The Relationship between the Secretory IgA, pH and Salivary Flow Rate with the Occurrence of Early Childhood Caries

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Abstract

Salivary secretory immunoglobulin A (SIgA) has immunological control over dental caries and presumably prevents the adherence of cariogenic microorganisms to the hard surfaces, relation between SIgA and dental caries still in controversy. This study aims to investigate the relationship of SIgA, salivary flow rate and pH with the occurrence of Early Childhood Caries (ECC).

A total of 36 patients aged 2-5 years old were divided into 3 groups, according to their caries status (caries-free, ECC and Severe ECC). The subjects were selected based on inclusive and exclusive criteria needed. ECC and Severe ECC were diagnosed based on AAPD guidelines. Score of dmft was recorded. Unstimulated salivary flow rate and pH were measured. The samples were analyzed for determining the SIgA level.

Mean value of IgA levels in ECC group (2404.6516 ng/mL ± 395.03208 ng/mL) was higher compared to other caries status group, although there was no significant relationship as the p-value is 0.404. Median values of salivary flow rate & salivary pH showed no significant difference between 3 caries status groups which p-value is 0.701 & 0.227 respectively.

The result for this study showed higher level of secretory Immunoglobulin A (SIgA) in Early Childhood Caries (ECC) group although the result was not significant. Salivary flow rate and pH have not significant effects on the occurrence of ECC. However, the highest median value for flow rate and pH are seen in the Severe-ECC group.

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Introduction

Early Childhood Caries (ECC) is defined as any presence of decayed (non cavitated or cavitated lesions), missing (due to caries) or filled tooth in any primary tooth in a child 71 months or younger. [1] While, Severe- Early Childhood Caries (S-ECC) is defined by any signs of smooth–surface caries in child age younger than 3 years of age, dmft score more or equal to 4 for age 3, dmft score more or equal to 5 for age 4, and dmft score more or equal to 6 for age 5. [2,3]

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Secretory Immunoglobulin A (S-IgA) is a subclass of IgA, an antibody that play an active defence role against pathogens. It also prevent attachment of microbial, neutralizing the enzymes, toxins and also act synergistically with lactoferrins and lysozymes. It is predominantly found in mucous secretions from glands, such as salivary glands. [4]

Hemadi et. al. (2017) mentioned the roles of saliva in protecting human body specifically the teeth by remineralisation process, buffering the low ph oral environment, cleanse food debris and plaque and acts as antibacterial properties.[5]

Most of previous studies provided the evidence of higher levels of Immunoglobulin A (IgA) in children with ECC, and suggest its

likelihood as a caries biomarker. The controversy remains regarding the relationship between salivary Immunoglobulin A and dental caries. [5]

The systematic review and meta-analysis study by Fidalgo et al. (2014) reported there were an interrelation between the high level of salivary IgA and elevated caries activity. [6] A cross-sectional study by Bagherian A, Asadikaram G. (2012) revealed higher mean value of SIgA levels in ECC group. [7] On the other hand, study by Omar et al (2012) reported an inverse relationship between salivary IgA levels and caries experience in children 3-6 years of age. [8] Meanwhile, Shifa et al.,(2008) proved that there was no correlation between salivary IgA and caries activity among children 3-6 years old. [9] Addition to this, systematic review by Hedge M, et al., (2019) expressed 7 out of 11 studies have significant differences between caries-free and caries-active groups, for various salivary components such as pH, buffering capacity, proteins, electrolyte, antioxidant, enzymes, and minerals. [10]

The purpose of the present study was to investigate the relationship between Secretory Immunoglobulin A (SIgA) levels, salivary flow rate and pH with the occurrence of early childhood caries. Specifically, to evaluate the effect of salivary flow rate and pH on the insistence of ECC and to investigate the relationship between levels of secretory IgA with the severity of ECC.

It was hypothesized that secretory immunoglobulin A (SIgA) level, salivary flow rate and pH have significant relationship with the occurrence of early childhood caries.

Based on previous studies done, Immunoglobulin A can be one of the biomarker for the caries. The higher level of IgA indicates the higher caries risk. It is then supported by the alteration of salivary flow rate and salivary pH. By knowing these parameters, dentist has good indicators that can be used in preventive measures of dental caries.

Materials and methods

Subject Recruitment and Oral Examination

Ethical approval was obtained from IIUM Research Ethics Committee, IREC in April 2019 (IREC NO: IREC 2019-028). This cross-sectional study consisted a total of 36 subjects obtained from patients in Kulliyyah of Dentistry, International Islamic University Malaysia (IIUM), Kuantan Campus and kindergartens in Indera Mahkota area Kuantan, Pahang with the age range from 2-5 years old. All patients' parents or guardians were gave a written consent prior to participation.

The subjects were excluded if they have congenital abnormalities, undergone chemotherapy and radiotherapy, mentally illness, on antibiotic treatment within 3 previous months, on chronic medications that cause the hyposalivation or suffer from upper respiratory tract infection in past one week.

The number of carious teeth and surfaces was charted. The subjects were then divided into 3 caries status groups (Caries Free, ECC, Severe ECC) based on definition by AAPD Guidelines 2016. 3

Saliva pH Determination and Saliva Collection

Salivary pH was determined using litmus paper (MACHEREY-NAGEL is certified according to the medical device directive ISO 13485) placed on the floor of the mouth for 5 seconds.

Unstimulated whole salivary samples were collected between 0830 to 1130 in the morning. Patient was advised not to eat/drink/brush teeth 30 minutes prior to the procedure. Patient was asked not to swallow the saliva once the time is recorded.

Pre-weighed sterile cotton rolls were placed at the buccal sulcus (right and left) and sublingual area for 2 minutes and 30 seconds, and it was repeated twice. Once 5 minutes reached, cotton rolls were removed and transferred into pre-weighed sterile saliva container. The container were then kept in the icebox up to 2 hours.

The weight of the container and cotton rolls were determined in the lab. Flow rate was determined by measuring the collected saliva weight divided by duration of collection (mL/min). Saliva was then extracted from cotton rolls using syringe 10cc into micro centrifuge tube. It was then stored in the freezer at- 20 degree Celsius until analysed.

Lab Procedure

The samples then thawed and centrifuged for 5 minutes at 3.5 rpm at 9 degree Celsius to remove the mucin and debris. The supernatant

was collected and assay was carried out by Human s-IgA (Secretory Immunoglobulin A) ELISA Kit from Elabscience®

All reagents, standard solutions, and samples were prepared as instructed in the manual provided by the kit. Duplication was done for each Standard and samples. The process was followed as instructed in Kit's Manual. Optical Density was determined using a Microplate Reader at 450nm immediately. The results obtained were tabulated and analysed.

Graph Generation

Sigmoidal-4 parameter logistic graph was generated using Graphpad Prism software version 8.2.0 (435), by plotting known value of concentration serial diluted standard solution and their corresponding Absorbance value. The samples data obtained by interpolating from the standard curve to estimate the concentration of IgA in samples, as shown in Figure 1.

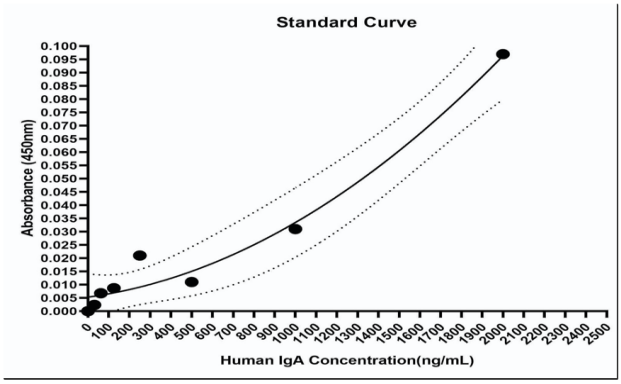

Figure 1. Human IgA Standard Curve.

Statistical Analysis

The data was then analysed using IBM SPSS Statistics Version 25. Normality test was run for salivary flow rate, pH and concentration of IgA. One-Way ANOVA test was used for concentration of IgA and Kruskal-Wallis Test were used for salivary flow rate and pH with p-value less than or equal to 0.005 was accepted as significant.

Results

Sample Characteristics

Our sample consisted of 58.33% boys and 41.67% girls. According to caries status, samples were divided almost equally, 30.56% for CF, 33.33% for ECC, and 36.11% for Severe-ECC. As in Figure 2 and Figure 3.

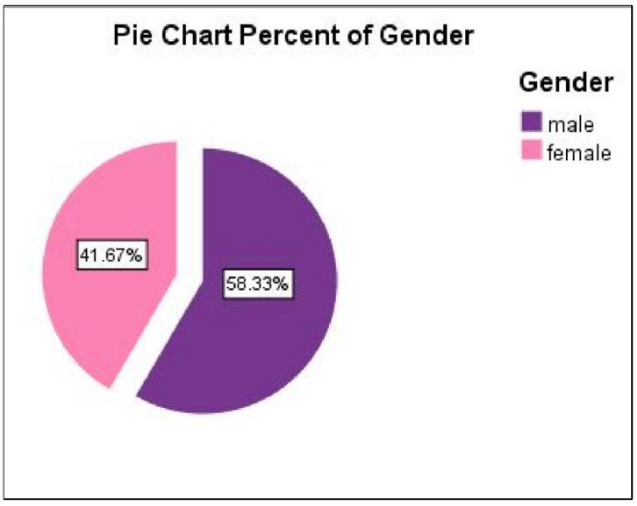

Figure 2. Compering sample percent of genders.

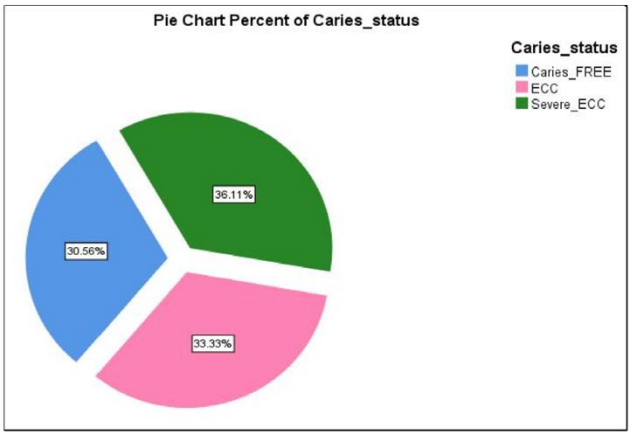

Figure 3. Compering sample percent of caries status.

Salivary Flow Rate, pH and IgA in 3 Different Caries Status Groups

The median value of Salivary Flow Rate shown no significant difference between 3 caries status groups which p-value is 0.701. However, the highest median value for flow rate are seen in the Severe-ECC group, which are 0.42660 ± 0.503.

The median value of salivary pH also shown the same, with no significant difference between 3 caries status groups, as the p-value is 0.227. The highest median value for pH are seen in the Severe-ECC group, pH 7 with the mean age of the samples 46.31 ± 12.093 months and mean dmft index of 7.92 ± 4.271.

Using ANOVA Statistical Test, the mean of IgA levels is not significant in 3 different caries status group as the p-value is 0.404. The highest mean value is seen in the ECC group, with the

mean age of the samples 48.45 ± 10.386 months and mean dmft index of 2.64 ± 1.286.

These are shown in the Figure 4 and Table 1 as below.

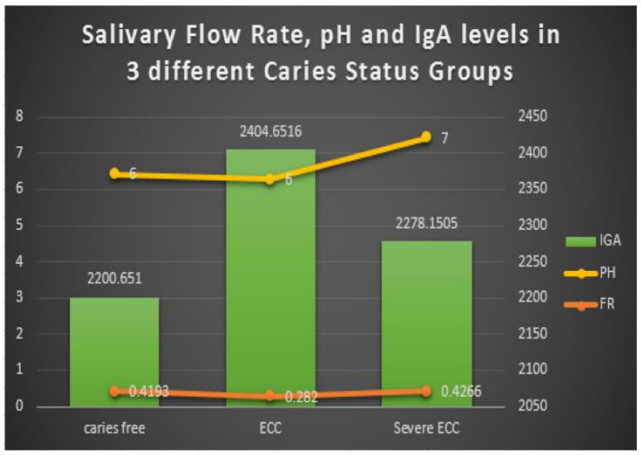

Figure 4. Salivary Flow Rate, pH and IgA Levels in 3 Different Caries Status Groups.

Caries Status	CF	ECC	Severe ECC
Mean Age (SD) (months)	41.70 (6.550)	48.45 (10.386)	46.31(12.093)
Mean dmft Index (SD)	0 (0)	2.64 (1.286)	7.92 (4.271)
Salivary Flow Rate			
Median (IQR) (ml/min)	0.41930 (0.494)	0.28200 (0.616)	0.42660 (0.503)
Chi Square Statistic (df)	0.501166 (2)		
p-value*	0.701 (>0.005)		
Salivary pH			
Median (IQR) (pH)	6.00 (1)	6.00 (1)	7.00 (1)
Chi Square Statistic (df)	3.074592 (2)		
p-value*	0.227 (>0.005)		
IgA level			
Mean (SD) (ng/mL)	2200.6510 (322.05943)	2404.6516 (395.03208)	2278.1505 (321.37762)
F Statistic (df)	0.934 (2,31)		
p-value*	0.404 (> 0.005)		

Table 1. Age, dmft Index, Salivary Flow Rate, pH, and IgA Levels and Caries Status.

Discussion

In this study, there are no significant differences between salivary flow rate, pH, and IgA concentration with the severity of the ECC. This was concurrent with the study by Pallavi P. et. al., 2015 proved that there were no significant correlation between caries activity, salivary flow rate and pH.[11] Study by Bagherian A, et al, 2012 also discovered there were no statistically significant correlations between dmft index in ECC group and sIgA concentration. [7]

However from present study, there is a high median value of salivary flow rate and pH in the Severe-ECC group. In contrast with the study by Dwitha A. et al, 2014, Caries-Free group had a significantly higher mean salivary flow rate compared to minimal and Nursing Caries groups.[12] Study by Muchandi, S et al, 2015 also acknowledged the mean salivary pH was higher in Caries-Free group.[13] This inconsistency may be due to the different method used in saliva collection. These two studies used drooling method, while present study used absorption method to collect saliva.

Present study also found that the highest mean value of IgA concentration was seen in ECC group. This is supported by the pooled meta-analysis by Fidalgo et al. 2014 demonstrated salivary IgA levels in dental caries concluding that high levels of IgA were higher in patients with caries.[6] On the other hand, study by Omar et al, 2012, found that IgA levels were high in caries-free children and significantly lower values were obtained in the high caries group.[8]

Saliva plays an important roles in protecting soft as well as hard tissue in the oral cavity. Components of saliva, such as IgA antibody are secreted by salivary gland to provide barriers against pathogens. It acts as first line defence in immune system, and can enhance its role by co-action with other protein such as lysozymes. High level of IgA indicates of having high risk of getting oral diseases, such as dental caries, as host immune response. As mentioned by Haeri-Araghi et al, 2018 in their study, salivary IgA is only a response of immune to control the microorganism, and it is not a caries prevention component.[14] This support our result stated that high level of IgA was seen in ECC group, even though there was no significant differences between groups.

In addition, saliva also function as dilution to sugars after food intake. This prevent the acidic oral environment that can leads to tooth decay. Decreased pH oral environment indicates the acidic condition that will cause demineralization of the dental hard tissue.

Saliva also acts as physical protection to the hard oral tissue, by “bathing” them, prevent the adherence of microbial onto the surface.

Reduction of salivary flow rate will increase the exposure of the tooth of having dental caries.

In management of xerostomia patients, the use of salivary flow stimulant, such as sugarless chewing gums can prevent the caries progression, [15] as proposed by Nan Su et al, 2011. However, our result showed the high mean value of both salivary flow rate and pH were seen in Severe-ECC group which are not consistent with this theory.

One difficult factors for the present study was during saliva collection. Subjects aged range from 2-5 years old were difficult to follow the instructions given. They even cannot spilled or drooled saliva on their own. It was impossible to determine how accurate the flow rate measured. Therefore, this study favoured absorption method in collecting saliva. In future studies, it is recommended to use drooling technique in saliva collection considering on subjects' age and also other factors.

Our study findings show that salivary flow rate and pH had weak association with the occurrence of dental caries in children. This may due to the small sample size and improper technique during saliva collection and pipetting during ELISA Kit. Thus, future studies should interpret with caution an assessment of salivary pH or flow rate to determine the caries risk of all patients.

As stated in the results of the current study, the salivary IgA can be considered as an indicator for the immune system function, which may be increased by the number of decayed teeth. Further studies are needed in large scale population setting to understand our conflicting results. It is also recommended to assess other factors that can contribute to the development of dental caries such as child's age, social/biological factors, protective factors, and clinical findings as recommended by AAPD (2019).[16]

Addition to that, we do not include radiographic examination as one of the tools to diagnose caries. In future study, this radiographic examination may be include to diagnose interproximal caries that difficult to be seen clinically. As the result may become more significant and more reliable.

Conclusions

The result for this study showed higher level of secretory Immunoglobulin A (SIgA) in Early Childhood Caries (ECC) group although the result was not significant. Salivary flow rate and pH have not significant effects on the occurrence of ECC. However, the highest median value for flow rate and pH are seen in the Severe-ECC group.

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Declaration of Interest

The authors declare that they have no conflict of interest.

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