme levels in body fluids may be helpful in diagnosing several diseases such as atherosclerotic cardiovascular [22] and infections such as periodontal disease [23] as well as micro vascular trauma in smokers’ lungs [24]. Lysozyme also can be used as an important marker for the diagnosis and development of dental caries. Study found that there was an association between lysozyme value and the number of S. mutans and lactobacillus [25]. Other studies reported that lysozyme concentration in unstimulated saliva was significantly higher in the caries-free group compared with ECC group [26].

The purpose of this article is to present the relationship between sIgA and salivary lysozyme with the incidence of ECC and the development of sIgA and saliva lysozyme as one of the biomarkers for detecting, risk assessment, diagnosis, prognosis and disease monitoring and evaluation of early childhood caries (ECC).

II. LITERATURE REVIEW

A. Secretory immunoglobulin a and the relationship with ECC risk

sIgA is synthesized by B lymphocyte cells located around the secretory epithelium. Once secreted in the interstitial fluid, sIgA it is taken by acinar cells and salivary gland duct cells and then secreted into saliva [18]. The structure of sIgA is dimer [27], because two molecules of IgA (weight 300 kDa) are bonded into one complex by J chain binding protein (Joining Piece, weight 15 kDa). In addition, this complex has a protein in the form of a secretory component (secretory component, weight 70 kDa) that serves to stabilize the molecule and reduce susceptibility to proteolytic enzymes [28].

Salivary immunoglobulin is formed due to an antigen microorganisms in the oral cavity. The sIgA anti-microorganisms are able to interact by antibody bonds on specific antigens in the bacterial cell wall resulting in bacterial agglutination. Bacterial agglutination causes bacteria cannot make colonies again in the oral cavity and the results will be transported to the stomach. In addition, microorganisms that are covered by sIgA are more easily phagocytosed by leukocytes [20,29]. Another mechanism of sIgA protection against oral microorganisms is that the sIgA anti-microorganisms will mask the adhesion of microorganisms or antigen molecular receptors so that they cannot attach to salivary aglutinin glycoprotein receptors (SAGs) on tooth surfaces [30]. The sIgA is also capable to neutralize extracellular enzymes produced by microorganisms [31]. This can cause the microorganisms not attached to the tooth surface [29].

IgA has not yet been discovered in baby after birth. It is secreted by a month-old infant, that subsequently undergo maturation process from mucosal immune response that occurs at the end of one years old [28]. In child’s saliva, the sIgA concentration contains half of the sIgA concentration in adult and this value will remain low until the end of the first year or even past that age [20]. Khan et.al [29] showed that age affect changes in sIgA concentration. The mean of sIgA was higher in individuals age 11-20 years old (7.47 ± 1.48 mg/dl) than children age 1-10 years old (5.20 ± 0.94 mg/dl), but the mean of sIgA will decrease at individuals age 61-70 years old [17].

Saliva’ immune response to streptococci mutants showed an individual characteristics in childhood. Each child responds differently to streptococci mutants infection, a condition that results from an extension of infection (antigen dose) or age at the time of infection (maturation of the immune response) [32].

Antibodies to S. mutans can be detected in saliva, but their relationship with immunity to caries is still a contradiction. Research in children age 4-8 years old found that the caries-free group had highest total of sIgA compared to groups of children with caries experience 1-5 teeth and 6-10 tooth [21]. Same results were also reported by Pal et al. [33], that the highest total sIgA in caries-free group were 213.67 ± 28.17 μg/ml, followed with moderate caries children (1-4 teeth) 189.13 ± 26.74 μg/ml and lowest in children with high caries (5-15 teeth) which was 144.13 ± 20.85 μg/ml.

Different results reported that there was no difference in total sIgA concentrations in caries-free children with children having caries experience≥ 5 [27]. Bagherian & Asadikaram [34] reported the opposite results that the highest total sIgA concentration was owned by ECC children (196.14 ± 100.07 mg/dl) than in caries-free children (148.45 ± 81.26 mg/dl; p=0.015).

From several studies, it can be concluded that there was a relationship between sIgA concentration and caries incidence [21, 33, 34], therefore sIgA concentrations could be developed as an ECC risk assessment biomarker.

B. Salivary lysozyme relationship with ECC

The lysozyme (or muramidase) (EC 3.2.1.17) is a small protein (14.6 kDa) with isoelectric points (PI) 10-11 and is cationic [35]. Lysozyme can be divided into several types based on the sequence of similarities and its three dimensional structure: 1). Type c (chicken-type lysozyme); 2). Type g (goose-type lysozyme); 3). Type i (invertebrate-type); 4). T4 phage lysozyme (phage-type); 5). Bacterial lysozyme; 6). Plants lysozyme. Human lysozyme is included in type c [36].
Human lysozyme has 130 single polypeptide chain amino acids [36]. There are four disulfide bridges that serve to stabilize the molecule in a compact ellipsoidal form [37]. Human lysozyme genes are the LYZ gene, with the length of the gene structure is 5856 bp. The lysozyme gene is a small gene with 4 exons structure and 3 sequence interventions that is very similar to the chicken lysozyme genes. The main differences between human and chicken lysozyme genes is in the size of introns and 3 non-coding regions [38].

Human lysozyme is produced by monocytes, neutrophils, Paneth cells and salivary glands [39,40]. Lysozyme is a non-specific immune cell response, and lies between blood system cells especially leukocytes [37].

The lysozyme enzyme has a small concentration in the salivary glands, but has significant biological activity [18]. Lysozyme concentration in saliva is reported vary from 2 to 60 µg/ml. The fluid in dental plaque is reported to contain up to 15 times the amount of saliva lysozyme [41]. Lysozyme concentration in breast milk is about 100 µg/mL, and this concentration will increase at the end of lactation [40].

Lysozyme acts as an antibacterial, antiviral, and antitumor and immune modulator activity [42]. Lysozyme activity as antimicrobial relates to its ability in lytic action in bacteria [43] by hydrolyzing β (1-4) bonds between N-acetylmuramic acid and N-acetylglucosamine in peptidoglycan layer of bacterial cell wall [26]. Glycosidic bond hydrolysis results will cause the occurrence of small pores inside bacterial cell wall so that bacteria will die. Lysozyme primarily hydrolyze cell walls of Gram-positive microorganisms, such as S. mutans [44]. Gram-negative bacteria are generally much less sensitive to lysozyme because the murein content in the cell wall is only 10%, whereas in Gram-positive it is 50% [18].

Lysozyme as a strong cationic protein, can also mediate bacterial aggregation and inhibit bacterial attachment and also activate bacterial autolysis by destroying bacterial cell walls [26,43]. The lysozyme activity will survive when the enzyme is absorbed in the pellicle [45]. The absorption of cationic molecules against bacterial walls is highly dependent on pH and ionic strength [43].

The concentration of saliva lysozyme in humans varies with age. A longitudinal study by Hyypp et al. [46] found that the concentrations of lysozyme did not differ between pre-dente groups (age 2-6 months, mean age 4.3 months) with dentate phase group (12-19 months, mean age 12.7 months). Lysozyme concentrations in these two groups of children were lower than in the adult group (age 21-31 years, mean age 23.3 years).

Researcher de Andrade FB et al. [19] found that bactericidal and bacteriostatic effects of lysozyme against S. mutans were 68.5 mg/mL (MBC concentrations) and 58.7 mg/mL. Other studies found that concentrations of lysozyme required to inhibit biofilm formation is 50-200 µg/mL [47].

Development of salivary lysozyme as one of ECC risk assessment biomarkers can be done, Mass et al. [25] had found a link between lysozyme value and the number of S. mutans. Other investigators reported that the concentration of unstimulated salivary lysozymes in ECC children was higher than in caries-free children [48]. Similarly, Letsirivorakul et.al. [49] obtained similar results, unstimulated saliva sample taken from 32 caries-free children and 32 children with caries showed the concentration and lysozyme activity in children with caries were higher than in caries-free children (p = 0.008). Different results reported by Felizardo et.al [50] that found lysozyme unrelated to the caries experience. Hao & Lin’s study [51] also states the same, there were no differences in lysozyme concentrations between caries groups and children have caries (dmft> 5).

In a study of 21 children age 36-71 months caries-free compared to 21 children with ECC, the concentration of lysozyme in unstimulated saliva was significantly higher in the caries-free group compared with the ECC group (p = 0.04). The study also evaluated 3 months later, after all carious teeth performed dental treatment. There was no difference in lysozyme value between baseline values before dental treatment compared with after dental treatment, although substantially higher lysozyme value was in after dental treatment than baseline [26]. Bhalla's study [52] also obtained a DMF-T index decreasing as the concentration of lysozyme increased.

III. DISCUSSION

The study of total slgA concentrations associated with caries occurrences has been widely reported, and more recent research that specify slgA on S. mutans has been widely developed. Research has reported a tendency of higher intensity and a more complex pattern of serotype c anti-S. mutans responses in low caries individuals compared with caries-free individuals [53]. Other literature states that higher serotype c anti-S. mutans response in caries-free individuals than individuals with active caries [20]. SlgA response to low effect of anti-serotype c S. mutans in caries development is not very clear, it may be due to a wide range of specificities slgA against the antigen virulence of microorganisms [54].

S. mutans serotype c is reported to be most commonly about found serotype which is 70-80%, followed by serotypes e, f and k [55]. In Finnish children samples found 75-90% S mutans with serotype c, 10-20% serotype e, and few serotypes f [56]. In various studies it was found that serotype f is rare in individuals [57,58,59]. However, research in Jakarta in children age 3-5 years old, found that serotype f is more dominant bacteria that is equal to 91%, followed by serotype c 79% and serotype e of 29% [60]. The study reported that children with dental caries had a higher anti-S. mutans serotype f than a caries-free child, although statistically this difference was not significant [61]. Serotype f had possibility to plays a role in the incidence of caries at an early childhood.
Lysozyme antibacterial effect depends on the lysozyme concentration and activity in saliva. Results reported that many studies try to relate lysozyme concentrations with ECC events, but lysozyme activity level may be more relevant to explain the defensive role of the protein. Research that distinguishes between active and inactive lysozymes in saliva will yield better results than simply describe concentrations [62].

The type of lysozyme used in the biomolecular examination may also affect a study result. Iacono et al. [63] found that human lysozyme 3–4 times more reactive than hen egg white lysozyme (HEWL). HEWL is very similar to human lysozyme and has 129 amino acids (14.3 kDa) [36]. HEWL is not expensive and it has good correlation to human lysozyme, therefore HEWL is often used in research as a substitute for human lysozyme. This is likely to lead some researchers to report that most microorganisms are not susceptible to lysozyme. This is effective against streptococci [63].

Lysozyme抗菌活性依赖于唾液中溶菌酶的浓度和活性。结果表明，许多研究试图将唾液溶菌酶浓度与早期儿童龋病事件相关联，但溶菌酶活性水平可能更有利于解释其防御性作用。研究区分了活性和非活性唾液溶菌酶在唾液中的作用，这将提供比单纯描述浓度更好的效果 [62]。

在生物质分子中，溶菌酶的类型也会影响到研究结果。Iacono等[63]发现人溶菌酶与蛋清溶菌酶（HEWL）之间存在3–4倍的活性，HEWL与人溶菌酶相似，有129个氨基酸（14.3 kDa）[36]。HEWL不贵且与人溶菌酶有良好的相关性，因此经常被用作代替品。这可能会导致一些研究者认为大多数微生物对溶菌酶不敏感。这种活性对链球菌有效 [63]。

研究指出，最敏感的HEWL与人类溶菌酶和有129个氨基酸相似，有129个氨基酸，因此被用作DAC的替代品。这可能会导致一些研究者认为大多数微生物对溶菌酶不敏感。这种活性对链球菌有效 [63]。

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