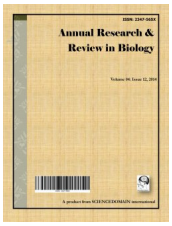

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Analysis of IgA Anti-*S mutans* Serotype c and e in ECC Patients and Its Correlation towards Saliva Viscosity and DMFT Score

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Authors' contributions

This work was carried out in collaboration between all authors. Author EWB designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors AW and SJ carried out laboratory work and performed the statistical analysis, when they conducted scientific work in their studentship at Faculty of Dentistry, Universitas Indonesia. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To analyze Immunoglobulin A anti-*S mutans* serotype c and e level and the correlation towards saliva viscosity and decayed, missing, and filled teeth (DMFT) score of Early Childhood caries (ECC) patients.
Study Design: Titer of IgA was measured from saliva samples of ECC patients using ELISA methods.
Place and Duration of Study: Laboratory of Oral Biology Faculty of Dentistry Universitas Indonesia. Jakarta 10430 Indonesia. September-December 2016.
Methodology: Titer of IgA anti-*S mutans* serotype c and e and the correlation between IgA titer and saliva's viscosity as well as DMFT score were analyzed.
Results: The titer of IgA anti-*S mutans* serotype c and e were 6 and 15.1, the Mann-Whitney test showed $P<0.05$. Levels of IgA anti-*S mutans* serotype c and e were in line with the saliva's viscosity in which increasing viscosity resulted in higher level of IgA titer. Further regression

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analysis reveals that the titer of IgA with high viscosity of saliva contain higher titer of IgA anti-*S mutans* compare those lower viscosity. In addition, the data shows that DMFT score have a negative correlation with IgA anti-*S mutans* serotype c and e levels.
Conclusion: In ECC patients, *S mutans* serotype e may be more immunogenic than serotype c and higher salivary viscosity may be due to its high IgA content.

Keywords: S mutans serotype c; saliva; Immunoglobulin A; viscosity; DMFT; early childhood caries.

1. INTRODUCTION

Early Childhood Caries (ECC) is the presence of one or more decayed, missing or filled tooth in a child 71 months of age or younger [1]. ECC started with white patches, that becoming caries on tooth surfaces [2]. ECC is concerned as oral health problem in Indonesia In 2015, ECC prevalence in Pineleng, North Sulawesi was 66.21% and ECC prevalence in Gunung Anyar, Surabaya was 60% [3]. *S mutans* is the main cariogenic microorganism that play important role in ECC pathogenesis [4]. This microorganism can colonize the tooth surfaces, fermenting carbohydrate, then produces acid that could undermine tooth surfaces [4]. Based on their chemical structure, *S mutans* classified into four serotype, c, e, f, and k [5]. The most dominant *S mutans* serotype that found in oral cavity is serotype c (70-80%), followed by serotype e (20%), f (<5%) and k (2-5%) [5]. *S mutans* population in oral cavity were affected by saliva condition [6,7]. Körber et al. [8] reported that in the distribution of serotypes of *S mutans* in the caries population was predominated by serotype c (53.2%), followed by e (31.9%), f (8.5%) and k (6.4%).

Saliva consist of 99% water and various electrolytes (Na, K, Ca, Mg, bicarbonate, phosphate), immunoglobulin, proteins, enzymes, mucins, and nitrogen products [9]. Saliva has a multi-function such as a taste enhancement and digestion, lubrication and protection, buffering and clearance, maintenance of tooth integrity, and as the first line of defense against bacterial and viral attack [6]. Specific humoral immunity components are IgA, IgM, and IgG, while non-specific immunity factor consist of mucins, lactoferrin, lysozyme, peroxidase, cystatins, and hystatins [9]. The physicochemical properties of saliva, such as viscosity, pH, salivary flow rate, and buffering capacity has a relation with caries activity in children and act as markers of caries activity [8]. The protein and ion components in saliva make a solution into a viscoelastic solution [10] IgA, the predominant immunologic component of saliva act to maintain the integrity of oral surfaces, by limiting the formation of *S mutans* biofilm through binding the pellicle surfaces or by agglutinating the bacteria [9]. Immunoglobulin A has a role in preventing the formation *S mutans* biofilm [11], which is known as the main causes of ECC. Therefore, we were interested to analyse IgA anti-*S mutans* serotype c and e level in saliva of ECC patients, and its correlation with DMFT score and saliva viscosity in ECC patients. Here we evaluated the relationship between level IgA anti-*S mutans* serotype c and e and the salivary flow rate, viscosity as well as the DMFT scores in saliva of patients ECC.

2. MATERIALS AND METHODS

This study obtains ethical approval from Ethical Research Committee Faculty of Dentistry Universitas Indonesia (No:49/Ethical Approoval/FKGUI/IX/2015).

Clinical examination of subjects was carried out by single examiner. Decay, missing and fillings (DMFT) score determined according to World Health Organization (1986) diagnostic criteria. Resting whole saliva was collected in the morning time according to Fédération dentaire internationale (FDI). The subjects were 20 children in Early Childhood Education (ECD) Al-Ikhlas Paseban Village, Senen Sub-district, Central Jakarta, aged 3-5 years who have diagnosed as ECC. Subjects were instructed to accumulate saliva in the mouth for 2 min and then spit in the sterile container. This procedure was repeated three times and the salivary flow rates were calculated. In addition, the viscosity of saliva also recorded as high or low.

2.1 ELISA

Levels of IgA anti-*S mutans* serotype c and e were measured by enzyme-linked immunosorbent assay (ELISA). Wash buffer was consist of PBS solution and 0.05% Tween-20. Coating buffer consist of 16 mM Na2CO3 (BD) and 34 mM NaHCO3 (pH 9.6) (BD). 5% (w/v) Blocking buffer contains skim milk in PBS

(Oxoid), 0.1% (v/v) Tween 20 (Sigma), PBS with 0.1% (v/v) Tween 20 (Sigma). Secondary antibody, Anti-Human IgA HRP (AB CAM) was used at 1:1000 concentration [12,13].

2.2 Bacterial Culture and Antigen Preparation

Glycerol stock of *S mutans* serotype c and e bacteria from clinical isolates was obtained from Oral Biology Laboratory Faculty of Dentistry, Universitas Indonesia. Bacteria were grown in TYS20B medium with anaerobic condition in 37℃ for 72 hours. Cultures of *S mutans* serotype c and e bacteria then sonicated and centrifuged to get the antigen. *S mutans* serotype c and e antigen concentration than measured using Bradford methods. After antigen concentration obtained, then dilute the antigen with coating buffer until the concentration become 10 µg/mL [12].

100 µL of antigen of *S mutans* serotype c and e were coated at concentration of 10 µg/mL in each well of ELISA plate, then incubated in 4℃ overnight. Wash all the well using PBST, and then 100 µL blotto as blocking solution and incubated in 37℃ for one hour. After that, wash the well using PBST. Each saliva sample then diluted serially from 1:2 to 1:16. Followed by incubated in 37℃ for one hour. After each well was washed using PBST, then add 100 µL of 1:2000 secondary antibody into each well and incubate the plate in 37℃ for 1 hour. The plates were washed with PBS prior added by 50 µL TMB substrate. ELISA plates than were shaked in orbital shaker for 15 minutes. The enzyme reaction was stopped by adding 50 µL HCl 1 N each well, and the color that developed read at 405 nm with ELISA reader [12].

2.3 Statistical Analysis

The differences of titer IgA anti-*S mutans* serotype c and e were statistically tested with Mann-Whitney U Test. In addition the correlation of DMFT score and titers of IgA anti-*S mutans* serotype c and e were tested with simple linear regression analysis.

3. RESULTS

3.1 The Relationship between DMFT Score with Salivary Flow Rate with Salivary Viscosity

Fig. 1 shows that the salivary flow rate in ECC subject with DMFT score 4 was higher (4.7 mL/min) than those have DMFT score 5 and score 6 (4,6 mL/min, 3.6 mL/min and 4 mL/min) respectively ($p>0.05$).

Observation in viscosity of saliva reveals that 8 ECC subjects with DMFT score 5 have high viscosity and only 2 of them have low viscosity. Subjects with DMFT score 4 seems to be equal number in the viscosity of saliva (4 vs 4). Interestingly all subject with DMFT score 6 have high viscosity in their saliva (Fig. 2). It seems that in ECC high viscosity saliva related to the severity of caries.

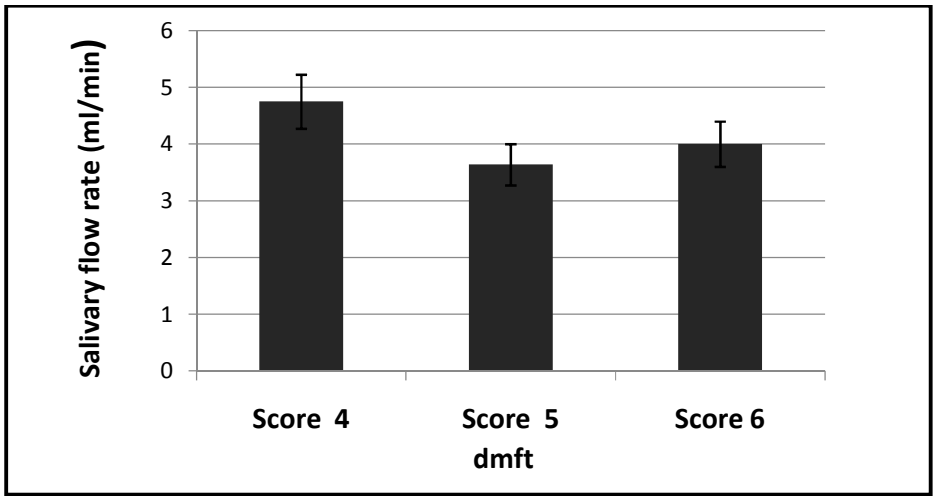

Fig. 1. The distribution of DMFT in ECC subject based on their salivary flow rate

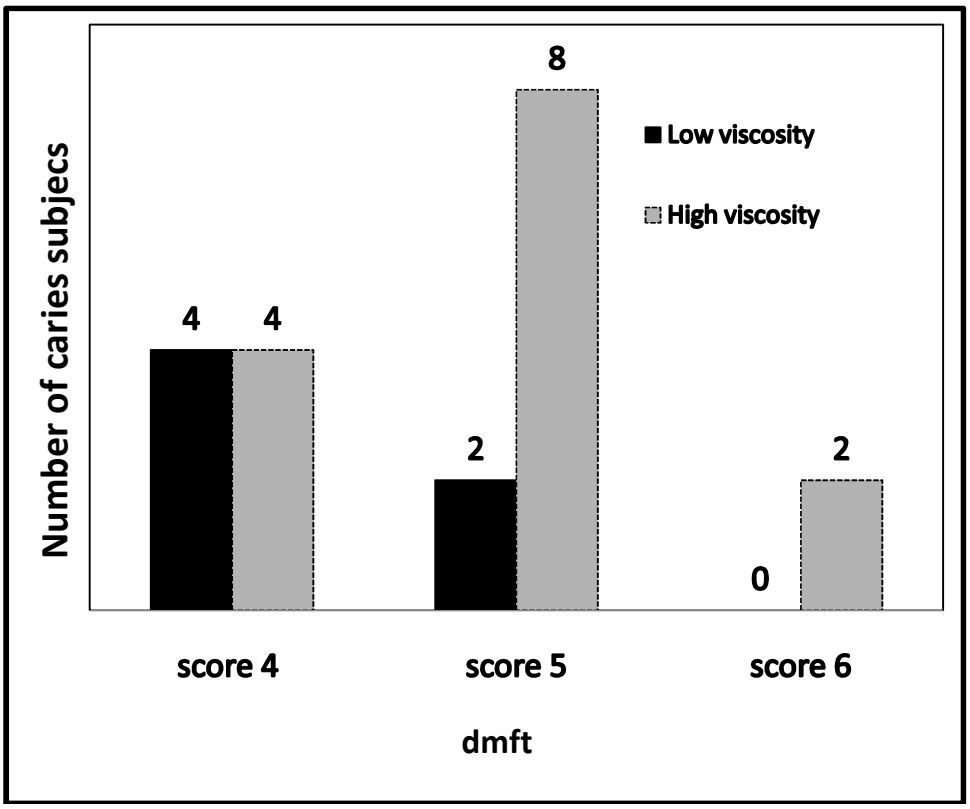

Fig. 2. The distribution of DMFT score based on viscosity of saliva in ECC subject

3.2 Titer of IgA Anti-*S mutans* Serotype c and e in ECC Patients

IgA titer was determined as the higher dilution saliva sample that reach optical density (OD) 0.2 at 450 nm absorbance in ELISA reader. As shown in Fig. 3. The data reveals that in saliva of ECC subject, the average titer of IgA anti-*S mutans* serotype c and were 1/6 and 1/9.8 (P<0.05).

We analyse data of IgA anti *S mutans* of ECC's saliva subject in their relationship to DMFT score. The results show that IgA anti-*S mutans* serotype has stronger negative correlation with DMFT (r= -0.978) than serotype c (r= -0.577) with P <0.05. (Fig. 4).

Further analysis of IgA anti-*S mutans* in saliva of ECC subject and the correlation with viscosity of their saliva reveals that highers titers of IgA anti-*S mutans* in high viscosity of saliva both in serotype c and e. However, IgA anti-*S mutans* serotype e has the highest one (Fig. 5).

4. DISCUSSION

The main bacteria that causes caries is *S mutans* classified into 4 serotype, one of them is serotype c [12]. Previous study reported that *S mutans* serotype c is the most commonly found bacteria in oral cavity [5]. In Indonesia *S mutans* serotype f is the most commonly found bacteria in child's oral cavity [14].

This study found that that in saliva of ECC subject, the average titer of IgA anti-*S mutans* serotype c is lower than IgA anti-*S mutans* serotype e. This may indicated that due to *S mutans* serotype e play predominant role in ECC patient. Level IgA against antigens of *S mutans* have been studied previously, and low levels of IgA are associated with a high risk of caries in children. IgA antibody specificities may be critical in modulating initial *S mutans* infection [15].

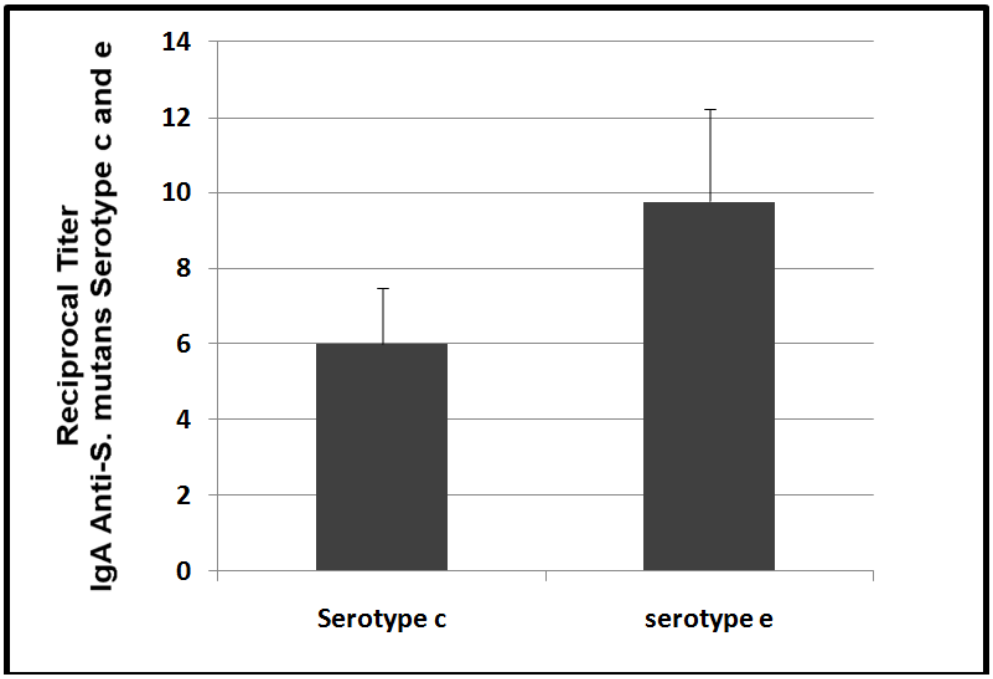

Fig. 3. Titers of IgA anti-*S mutans* serotype e is higher than those serotype c in saliva of ECC subjects (*P*<0.05)

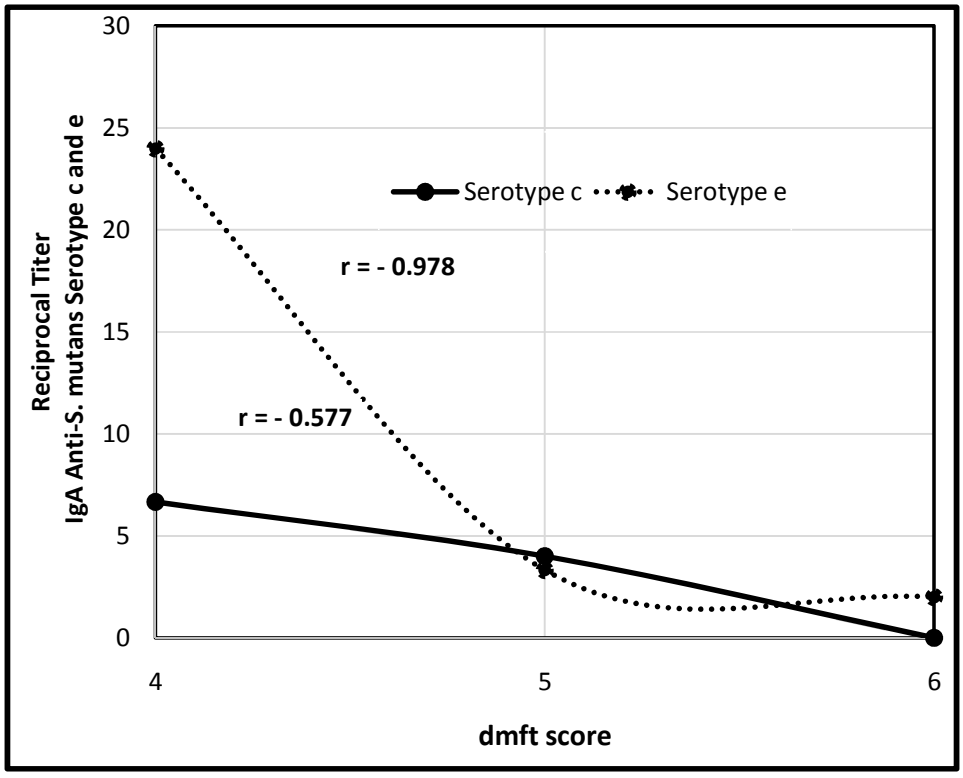

Fig. 4. The correlation of DMFT score and titers of IgA anti-*S mutans* serotype c and e in saliva of ECC subject. Both IgA anti-*S mutans* serotype c and e have negative correlation with DMFT score, with (r= -0.978) in serotype e and (r= -0.577) in serotype c respectively

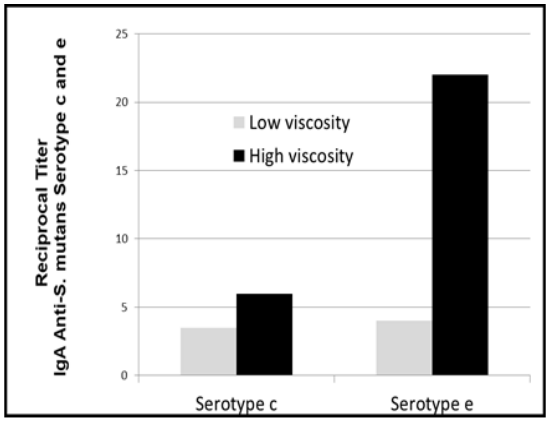

Fig. 5. The average of IgA anti-*S mutans* serotype c and e titers of ECC subject with high and low saliva viscosity. The highest antibody titer is IgA anti-*S mutans* serotype e in subject with high viscosity of saliva (*P* < 0.05)

This study showed that low levels of IgA anti- *S mutans* serotype e of in patients with high DMFT scores. With the decrease of the level of IgA anti-*S mutans* serotype e at high DMFT scores can be seen that the risk of caries owned is certainly higher. Meanwhile, According to Ranadheer et al. [15] provide mechanical protection against bacteria *S mutans* on the teeth causing caries is the purpose of the IgA so it would be good if in the salivary IgA level is higher than the score DMFT. Besides the observations that have been obtained is also consistent with studies that have been done before by Doifode D. [16] which states that the IgA antibody contained in saliva naturally have an important role in the immunologic control of dental caries that occurs in children such as ECC. Koga-Ito Cristiane et al. [17]. reported that anti-*S mutans* IgA levels are higher in caries-free individuals than in individuals with karies and low level of IgA have a correlation towards the high risk of caries in children.

In this study found there is an association between high viscosity and the high levels of IgA. While, a negative correlation between IgA anti-*S mutans* serotype c levels with saliva viscosity and DMFT score founded in this study. The caries risk assessment using saliva in terms of pH (stimulated and unstimulated), consistency and buffering capacity assists dental professionals in determining low, moderate, high or extreme high caries risk caries [18]. Some study supported about this study results, that the higher DMFT score, the lower IgA anti-*S mutans* serotype c levels [19]. Decreased IgA levels, reflect the high caries risk factor in patients [20,21]. More appropriate protein intake might be need for patient with ECC. As reported by previous study that multivitamin usage and tooth decay relation is not a widely questioned phenomena in ECC [22]. Our finding might be valuable for the strategy to prevent ECC by focusing on *S mutans* serotype c and the role of specific IgA against this *S mutans* serotype in saliva.

5. CONCLUSION

In ECC patients, *S mutans* serotype e may be more immunogenic than serotype c and higher salivary viscosity may be due to its high protein contain as well as IgA content.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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