Mutans Streptococci counts from saliva and its protein profile in early childhood caries

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Abstract: *Aim:* This study aims to analyze the number Mutans Streptococci (MS) and its protein profile from the saliva of early childhood caries (ECC) and caries-free subjects. *Methods:* MS counts were cultured from saliva samples, and the protein profile of MS was determined from ECC and caries-free subjects. The number of colonies were counted, and the protein bands with the molecular weight of 13, 29, 39, 41.3, 74, and 95 kDa were determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis method. *Results:* We found that the number of colonies from saliva of ECC patients was higher than those caries-free (22.20×106 CFU/ml vs. 19.16×106 CFU/ml, $p < 0.05$). There are higher expression frequencies in protein 29, 39, 41.3, and 74 kDa of MS in ECC than caries-free subjects. *Conclusions:* There is the higher number of MS colonies and difference of MS protein profile isolated from saliva among children with ECC and caries-free counterparts.

Keywords: Mutans Streptococci, protein profile, saliva, early childhood caries, dmft

Introduction

Early childhood caries (ECC) is defined as a condition of one or more of decayed, missing, and filling in teeth (dmft) of children aging less than 71 months or even younger [1]. Prevalence and severity of ECC is still high in some countries. According to Indonesian Basic Health Survey (RISKESDAS) in 2013, national dmft index in Indonesia reaches 4, 6 [2]. In 1988, the prevalence of ECC at preschool age in DKI Jakarta and its surrounding area was 85.17%. The definition of ECC is the presence of one or more decayed (non-cavitated or cavitated lesions), missing (due to caries), or filled tooth surfaces in any primary tooth in a child under the age of 6 years [3]. This condition will lead to a decrease in children's health quality, where ECC can decrease the chewing ability of the children, so that it can interfere in growth and development of children [4, 5].

ECC is a multifactorial disease that occurs due to host, environment, and microorganism as the etiology factors [5, 6]. Mutans Streptococci (MS) is the main microorganism that causes ECC. In the past decade, several researches conducted in the relationship between the occurrence of caries and the presence of salivary MS [7]. Saliva may transmit the bacteria and plays a role as a reservoir for the colonization of the bacteria. If the cariogenic bacteria predominate in saliva and plaque, it will increase the acids, which are produced by them through the fermentation process of carbohydrate. This will also increase the colony of the bacteria and start creating virulence biofilm on tooth surface by quorum-sensing mechanism [8].

One of the main factors of MS virulence is the ability to produce glucan synthesized by glusyltransferase, which mediated microorganism attachment to the tooth surface along with other protein, such as I/II antigen 185, PAc 190, GbpA 74, GbpB 41,3 kDa, etc. The proteins affect biofilm formation and increase caries activity in children (ECC) [9]. Hence, we are interested to observe the difference of MS amount and protein profile isolated from saliva in ECC subjects compared to caries-free children.

Materials and Methods

This study obtained ethical approval from ethical research committee of Faculty of Dentistry, Universitas Indonesia (no.: 24/Ethical Approval/FKGUI/V/2017). An examination was carried out by measuring the number of dmft teeth of children.

MS was isolated from saliva sample on the floor of the mouth, mandibular buccal, and labial vestibular using transfer pipette. The saliva samples were collected in 1.5 ml microcentrifuge tube, which contained phosphate-buffered saline and 0.01 mM phenylmethylsulfonyl fluoride. The bacteria were collected from 16 children of ECC and 16 caries-free children, aged below 71 months in Al-Mutazam Kindergarten, Depok, West Java. Samples were cultured with trypticase soy agar and incubated in an anaerobic condition at 37 °C for 72 h. Then, the bacterial culture colonies were counted prior subculture in tryptic soy broth for further incubation in an anaerobic condition in 370 °C for 72 h. Preparation of MS protein was carried out by bacterial cell lysate method [9]. The protein was added with 0, 3 ml Tris, EDTA and NaCl buffer and 0.065 sodium dodecyl sulfate (SDS; 10%). It was incubated for 10 min at 37 °C. The protein concentration was measured by Bradford protein assay methods.

SDS polyacrylamide gel electrophoresis methods

Resolving gel and stacking gel were made until wells are formed. Samples and protein marker were run in each well to electrophoresis procedure. Electrophoresis tank was connected to the power source and had been set about 150 V, 80 mA for 70 min [10].

Results

MS count

The number of MS from the saliva of ECC patients was higher than caries-free children; there were 22.20×106 and 19.16×106 CFU/ml, respectively *(Fig. 1)*. We also analyzed the dmft score of MS count. The data show that the severity of dmft correlates with the number of MS in which the higher dmft value is in line with MS amount in the saliva ($p < 0.05$) *(Fig. 2)*.

Protein profile of MS in ECC and caries-free subjects

Figure 3 shows that the expression of the protein with molecular weight 29 kDa is found higher in ECC subjects (100%) than caries-free (56.25%); the 39-kDa protein is also found higher in ECC subjects (93.7%) than caries-free subjects (31.25%). The appearance of protein that has molecular weight 41.3 kDa found higher in ECC subjects (43.75%) than caries-free subjects (31.25%). The 74-kDa protein band appears higher in ECC subjects

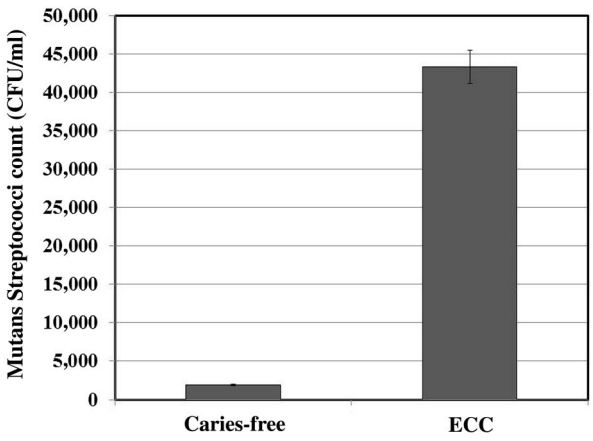

Fig. 1. The quantity of Mutans Streptococci in saliva of ECC patients and caries-free children

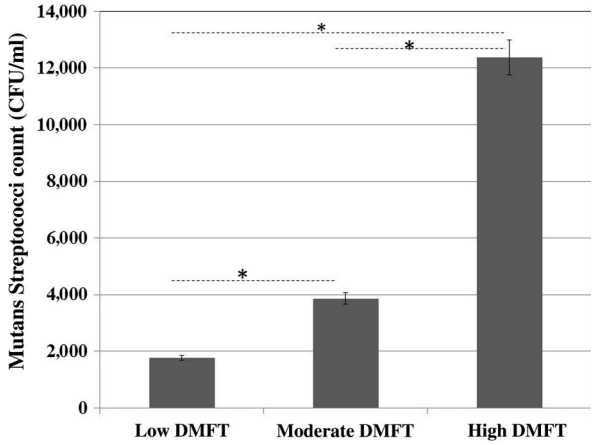

Fig. 2. Distribution of the quantity of Mutans Streptococci in saliva of low, moderate, and high dmft of patients. $*p < 0.05$

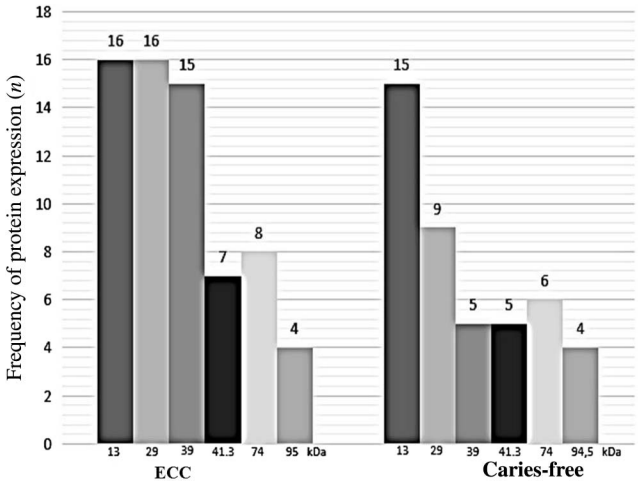

Fig. 3. The expression of protein with molecular weight of Mutans Streptococci in ECC and caries-free subjects

(50%) than caries-free subjects (37.5%). Finally, the protein with molecular weight 95 kDa appears at same frequency between ECC and caries-free subjects (25%). There was the difference of MS protein profile, which was isolated from saliva in ECC and caries-free subjects.

Discussion

A large number of studies previously have established a positive correlation between MS and ECC [11, 12]. The results of this study also confirmed that MS was associated with ECC as indicated by the number of colonies (ECC children had more MS colonies). A positive correlation was also found between severity of ECC as measured by the dmft index and high levels of MS counts, indicating that as the number of colonies increased, the number of teeth and surfaces affected by caries also increased.

Protein with molecular weight 13 kDa was suspected as antigen D, 29 kDa as antigen A, 39 kDa as antigen III (AgIII), 41.3 kDa as GbpB, 74 kDa as GbpA, and 94.5 kDa as dextranase (DexA) [13–18].

The frequency of 13 kDa antigen D, collected from saliva, that is detected in this research is not much different even though higher in ECC than caries-free subjects. According to Russel in 1993, this protein had been identified before as a protein with a low molecular mass. It was explained more by Roman et al. in 2013 that those proteins with a low molecular mass responded to enzymes, which involve in amino acid production. This antigen will interact with another protein that resides in the human body for which it will take part in the early process of active caries. Thus, it is assumed that protein whose molecule weighs 13 kDa presents both in ECC and caries-free subjects [13, 19].

Another protein, which has molecular weight 29 kDa, suspected as an antigen A [15]. This antigen does not involve in dextran bond, which may take part in early bacterial colonization [19]. Another research reported that even though the specific role of antigen A remains unclear, individuals who received this antigen immunization may be protected from the caries process [20]. It happened because antigen A induces IgA antibody that may disrupt the adherence and bacterial colonization, which initiates the caries process [13, 15]. But the result in this research reports that the expression in ECC is higher than caries-free subjects; this may be caused by the caries risk factor more dominant like salivary flow rate or buffer capacity than the immunologic factor, such as level of IgA antibody [13].

Protein with molecular weight 39 kDa is suspected as AgIII, whose frequency has been detected more on ECC subject. This protein found on the whole surface of the cell. The function of this protein remains unclear [13]. This protein is one of cell surface antigens on MS, which is immunogenic for some population that induced IgG antibody in caries-active groups or with the history of caries. On the other hand, based on some literature and research, IgG also found less sensitive in the role of protection to caries compare with IgA [15].

GbpB protein, which molecule protein weight assumed is 41.3 kDa, isolated from saliva and the result of this research is higher in ECC subject. According to Mattos-Graner et al. in 2001, GbpB has a positive correlation to biofilm formation. GbpB also may have a role in cell walls formation, which influences their growth and lives [16, 21]. In summary, if there is no GbpB presence in the cell of MS, these bacteria cannot grow and survive. The caries-free subject may be as the result of less GbpB expression in MS, followed by the disruption of the bacterial growth. Thus, it could be concluded that GbpB maintains the integrity of the bacteria cell formation and leads to biofilm increase [16].

Protein with molecule weight 74 kDa was suspected as GbpA protein. As functionally, GbpA contribute to adherence and cohesivity of MS. Hazlett et al. reported that GbpA protein has a hypercariogenic affinity resulted from plaque structure changes raise acid production on enamel and barrier made between tooth surfaces and saliva. A hypercariogenic affinity leads to demineralization and also decreases saliva buffering capacity. GbpA protein also found to enhance bacterial adhesion to the tooth enamel via sucrose [22]. Therefore, this molecular weight is suspected that it directly contributes to form caries lesion.

Protein with molecular weight 94.5 kDa has the same frequency of expression both in ECC and caries-free subjects in this research, suspected as DexA [18]. The virulence properties of this protein remain unclear. From several studies, DexA not only able to hydrolyze water insoluble glucan, which reduces the adherence of MS to enamel surfaces, but also inhibits biofilm forming on tooth surface [23]. Other research also reported that DexA together with glucanohydrolase would impact in reducing the total glucans [24–26].

Conclusion

The number of MS and the expression of some protein bands were higher in ECC subjects compared to caries-free subjects.

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Authors' contribution: EWB designed the study and wrote the first draft of the manuscript. BBM wrote the protocol. AR and FPG carried out sample collection and laboratory work. All authors read and approved the final version of the manuscript.

Conflict of interest: The authors declared no competing interests exist.

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