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ORIGINAL RESEARCH

The role of salivary sIgA as protection for dental caries activity in Indonesian children

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Purpose: The aim of this study is to asses the relationship between the level of sIgA and dental caries experience in healthy children who are 6- to 9-years-old from Indonesia. The case-control study is conducted to determine the protective role of salivary secretory immunoglobulin A (sIgA) levels in the stimulated whole saliva of dental caries-active and caries-free children.

Methods: This research was done by stimulating the whole saliva which had been collected from 6- to 9-years-old children with the index *def-t*≥3 of 30 children as the caries-active children group and 30 children with *def-t*<3 as the low caries-active children group. Saliva samples were collected in sterile vials between 10 am-12 pm due to the circadian rhythm, which is at least one hour after last meal. 1,5 ml of collected salivary sample was centrifuged, then the supernatants was transferred to other tube and stored immediately to the laboratory at a temperature of –20 °C. The estimation of sIgA concentration was done by using ELISA. The differences in the level of sIgA between the two groups with caries were analyzed using the *t*-test afterward.

Results: The total salivary concentration of sIgA was statistically significantly higher in the low caries-active children group than in the caries-active children group.

Conclusion: The total salivary concentration of sIgA was statistically and significantly higher in the low caries-active children group than caries-active children Group. There is a negative correlation between sIgA level and dental caries activity of 6 to 9-years-old children.

Keywords: sIgA level, ELISA, dental caries, Indonesia

Introduction

Dental caries is an infectious disease that causes health problems in some developed and developing countries. Based on Republic of Indonesia Basic Health Research in 2018, the *def-t* index in Indonesian children who are 6- to 9-years-old is 92,746.[1]

The risk of dental caries is controlled by saliva due to the presence of *Secretory Immunoglobulin A* (sIgA) as an antibacterial substance. Factors that play a role in the development of dental caries are the host response, bacteria in plaque as antigen, quality and quantity of diet, and time. Genetic and environmental factors are considerated to contribute to an increased risk of dental caries. A previous research has shown that there is a relationship between the genetic aspect and the immune response to dental caries.[2] Genetic factors have an impact on the introduction of antigen, immune response and dietary patterns. A research on humans and animals proves that genetic differences causes immunomodulatory deviations from antigens in which they play a role in dental caries.[4–11]

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SIgA in saliva has the same role as sIgA in the mucosal immune system. The roles of sIgA includes viral neutralization, neutralization of toxins, as well as growth and colonization of microorganisms in the epithelium or tooth surfaces.[7,12,13,14] Rashkova[34] classifies sIgA values of examined children with these criteria, up to 100 µg/ml as low sIgA group, medium sIgA in range 100–300 µg/ml and >300 µg/ml as high sIgA group.

Indonesia shows a high prevalence of dental caries with growth tending to increase. The Household Health Survey (SKRT) in 2004 showed that 39% of Indonesians suffered from dental and oral diseases. This study was conducted to predict the response of mucosal immunity to cariogenic bacteria through measurement of salivary titer in 6- to 9-years-old children from Java population in Surabaya Indonesia.

Methods

Study sample

Elementary students who were 6- to 9-years-old in Surabaya, East Java, Indonesia. The subjects were randomly selected from all areas of Surabaya, by dividing the municipality into Central, West, East, North and South region of Surabaya, it was based on the data from the Ministry of National Education in Surabaya. Judgment sample was taken in second grade elementary school in each region.

Clinical examination and evaluation of dental caries

The caries-active population were students with *def-t*≥3 and low caries-active population was students with *def-t*<3.[3] The sample was 60 children, They were divided into 30 low caries-active children group samples and 30 caries-active children group samples. Samples of saliva were performed on all elementary school students aged 6–9 years who met the inclusion criteria for the *def-t* test. Then the results of measurements *def-t* were used as the basis for determining low caries-active children group and caries-active children group by matching the age and sex of two sample groups. If there were students who could not match the criteria, then the student would not be included in the sample group.

Ethical aspect

All students were taken from the same population and ethnicity, Javanese population in Surabaya city. This matching was expected to reduce the possibility of differences in final results. Written informed consent forms were distributed one day before the saliva collection and parents or legal guardians of all participating students were given the written informed consent. All samples have determined several sample criteria. Six- to 9-year-old children, children who had suffered from upper respiratory tract infections in the past week were excluded from the study due to development of IgA and lysozyme. This study was approved by the Health Reseach Ethical Clearance Commission (Universitas Airlangga Faculty of Dental Medicine Number 307/HERCC.FODM/XII/2017).

Method of saliva collection

Stimulated saliva was collected in the morning, between 10 am and 12 pm, one hour after the last meal, this was done in order to prevent circadian rhythm effects at the concentration of saliva samples. Before collecting saliva samples, oral hygiene instruction was given to all students who would be the respondents in this study. The instruction required them to brush their teeth regularly. Moreover, prospective respondents were instructed not to chew anything for an hour before saliva collecting.

ELISA method for determining sIgA level

The sIgA level was examined through indirect ELISA (Immun Diagnostik, K8870) to describe the antigen-antibody reaction in units of ng/ml. SIgA levels were grouped by *def-t* index. Based on the clinical examination and evaluation of dental caries, we obtain data that the *def-t* index in this population ranges from 0–5

Results

Total from 60 research subjects, the case group which consists of 30 subject (11 boys and 19 girls) with the mean age of 93.3 months (7.7 years). The control group contained 30 research subjects (11 boys and 19 girls) with the mean age of 9.33 months (7.9 years). The sex and age data distribution in the total subject research is shown in Table 1.

Def-t index in low caries-active children group and caries-active children group

The *def-t* index is the index that describes the severity of dental caries in the early teeth, it is measured using WHO standards. Based on results of intra-oral examination in 60 samples, we have grouped samples into caries-active children group with *def-* index *t*≥3 and low caries-active

Table 1 Distribution of sex and age in the low caries-active children group and the caries-active children group

Sex	Caries-active children group	Low caries-active children group
Boys	36.7%	36.7%
Girls	63.3%	63.3%
Age (months)	93.33	95.33

children group with *def-t* index <3. Data of *def-t* index from the two groups are shown in Table 2.

SIgA level on low caries-active children group and caries-active children group

The result of sIgA titer assessment through ELISA testing on saliva samples based on the variety of *def-t* index valued by intra-oral assessment is shown in Table 3.

Correlation between sIgA and def-t index

There is a significant correlation between sIgA level and *def-t* index by −0,784. This correlation value reveals an inverse relationship between sIgA level and *def-t* index, in which the higher the *def-t* index value the lower the sIgA level, and vice versa. Correlation between the two groups are shown in Table 4.

Discussion

The study has been conducted on 60 subjects who have met the Declaration of Helsinki standard with the saliva as an analysis unit. Dental caries is an infectious disease with multifactorial causes that occurs in many populations of both developing and industrialized countries. Caries is more common in low socioeconomic groups, this is influenced by educational background and opportunities for health care15 The etiology of caries is influenced by host, environmental, and time factors. In particular, environmental factors are influenced by the accumulation of oral and dietary bacteria. Host factors are influenced by genetic variations of control of antibody secretion in saliva, and genetic variations in locus which takes control of the formation of the hard tissue of teeth, including amelogenin, enamelin and tuftelin.[16]

The role of sIgA in the oral cavity is to prevent the adhesion of *S. mutans* to the surface of the tooth, thus glucan is not formed and inhibits the demineralization process of hard tooth tissue. Various studies have shown that low sIgA levels in the oral cavity has implications for high caries risk, whereas high sIgA levels lead to low caries risk.[1,17,18]

The use of cut off in this study was *def-t* <3 in the low caries-active children group and *def-t*≥3 in the caries-active children group. It is based on previous studies that the increased levels of s-IgA in caries-active children might be a defensive mechanism to the number of *S. mutans* in whole saliva of caries-active children.[32]

As previously explained, the correlations relate to the role of saliva in dental caries. The secretion of sIgA from gingival crevicular fluid and the presence of sIgA in saliva play a role in the caries pathogenesis. Salivary gland hypophysis has an effect on the rate of saliva flow and also affects the development of dental caries. In addition, Several studies have reported the fact that treatment with psychopharmaceutic drugs and unregulated diabetes treatment have an effect on the decrease of saliva flow rate.[19–21]

The relationship between carbohydrate diet and dental caries is difficult to predict. If a person consumes a large amount of sugar while at the same time also consuming fluoride treatments, the sugar consumption does not damage the tooth tissue.[22–25]

The history of dental caries for mothers might increase the risk of caries for their children and the caries history in the early tooth phase is a prediction of caries in the permanent dental phase.[26–30] Age affects dental caries due to mucosal immunity to cariogenic bacteria played by sIgA which is in line with body immunity maturity. The measurement of child immunity is recommended over

Table 2 *Def-t* index in low caries-active children group and caries-active children group

Group	n	*def-t index*				Wilcoxon-Mann-Whitney test
		Mean	SD	Minimal	Maximum	
Low caries-active children group	30	0,7	0,651	0	2	*p*=0,000
Caries-active children group	30	4,17	0,592	3	5	

Table 3 SIgA titer assessment through ELISA testing on saliva samples based on the variety of *def-t* index value

Group	*def-t*	n	sIgA		*p*-value
			Mean	SD	
Low caries-active children group	0 1 2	12 15 3	545.833 502.667 490.000	90.298 115.571 105.000	$p<0.001$
Caries-active children group	3 4 5 ∑	3 19 8 60	138.334 178.263 172.250	37.527 72.647 94.803	

Table 4 Correlation between the sIgA level and *def-t* index

	sIgA	*def-t*	*p*-value
sIgA	–	−0,784	$P<0,000$
def-t	−0,784	–	

6-years-old because the immune system is considered to be complete at this age.[31]

Dental caries remains a common dental health problem and often found in early childhood, between the ages of one and six years. Children of preschool age are one of the most vulnerable groups to dental and oral disease because generally they do not properly maintain their oral health. Parental care is an important factor as it serves as the basis of the formation of behaviors that support, or not, childhood oral hygiene. Prevention and management of dental caries in children need special concern. Mothers' beliefs about the importance of maintaining children's dental health is the main principle in the development of early childhood caries.[35,36]

Conclusion

The total salivary concentration of sIgA is statistically and significantly higher in the low caries-active children group than caries-active children group. There is a negative correlation between sIgA level and dental caries activity in 6- to 9-year-old children. As can be seen, sIgA levels of stimulated saliva has some roles, one of them is as protection against dental caries. Further research is needed on the effect of sIgA as protection against the activity of dental caries, with various tests and increasing the number of samples. Long term studies should be conducted to investigate sIgA levels and dental caries with different *def-t* indexes.

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Disclosure

The authors report no conflicts of interest in this work.

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