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**THE DIFFERENCE OF SALIVARY SECRETORY IMMUNOGLOBULIN A (sIgA)
 LEVEL BETWEEN MALE ASTHMATIC CHILDREN AND FEMALE ASTHMATIC
 CHILDREN**

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ABSTRACT

Background: Asthma is chronic airway inflammatory disorder where mast cells, eosinophils, and T lymphocytes play important role. The symptoms include wheezing, recurrent cough, shortness of breath, and suppressed chest. The age prevalence of asthma children is quite high and increases annually. Asthma affects boys twice the rate of the asthma as girl. Asthmatic patients need local immunity and antibodies so infections that occur in the oral cavity as a result of inhalation medication use can be avoided. The antibody of the oral defense system is secretory immunoglobulin A (sIgA). **Objective:** To explain the difference in salivary sIgA levels in male asthmatic children and female asthmatic children. **Methods:** The study was conducted on 21 subjects of asthmatic children consisted of 11 male and 10 female with the age range 9-11 years. Saliva sampling was taken in RSKP Respira Bantul, then it was taken to Laboratory of Molecular Biology FK UGM for ELISA test. Saliva was taken by Spitting method for 1.5 mL and done in the morning from 8-11 hours. Subjects were not allowed to eat 1 hour prior to salivary taking. Anamnesis and clinical examination were performed to see the condition of oral cavity, social status, and nutritional status. Saliva measurement using Anti-human sIgA ELISA kit (Elebscience). Data were analyzed using Independent T test. **Results:** The mean of sIgA level in males was $0.18 \times 10 \pm 0.06$ and females was $0.15 \times 10 \pm 0.03$. Based on the results of the Independent T test, it was found that the p value = 0.283 ($p > 0.05$). **Conclusion:** There is no difference in sIgA levels between male and female patients.

Keywords: Asthma, sex, salivary sIgA

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PRELIMINARY

Asthma is a global health problem that mostly occurred in developed and developing countries. Increased asthma associated with air pollution from industry and automotive, home interiors, lifestyle, smoking habits, diet, use of bottle milk and exposure to early allergens. Asthma has a negative impact on the lives of sufferers, especially children. Asthma is a chronic airway inflammatory disorder with many cells that play roles, especially mast cells, eosinophils, and T lymphocytes. Symptoms include wheezing, recurrent cough, shortness of breath, and suppressed chest. ¹ The prevalence of asthma continues to increase each year in both developed and developing countries. ² Asthma is

divided into 3 classifications, which are: 1) mild asthma, is an asthma condition with frequency of attacks that are very rare to arise so it doesn't require control drugs. 2) Moderate asthma, is a condition of asthma that is quite often arise, disrupt the activity, and require anti-inflammatory drugs. 3) Severe asthma, an asthma condition that often arises, disrupt daily activity and requires anti-asthma treatment

In childhood, boys have a higher risk of asthma than girls. Differences in physical conditions, activity, hormonal, and growth spurt also contribute to the risk of asthma trigger. ⁴ Usually asthma that begins from childhood to adulthood resulted in the long term use of anti-

asthma medication. Medication of asthma has a negative impact in the oral cavity as reflected in the increased incidence of caries, dental erosion and periodontal disease.⁴ Inhalation medication of anti-asthma used for a long time can cause inflammation of the parotid gland resulting in decreased salivary amount and reduced secretory IgA which represent as an important antibacterial component.⁵

Asthma sufferers require local immunity and antibodies so that infections that occur in the oral cavity as a result of inhalation medication use can be avoided. IgA secretory saliva is one of the antibodies present in saliva.⁶ The sIgA antibody can help oral immunity in preventing microbial attachment, neutralizing enzymes, toxins, and viruses or creating synergies with other factors such as lysozyme and lactoferrin. The sIgA concentration is directly and positively correlated with the severity of periodontal tissue inflammation

Secretory Immunoglobulin A (sIgA) saliva level may be elevated in the presence of local stimulation by antigens, and decreased by various factors such as environmental factors and lifestyle, physical activity, nutritional deficiencies, drugs, viruses or impaired immune function.³ Classification of SA levels saliva in children based on research ie; (1) low (0-100 $\mu\text{g} / \text{mL}$). (2) medium (100-300 $\mu\text{g} / \text{mL}$). (3) high sIgA (> 300 $\mu\text{g} / \text{mL}$).⁸

This study aims to determine differences in salivary sIgA levels in male and female children with asthma. This study is expected to be useful in providing information to the dentist about the problem of oral cavity that often occurs in children with asthma, so that the dentist's child can provide prevention efforts by increasing sIgA saliva levels to overcome or prevent the oral cavity problem.

RESEARCH METHODS

This study was a clinical epidemiological study using survey-analytic method with cross sectional design. The study used nonprobability sampling method, ie consecutive sampling.¹⁰ The subjects of the study were children age 9-11 years who were examined at RSKP Respira Bantul. Saliva samples were obtained from 21 samples consisted of 11 boys and 10 girls with asthma. Saliva samples were then taken to the Laboratory of Molecular Biology FK UGM. Research period was from May to July 2016. Ethical clearance of research explanation (Ethical Clearance) was No. 0052 / KKEP / FKG-UGM / EC / 2016.

Preparations to be undertaken prior to the research were: (a) Ethical clearance: Application for approval of research from the Dentistry Ethics Committee of Gadjah Mada University, Yogyakarta. (b) Applications for permission for research are conducted at RSKP Respira and molecular biology laboratory Faculty of Medicine,

Gadjah Mada University, Yogyakarta. (c) selection of research subjects based on the inclusion criteria. (d) The researcher provides information both orally and in writing about the research that would be conducted to the child's parents. Those who were willing to be the subject of research would be given informed consent (informed consent) which must be filled and signed by parents of the research subjects. (e) During the study, the subject would be accompanied by a parent / guardian and explained briefly about the research procedure. (f) Collection of saliva was performed 1.5-2 hours after the last eating subject.

The collection of saliva was using spitting method. The subject of the research was put in a calm and silent sitting position while bowing the head and holding a measuring cup of saliva container on the right hand (figures 1 and 2). Salivary collection was performed for 10 minutes, then every 1 minute interval subjects were asked to remove saliva accumulated in the mouth into the measuring tube through the measuring glass.

Measurement of salivary flow rate was done by looking at the amount of saliva that was collected and then continued with the calculation of the average value and recorded. Saliva flow rate <0.1 ml / min is salivary flow rate in asthmatics, whereas in healthy individuals salivary flow rate \geq 0.3 -0.7 ml / min.¹¹ The collected saliva was directly inserted into the Tube Separator Sample (SST) and stored in a cupboard cooler (freezer) - 80°C in Molecular Biology Laboratory FK UGM Yogyakarta.



Figures 1 and 2. The subjects performed the saliva taking with the Spitting method

Processed sample in the laboratory was sample originally stored in a frozen state and then melted at room temperature. Then all the samples were centrifuged at 1500 \times g (@ 3000 rpm) for 15

min at 22 ° C. After that, 25 µL supernatant from saliva was taken and inserted into prepared sterile and previously labeled.

Measurement of sIgA with ELISA kit (ELABSCIENCE): microplate was prepared, samples and all reagents consist of: sIgA Antibodies-Enzyme Conjugate, standard sIgA, Diluent, wash buffer, Tetramethylbenzidine (TMB) and stop solution, determining microplate design, µg / mL, 200 µg / mL, 66.7 µg / mL, 22.2 µg / mL, 7.4 µg / mL, and 2.5 µg / mL. Put 3 mL SIX diluent 1X into one tube, saliva sample was diluted by inserted 100 µL SIgA diluent 1X into sterile pipes. Then 25 µL saliva from each sample was put into one tube for each samples, one tube of 12 x 75 mm caps then labelled for each unknown standard, control, and sample, and one tube for zero. 4 mL SIgA diluent was added 1X into each tube.

10 µL standard (from step 3) was added, control or unknown saliva sample (from step 4) to the appropriate tube. 10 µL SIgA diluent was added 1X to the zero tube, the 1: 120 conjugate antibodies was diluted by adding 25 µL conjugate to 3 mL of 1 µg diluent 1X prepared in Step 3. Mix up and average 50 µL conjugate antibodies has been diluted into all the tubes using a pipette. Each tube was mixed slowly by reversing and incubating it for 90 minutes at room temperature, inversion and adding 50 µL of solution from step 6 to microtitre plate. The adhesive plate was closed and incubated at room temperature by continuously mixing at 100 rpm for 90 min, washing the plate with a wash buffer. Each well on plate was washed with 250 µL wash buffer. After each well was washed, cleaned it with tissue paper before placing the plate reversed in an upright position.

50 µL TMB solution was added into each well using multichannel pipette. The plate rotator was mixed for 5 minutes at 100 rpm and incubated the plate in the dark room at room temperature for 40 minutes. Exposure to light was avoided because it was very sensitive to light

50 µL stop solution was added using multichannel pipette. Then placed on plate rotator for 3 minutes at 500 rpm. Make sure the whole well has changed to yellow. If the color is still green, continue mixing. The bottom of the plate was wiped with a cloth soaked with water and dried. Plate reader was readat 450 nm. The plate was read within 10 minutes of adding stop solution

After the data collection, then statistical analysis were performed using the Independent T test to see the significance of salivary sIgA levels in children with asthma male and female asthma.

RESEARCH RESULT

This study was conducted to see the influence of asthma severity on secretory levels of immunoglobulin A in saliva .The subjects of asthma research that met the inclusion and

exclusion criteria were taken. They consisted of 21 patients which were presented in Table 1

Table 1. Distribution of Research Subjects (n = 21)

Characteristic	Frequency	Percentage
Sex		
Female	10	47,6
Male	11	52,4

Table 1 shows that the number of male asthma patients in this study is higher than the number of female patients, where male patients consist of 11 children and female patients of 10 children.

Levels of secretory immunoglobulin A (sIgA) saliva patients by sex. A description of the secretory levels of immunoglobulin A (sIgA) of patient saliva by sex is presented in Table 2.

Table 2. Mean saliva sIgA levels by sex (µg / mL)

Sex	n	Level of salivary sIgA (mean±SD)
Male	11	0,18x10 ³ ± 0,06
Female	10	0,15x10 ³ ± 0,03

Table 2 above shows that the mean sIgA level in male patients is 0.18x10³ with standard deviation 0.06 and in female patients is 0.15x10³ with standard deviation 0.03. This shows that the means of IgA level in men is higher than in women

The p value of Shapiro-Wilk test on saliva sIgA level by sex was p> 0.05 which means that the data was normally distributed. This was followed by an Independent T test with p value of the equal variances not assumed in the column because the variant was not in the same value as the result shown in table 5. The similarity of variance was obtained from Levene's test with p <0.05.

Table 3. Independent T test levels of Saliva male and female Patients

Variable	t count	t table	P
Sex	1,118	2,093	0,283

Based on the results of the Independent T test above, it is obtained that p-value equal to 0.238 (p>0.05) which means that there was no difference in sIgA level between male and female patients.

DISCUSSION

Many factors affect the value of a salivary sIgA level which cause the result of this study to become meaningless. One of them is the lack of strict borders in determining the inclusion criteria of socio-economic background. This is caused by the small number of the samples which can not meet the desired large number of samples within a certain time. Patients who came with diverse socioeconomic and educational backgrounds can

illustrate the difference in nutritional intake and the condition of the oral cavity.

At the time of this study, the researchers examined the oral cavity of the subjects, of which only 8 children of 21 subjects who had problems in the oral cavity such as caries, and gingivitis. This indicates that the parents were concerned enough with the hygiene of the child's oral cavity. Based on the anamnesis of the socio-economic background, it can be found that the average number of subjects were came from lower to middle economic families, indicating the subjects to get a variety of nutrients. Nutritional intake and OH also affect salivary sIgA levels.

The condition of the child in mixed dentition also affects the level of IgA in the gum fluid clearance, whereas when the tooth fluid can come out from the gap and mixed with saliva fluid.¹² The use of direct inhaler or nebulizer during the first asthma attack can also inhibit the increase in salivary sIgA levels, so that sIgA can not protect the body from inflammation maximally. Taking saliva during asthma attacks has not been able to determine salivary secretory immunoglobulin A (sIgA) levels.

In this study, the number of male patients are higher than the female due to the higher visit rate of asthma male patients in RSKP Respira Bantul. This can be seen in the data of asthma patient's visit from January 2016-July 2016 in general hospital RSKP Respira Bantul. Based on the number of visits, this study supports the statement Almqvist et al (2007) which mentions that boys have a higher risk of asthma than girls. This is because boys have narrower airways, many allergic trigger cells which are eosinophils that act as important mediators of the cause of asthma. Whereas for severe asthma, there is no different incidence between boys and girls affected by asthma. The results of this study also support the Javarzadeh et al (2010) study which states that there is no significant difference between salivary sIgA levels in boys and girls.

The results of this study concluded that there is no difference in levels of salivary secretion immunoglobulin A (sIgA) in male asthmatic children and female asthmatic children. Therefore, it is necessary to develop research with a wider range of age and environment with a number of customized samples. The existence of further research on the use of anti-asthma inhalation drugs as inclusion criteria is needed for more accurate research results.

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