Original Article

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Dentistry Section

Analysis of Salivary Protein Profiles and its Viscosity in Early Childhood Caries (A Cross-Sectional Study)

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ABSTRACT

Introduction: Early Childhood Caries (ECC) affects a significant proportion of the child population worldwide. Salivary proteins contain a host defense system that plays a role in the caries pathogenesis.

Aim: To analyse the protein profile and salivary viscosity from ECC and caries-free subjects.

Materials and Methods: The number of subjects included in the study was 16 ECC and 16 caries-free. Salivary viscosity was assessed visually and salivary protein profile was analysed using SDS PAGE.

Results: The five most prominent protein bands with molecular mass 15 kDa, 25 kDa, 60 kDa, 65 kDa and 95 kDa were found in ECC patients with the frequency of occurrences in order 8, 3, 16, 6, 2 and caries-free subjects 14, 5, 16, 16, 9. The saliva viscosity of ECC patient was higher than the caries-free subjects.

Conclusion: In ECC patients, the frequency of occurrences of salivary protein profile 15 kDa, 25 kDa, 65 kDa and 95 kDa were found less than in caries-free subjects. Meanwhile, protein profile 60 kDa has the same frequency of occurrence in ECC and caries-free subjects. Viscosity of ECC patient was higher than the caries-free subject.

Keywords: Albumin, Amylase, Lactoferrin, Salivary Proline-Rich Proteins (PRPs), sIgA

INTRODUCTION

Early childhood caries is a condition in which there is presence of one or more decayed tooth with non-cavitated or cavitated lesions, missing due to caries, or filled tooth surfaces in any primary tooth of a child under the age of six years based on American Association of Paediatric Dentistry (AAPD) [1].

In most developed countries the prevalence rate of ECC is between 1 and 12% [2]. In less developed countries and among the disadvantaged groups in the developed countries, the prevalence has been reported to be as high as 70%. ECC has been found to be more prevalent in low socioeconomic groups [3,4]. Prevalence of ECC in DKI Jakarta and its surrounding area in 1988 was 85.17% and in 2001 was 81.2% [5]. As a result of the development of caries, it can cause pain and affect the ability to chew and eat, which can also cause iron deficiency due to malnutrition [6].

Dental caries is a chronic disease with complex and multifactorial causes resulting from an interaction between pathogenic microorganisms, carbohydrate fermentation substrate, host conditions and at a certain period [7]. One of the host factors in ECC is saliva which plays a crucial role in maintaining oral health and inhibiting the growth of pathogenic microorganisms.

Saliva has a physiochemical characteristic, one of which is viscosity-that affects performing one of saliva function which is self-cleansing that can reduce the risk of ECC [8]. There are specific defense proteins such as immunoglobulins, as well as many other proteins that work nonspecifically such as Proline Rich-Proteins (PRPs), mucin, agglutinin, lactoferrin, cystatin, lysozyme, etc., [9,10]. Protein helps saliva function in protecting and maintaining the oral health.

Differences in metaproteomic profiles of saliva and the salivary viscosity influence caries formation. This study aims to analyse the salivary protein profile by SDS-PAGE and salivary viscosity in children with ECC.

MATERIALS AND METHODS

The sample size was determined by binomial proportions as this study was a cross-sectional. Total 32 saliva samples from children aged 3-5 years consisting of 16 ECC patients and 16 caries-free subjects were included. Samples were taken in Al-Multazam Kindergarten, Depok-West Java, with ethical approval from the Ethical Research Committee Faculty of Dentistry University of Indonesia (No:24/Ethical Approval/FKGUI/V/2017).

Sampling and Sample Preparation

Unstimulated saliva was taken using the sterile pipette and accommodated in the 1.5 mL microcentrifuge tube containing Phosphate Buffer Saline (PBS) and Phenylmethanesulfonylfluoride (PMSF) solution. Saliva samples were homogenised and then centrifuged for 10 minutes at 4°C with 12000 rpm. The salivary supernatant was taken and transferred to a new microcentrifuge tube. The concentration of the protein was measured using Bradford methods. Standard protein and saliva were added to 96 well plate as much as 10 µL. Each of the well containing standard protein and saliva was added 190 µL. Bradford reagents and then read by microplate reader with optical density 595 nm.

SDS Page

Saliva concentration was synchronised to 100 µg/mL based on Bradford for the SDS PAGE methods. Ten µL buffer sample was added to the synchronised saliva sample and then put on the thermoblock at 95°C for 5 minutes. Resolving gel and stacking gel were made until wells were formed. Then, 15 µL of sample and 5 µL of protein markers (PeqGold VI Prestained) were put in each well of stacking gel for electrophoresis procedure. Electrophoresis tank was connected with the power source with 150 V and 80 mA for 70 minutes [11].

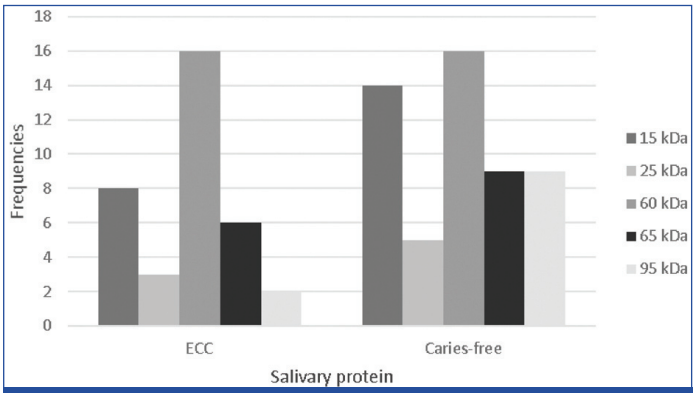
After the electrophoresis procedure was done, the gel was taken and put in the container containing Page Blue as a staining solution. The container was put on the shaker at 60 rpm for overnight. Then, the gel was destained using aquades every 30 minutes. The result of SDS PAGE was read by the scanner, and each of protein bands was noted in table and charts.

RESULTS

Protein Profile

Five most prominent protein band were found that are 15 kDa, 25 Da, 60 kDa, 65 kDa and 95 kDa.

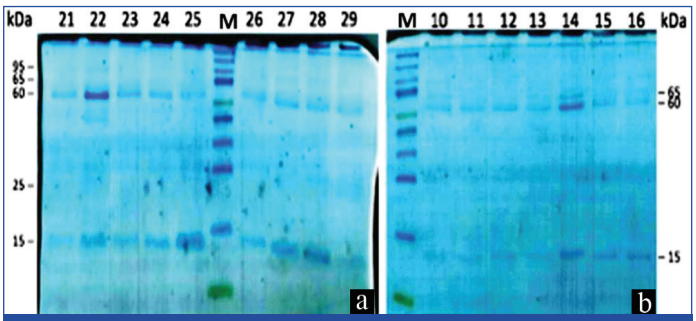
As shown in [Table/Fig-1-3], there are differences in protein profile occurrences in ECC patients and caries-free subjects. Protein with molecular weight 15 kDa, 25 kDa, 65 kDa and 95 kDa were found more frequently in the caries-free subject compared to ECC patients.



[Table/Fig-1]: Salivary protein profile in ECC patient and caries-free subjects.

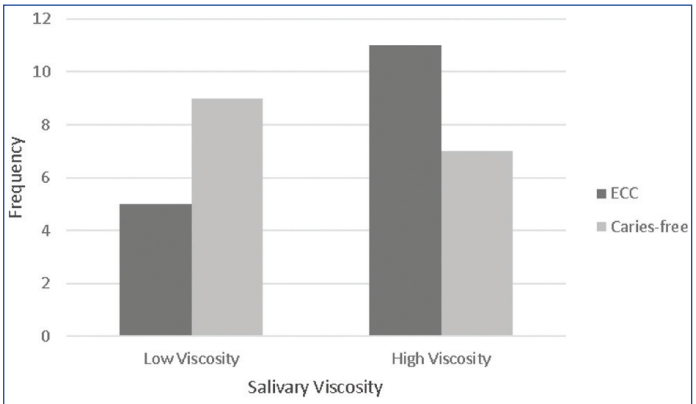
	15 kDa	25 kDa	60 kDa	65 kDa	95 kDa
ECC	8	3	16	6	2
Caries-Free	14	5	16	9	9

[Table/Fig-2]: The summary of the protein bands expression from the saliva of caries-free and ECC subjects.



[Table/Fig-3]: The representative data of SDS-PAGE protein bands of saliva from a) caries-free subject and b) ECC.
M: Marker

[Table/Fig-4] shows that on low viscosity, the frequency of ECC patients was lower (5) than the caries-free subjects (9). While on high viscosity, the frequency of ECC patients was higher (11) than the caries-free subjects (7).



[Table/Fig-4]: The saliva's viscosity in ECC patient and caries-free subject.

DISCUSSION

The 15 kDa molecular weight protein was suspected as cystatin [12,13]. Cystatin is a natural inhibitor of cysteine proteinase that also acts as antiviral and antibacterial by assisting in remineralisation [14,15]. Cystatin can be found in pellicle, which allows the penetration of minerals into the enamel to increase the enamel crystalline growth [16]. In this study, the 15 kDa protein was found more on the caries-free subjects than ECC patients which is in accordance to the previous studies [17,18].

Salivary PRPs are classified as acidic, basic or glycosylated [14,15,19]. In this study, the protein with molecular weight of 25 kDa was suspected as basic PRPs (bPRPs) [12,18] and has the frequency of occurrence higher on caries-free subjects than ECC patients. However, the differences are not significant. The bPRPs are derived from parotid glands and can be found in oral epithelial cells [20]. The function of these bPRPs is not well known [14,15] but allegedly binding polyphenols which are acidic and toxic, thus preventing the spread of toxic effects in the oral cavity. This protein also has antimicrobial properties through microbial aggregates and prevent binding to the surface of the oral cavity [13,14,21]. The previous study found that there was a significant difference between bPRPs on ECC and caries-free group, which was higher on the caries-free group. Although it is said that the function of bPRPs is not known clearly, but based on the evidences from various studies indicates that bPRPs contribute in protecting the oral cavity from caries [15,21-23].

Protein with molecular weight 60 kDa was found 100% on ECC patient and caries-free subject. This protein was suspected as α-amylase which is the most commonly secreted protein and salivary enzyme, primarily through the parotid gland (80%). Amylase works by lubricating the food and turns them into bolus and breakdown the starch into maltose and oligosaccharides. Amylase can act as an antibacterial agent but its ability is limited [14,24,25]. Amylase does not bind to epithelial cells and exhibits very minimal activity and can be found in pellicle at low level [22,23,26]. Based on this theory, amylase activity does not affect the occurrence of caries. This finding is supported by the previous study that there were no significant differences in salivary amylase expression between caries groups and caries-free group [22,27-29].

The protein with 65 kDa molecular weight was determined as serum albumin [12,13], that acts as a protein carrier and plays a role in salivary buffer system [15,29-32]. Based on this study which supported by previous studies, it appears that the serum albumin protein contributes in preventing the occurrence of caries as it was found to be more abundant in the caries-free subjects compared to ECC patients.

Protein with molecular weight 95 kDa was found more frequently in the caries-free subjects than ECC patients. Protein with molecular weight 80 kDa, 83 kDa, and 97 kDa are reported as the secretory component, which are the transporters of IgA in epithelial cells [12,20,30,31,32]. With this same range, 95 kDa protein can be estimated as the secretory component in saliva. Secretory IgA (s-IgA) is the primary immunoglobulin of saliva that serves as the first-line defense of the oral cavity that acts through inhibition of glucosyltransferase activity (Gtf), prevents bacterial adhesion, inactivates enzymes and bacterial toxins and acts synergistically with other salivary components [33-36]. The s-IgA has a relationship with dental caries even though there are different opinions about it. According to Chawda JG et al., s-IgA was found more significant in the caries-free subjects by providing localised immune protection against cariogenic microorganisms [34]. The result of this study found that the frequency of occurrence of 95 kDa protein was higher in the caries-free subjects compared to ECC patients. However, Ranadheer E, et al., mentioned that there was an increase of s-IgA level in the subjects with active caries [32]. This is supported by the previous study that s-IgA is more prevalent in caries subject providing an indication of an immune response to protect the oral cavity against pathogenic microorganism [35].

In addition to the protein profile of saliva, we also assessed the viscosity of saliva. Viscosity defined as a condensed salivary state, which is the rheological property of a complex salivary fluid [33,36]. In this study, the ECC patients had a high viscosity which is thick

and bubbly while the caries-free subjects had a low viscosity of clear and watery saliva. Increase in salivary viscosity might due to the decrease of water content in saliva [34] and may increase the risk of caries [14,16,36].

LIMITATION

There is some limitation of this study that needs further investigation such as determination of the proteins by Immunoblotting. The results of protein function analysis may have a benefit in future caries prevention.

CONCLUSION

The frequency of occurrences of the 15 kDa, 25 kDa, 65 kDa and 95 kDa protein profiles in the ECC patients was found lesser than in caries-free subjects. The 60 kDa protein profile has the same frequency in ECC patients and caries-free subjects. There is also a difference in the salivary viscosity which is high in ECC patients and low in caries-free subjects.

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