Serum IL-6 levels of atopic patients with recurrent aphthous stomatitis (RAS)

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ABSTRACT

Introduction: Atopy is a genetic tendency of allergic condition with specific clinical manifestations such as atopic dermatitis (AD), allergic rhinitis (AR), and bronchial asthma (BA). Atopic individuals might experience other health problems that coincide with atopic manifestations, including the appearance of recurrent aphthous stomatitis (RAS) which is an oral mucosal disease that can also be influenced by allergies. This study was aimed to be the preliminary study regarding the level of IL-6 serum in atopic patients who were also having RAS. **Methods:** Thirty subjects were recruited and grouped into three groups with ten subjects each were group of atopic (dermatitis, allergic rhinitis, or bronchial asthma) with RAS, atopic without RAS, and control. Blood specimens were obtained with an approved institutional board review protocol. This research was cross-sectional research with consecutive sampling method conducted at Dr Hasan Sadikin Hospital Bandung. **Results:** There were significant differences in IL-6 log levels in all three groups ($p < 0.001$). The higher IL-6 levels in the group of atopic with RAS might be due to the influence of atopy because this condition can increase the acute inflammation of RAS. IL-6 is a pro-inflammatory marker found in the circulation of atopic and RAS patients. **Conclusion:** Serum IL-6 levels in the group of atopic patients with RAS is higher than in other groups.

Keywords: Serum, IL-6, atopic, recurrent aphthous stomatitis

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INTRODUCTION

One of the most common oral mucosal diseases is recurrent aphthous stomatitis (RAS). RAS is a disease of the oral mucosa in the form of recurrent ulceration triggered by many factors, including genetic and allergies due to food.[1-3] Some allergic condition occurred in individuals with atopy, which is an abnormal type of hypersensitivity against environmental substances which was observed

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only in humans and tended to occur within families (genetic factor) without obvious prior sensitisation.[4] Many atopic patients complained about RAS, including pain, disturbing mastication, or difficulty when talking, and if this condition often occurs, their daily activities will be very disturbed. Research about RAS and atopy rarely get attention but our study in atopic population showed that as many as 31% atopic patients also having RAS, and there is a relationship between RAS and total serum IgE levels as a biomarker in atopy with the results of the point-biserial correlation coefficient rpbi was 0.534, which means that the correlation was quite strong. Atopy is characterised by an increase in IgE levels as a biomarker of hypersensitivity conditions or an allergic reaction in the body, but as we know the immune system of our body is very complex, there are many biomarkers involved in a disease condition.

Increasing level of IgE in atopy showed us there is an allergic condition occurred, and with complete examination such as skin prick test or patch test can show aetiology of the disease.[4] The relationship between risk factors for the occurrence of a disease can be determined through a biomarker of the body's immune system in an inflammatory response that is regulated by complex mediator networks and signalling patterns, for example, cytokines including interleukin (IL).[5] IL-6 is known as a pro-inflammatory cytokine while having regenerative or anti-inflammatory activity. IL-6 also has an important role during the immune process.[6] High levels of IL-6 as a proinflammatory cytokine are found in circulation or tissue ulcer in RAS patients, or atopy separately.[7,8] Based on our previous study showed that RAS is part of atopy, there is a mechanism for the pathogenesis of RAS that is affected by the same allergic conditions that trigger the emergence of atopy. Fornasa et al.[9] also published research about RAS and atopy, and found that familiar prevalence of atopy symptoms in patients with RAS was present with statistical significance indicates that RAS belongs to the heritage of an 'atopic background'. Rashkova and Peneva[2] also showed that 55% of the children with RAS have a case history with concurrent allergic conditions. It has been established that there is a statistically significant correlation between the frequency of the concurrent allergic conditions and the occurrence of RAS.[2] There have been no studies on biomarkers of RAS in atopic patients thus made us interested in examining serum IL-6 levels which are often found in RAS and atopy conditions separately. This study was aimed to be the preliminary study regarding the level of IL-6 serum in atopic patients who were also having RAS.

METHODS

This research was a cross sectional study. Sampling is in accordance with the length of the study (consecutive sampling) which is in a month. Research population are atopic patients include who have one of atopic disease such as atopic dermatitis, allergic rhinitis, or bronchial asthma. The study sample was patients who met the inclusion and exclusion criterias, and held at Dr. Hasan Sadikin Hospital Bandung in September 2018. The inclusion criterias were ages between 18-40 years old, male or female, having a RAS history with more than twice recurrence every year, and has atopic disease (atopic dermatitis/allergic rhinitis/bronchial asthma), and subject willing to join this research by signed the informed consent form. The exclusion criterias were done based on anamnesis and clinical examination to exclude subjects with herpetic stomatitis, traumatic ulcer, or other recurrent oral ulceration, having oral mucosal condition related allergy such as angioedema, oral allergy syndrome, or geographic tongue.

We were asked to fill out a questionnaire to find out the history of RAS and atopy. All atopic patients divided into two groups; atopy with RAS and atopy without RAS, then we collect a group of either not atopy or RAS as control. Then 5 ml blood samples were taken to determine serum levels of IL-6 using human enzyme-linked immunosorbent assay (ELISA) kit by laboratory assistants from the clinical pathology laboratory Dr. Hasan Sadikin Hospital. This investigation was approved by the Health Research Ethics Committee of Universitas Padjadjaran with Registry Number 990/UN6.KEP/EC/2018.

Data were analysed by R version 3.4.1 for Windows operating system. All statistical test were performed using level of significance 0.05. Data IL-6 was measure using one way ANOVA. To

fulfill the assumption of the one-way ANOVA test, the IL-6 variable transformation is done using natural logarithms. Then post-hoc test with the Tukey-HSD method to analyze each atopy group with healthy groups.

RESULTS

This study included 30 subjects. There were no significant differences in age and sex variables in the three groups (Table 1). Atopic manifestations in this study found that 10 subjects had atopic dermatitis history since childhood, 8 subjects with a history of allergic rhinitis since adolescence, and 2 subjects with bronchial asthma after adulthood.

The assumption of the one-way ANOVA test is that three groups have homogeneous variants and the distribution data in each group has data characteristics that are normally distributed, but with existing data, the two assumptions have not been fulfilled, so other efforts are needed, one of which is data transformation. We choose natural logarithms as an option that is quite often used for data on biological markers such as output in our study. The data analysis of IL-6 level using natural logarithms showed there were significant differences in IL-6 log levels in all three groups ($p < 0.001$) (Table 2).

Comparison log IL-6 (pg/mL) levels between various groups using post-hoc test with the Tukey-HSD method showed that there were significant differences in IL-6 log levels between groups of control and groups of atopic with and without RAS (Table 3).

Table 1. Characteristics of the subject groups

Variable	Atopy with RAS (n = 10)	Atopy without RAS (n = 10)	Control (n = 10)	p-value
Age (year); mean (SD)	29.4; (5.9)	32.5; (4.7)	30.4; (6.6)	0.481#
Sex; frequency				
Male	2	1	4	0.430$
Female	8	9	6	

SD= standard deviation; RAS =recurrent aphthous stomatitis; #One-way ANOVA test; $Fisher exact test

Table 2. Data analysis of IL-6 levels with natural logarithms

Variable	Atopy with RAS (n = 10)	Atopy without RAS (n = 10)	Control (n = 10)	p-value
Log IL-6 (pg/mL); mean (SD)	4.96; (1.51)	3.99; (1.59)	0.69; (0.51)	< 0.001#

SD= standard deviation; RAS =recurrent aphthous stomatitis; #One-way ANOVA test

Table 3. Comparison log IL-6 (pg/mL) levels between various groups

Log IL-6 levels (pg/mL)	Average difference	95% CI	Adjusted p-value
Control vs atopy with RAS	-3.30	-4.74; -1.86	<0.001*
Control vs atopy without RAS	-4.27	-5.72; -2.84	<0.001*
Atopy without RAS vs atopy with RAS	-0.98	-2.42; 0.46	0.231*

CI= confidence interval; *Post-hoc test with the Tukey-HSD method

DISCUSSION

Atopy is regulated through immune phenomena involving many cells, including mast cells, eosinophils, and T lymphocytes, and involves cytokines, chemokines and neuropeptides.[10] Excessive production of cytokines IL-4, IL-5, IL-6, IL-9, IL -10, and IL-13 can activate B lymphocytes to produce immunoglobulin E and cause atopic manifestations.[11-13] IL-6 is a key cytokine in the host mechanism that functions as a proinflammatory and anti-inflammatory cytokine. As a proinflammatory cytokine, IL-6 initiates the production of IL-4 and IL-5, the predominant Th2 cytokines in atopic dermatitis.[14] The most common atopic manifestations in this study are atopic dermatitis following allergic rhinitis and

bronchial asthma, this result are accordance with atopic march which is based on data from the age that first experienced atopic conditions.[15]

RAS is a common oral mucosal disease with the predilection onset before the age of 30 years, but the results of other studies in groups with age before 40 years.[16,17] This study showed that most patients with RAS in age range 20-40 years old, and the prevalence of female is more than male, as well as the results of previous studies.[17,18] Previous study showed that the level of IL-6 in serum from patients with asthma was significantly higher than that from controls.[19] IL-6 levels in this study showed that in a group of atopic with RAS have higher level than other group (Table 2).

There are two asthmatic patients in this study which are in group of atopy with and without RAS, they all had more higher serum IL-6 level compare with control group. This study also showed there is significant differences in IL-6 log levels in all three groups ($p < 0.001$). The higher IL-6 levels in first group (Log IL-6 level with mean = 4.96 pg/mL; SD = 1.51) might be due to the influence of atopy that this condition can increase the acute inflammation in RAS patient. Genetic and environmental factors in RAS may contribute to imbalance of the Th1/Th2 immune pathways.[20] Barros et al.[21] had published their study which showed factors that favor the onset of recurrent aphthous ulceration have been correlated with a Th1, but some recent studies have shown that gene polymorphism IL-10 found in RAS and indicated that IL10 that relesead by Th 2 have association with predisposition of individuals to RAS.[21,22] In our study also showed that the role of Th2 cells is more dominant with the presence of IL-6 secretion higher than other groups.

CONCLUSION

Serum IL-6 levels in group of atopic patients who having RAS is higher than other groups. Atopy can have an effect on increasing IL-6 levels in patients with RAS.

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