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DETERMINATION OF SERUM INTERLEUKIN 33 (IL-33) LEVELS IN ATOPIC ASTHMA PATIENTS USING ELISA KITS

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ABSTRACT

This study compares serum IL-33 levels in atopic asthma (AA) patients and normal controls to determine serum IL-33 levels. 5 mL of blood were withdrawn from subjects and centrifuged for 5 min at 2000 rpm to obtain the serum and were analyzed for IL-33 using kits. Data entry and analysis were done using SPSS version 20.0. The results showed serum IL-33 levels were higher in atopic asthma patients than in normal controls. This difference was not statistically significantwith a p-value of 0.218 determined by an independent-T test. The results also showed no significant difference in IL-33 levels according to AA severity with a p-value 0.396 determined by a Kruskal Wallis test. There was no significant difference of serum IL-33 levels between normal controls and AA patients. The levels of IL-33 and other predisposing factors showed significant associations in smoking status and occupational exposure.

Keywords: IL-33; atopic asthma; serum levels; severity; ELISA.

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1. INTRODUCTION

Atopy refers to an allergic disease due to overprodustion of IgE. It responds to one or several common environmental allergens [1]. House dust mites, Dermatophagoidespteronyssinus and Dermatophagoides farina are the most common allergens in Malaysia. These allergens trigger the symptoms of allergic disease [2]. Atopic diseases also consist of atopic dermatitis (AD), allergic rhinitis (AR) and atopic asthma (AA). A condition marked by recurrent attacks of airway inflammation and wheezing due to spasmodic construction of bronchi is reflected to asthma. AA is one of the common diseases of childhood causing substantial morbidity. Asthma is also linked with the immunity just like the other allergic reactions [3].

Asthmacan be identifiedintothree classesaccording to factors that caused the symptoms in contributing to this allergic response.Atopicasthma also can be divided into extrinsic and intrinsic asthma. Extrinsic asthma is reflected to an allergy to antigens that react with allergens. The allergensspread in theair in any types offormation such as pollen,dust,smoke,automobileexhaust or animaldander.Morethan50% of thecases in childrenandyoungadultsare consisted in this type of asthma. The other type of asthma is intrinsicasthma. It is usuallysecondary to chronic or recurrentinfections of thebronchi,sinusesandadenoids.Thistype of asthma developsfrom a hypersensitivity to the invader like bacteria, viruses and other causative factors causingtheinfection.The infections,emotionalfactorsandexposure to nonspecificirritants can be the common trigger that leads to this type of asthma.

The cytokines which are the mediators produced by the cells in the form of soluble molecules that act on other cells to respond on their function are the regulator of the immunity [4]. The development of immediate hypersensitivity reactions against common environmental antigens contributes from the cytokine. This action may be reflected form anaphylaxis response. IL-33 appears to be an important regulator of T_H2 responses. The studies done before in other types of cytokine demonstrated that IL-31 expression increased in allergic patients than normal person. However, there was no significant difference regarding to the levels of IL-31 in both groups of subject [5-6].

The clinical manifestation of asthma includes recurrent wheezing, respiratory problems, chest tightness, sleep disturbances, constraints in doing daily activity, lung function impairment and

the usage of rescue medications. There are other symptoms of asthma such as nasal congestion, runny nose and eye irritating problems.Exercise may leads to this symptom's occurrence. Some asthma attacks are triggered by cold, heat or stress. This often contributed to worsening of symptoms.Thesymptoms occur or worsen at night and sleep disturbance happened due to awakening the patient. Reversibility airflow obstruction is determined by an increase in FEV_1 of > 200 mL and ≥ 12% from baseline measure after inhalation of short-acting β_2-agonist (SABA).

Proliferation of smooth muscle cells and upregulation of the adhesion molecules ICAM-1 and VCAM, thereby favouring interaction with T cells as TNF released increased from an inflammatory cells.

Interleukin-33 is a new member of the IL-1 superfamily of cytokines. It expressed by mainly stromal cells like epithelial and endothelial cells. Its expression is upregulated following pro-inflammatory stimulation. IL-33 is a cytokine that functioned as a nuclear factor regulating gene transcription. IL-33 leads in alerting the functions of immunity to tissue damage, stress and other response after the cell necrosis.

The development of allergic diseases in an individual has both genetic and environmental components. The diagnosis of triad diseases (AD, AR and AA) are based on clinical history and physical examination. The association between IL-33 levels and AA is not well described in the literature. Thus, this research aims to determine the levels of serum IL-33 in a local population in Malaysia. We compared IL-33 levels between AA patients and normal controls as well as their severity. The associations between IL-33 levels and predisposing factors between AA and normal controls also done to relate all the factors study.

2. METHODOLOGY

2.1. Study Design

A cross-sectional study was conducted and included patients with AA who met the GINA classification criteria (Table 1).

The detailed explanation regarding the study protocol and a written consent form was given to all participants or their guardian before participated in the study. After getting the informed consent, basic data such as full name, registration number, full address, contact number, age,

sex, identification number, medications history, family history and smoking status were obtained by interviewer. The data also is getting from the patient's medical record. The patients were recruited from the chest clinic and paediatrics clinic at the Hospital UniversitiSains Malaysia (HUSM).The sample size was calculated to compare the mean level of IL-33 in controls and AA patients by using PS software. DD is based on expert opinion. DD = 100 pg/mL. The medium effect size was used to estimate the SD.

$$SD = \frac{DD}{d} = \frac{100}{0.5} = 200 pg/dL \quad (1)$$

where SD = standard deviation, DD = detectable difference and d = effect size. Based on PS software, t-test was used.

$\alpha = 0.05$ (2)

$1 - \beta = 0.8$ (3)

$DD = 100$

$SD = 200$

$m = 1$ (4)

$n = 63$

$63 + 10\% = 69$ per group ≈ 70 per group

2.2. Recruitment of Sample

In this study, 70 subjects were selected for normal controls and 70 subjects were enrolled for atopic asthma patients. Five mL of blood were collected from subjects and centrifuged for 5 min at 2000 rpm.

2.3. ELISA Procedure and Analysis

IL-33 levels were measured using a commercially available ELISA kit (R&D System). Each well of a 96-well microplate was immediately coated with 100 mL of the unlabeled monoclonal antibody specific for human IL-33. Then, the microplate was sealed or covered before incubated overnight at room temperature. After overnight incubation process, each well was aspirated and washed with wash buffer.

The process was repeated two times for a total of three washes. Then, the plate was blocked by 300 mL of reagent diluents added into the each well. Then, the microplate undergoes the incubation process at room temperature for a minimum of 1 hour. The repeated washes as in the previous washing step take place before get ready for sample addition.

A standard and one hundred microliters of a sample in reagent diluents was added to each well. After the sample was added into each well, the plate was incubated for 2 hours at room temperature. Then, the wash was repeated as similar process during plate preparation. One hundred microliters of biotin-labeled anti-human IL-33 detection antibody was added to each well. The plate was incubated at room temperature for 2 hours and the washing process was repeated. One hundred microliters of horseradish peroxidase (HRP) labelled anti-biotin antibody was added to each well. Then, the plate was incubated for 20 min and the washing process was repeated. After that, 100 mL of tetramethylbenzidine (TMB) substrate solution was added to each well. Then, the plate was incubated for 20 min at room temperature. After that, 50 mL of stop solution was added to each well. The color development was observed and the intensity was measured at 450 nm using ELISA reader.

2.4. Data Analysis

IL-33 serum levels of AA patients and normal controls were compared using the Independent-t test and IL-33 levels according to severity were determined using the Kruskal Wallis test in SPSS version 20.0 [7]. The associations between IL-33 levels and predisposing factors between normal controls and AA were determined by using simple logistic regression and multiple logistic regressions [8].

Table 1. GINA classification

Characteristic	Controlled	Partly Controlled	Uncontrolled
Daytime symptoms	None (twice or less/week)	More than twice/week	Three or more features of partly controlled asthma present in any week
Limitations of activities	None	Any	
Nocturnal symptoms/awakening	None	Any	
Need for reliever/rescue treatment	None (twice or less/week)	More than twice/week	
Lung function (PEF or FEV1)$^{++}$	Normal	< 80% predicted or personal best (if known)	
Exacerbation	None	One or more/year*	One in any week^{+}

*Any exacerbation should prompt review of maintenance treatment to ensure that it is

adequate

+By definition, an exacerbation in any week makes that an uncontrolled asthma week

++Lung function testing is not reliable for children 5 years and younger

3. RESULTS AND DISCUSSION

3.1. Demographic Data

A total of 140 subjects (70 atopic asthma patients and 70 normal controls) were enrolled in the study. Table 2 showed the demographic data of subjects between normal controls and AA patients. The mean (SD) age of AA patients was 40.93 (16.90) and controls was 32.49 (10.73). The majority of controls were in the age range of 18-35, which counted for 60.0%. For AA, majority of patients were over than 35 years old (70.0%). For patients, more than 50.0% were females. Majority of racial distribution were Malays for patients and controls with percentage range 87.1%-97.0%.

In this study, the mean age of the controls was 32.49 with a minimum age 18 years old and the maximum age was 54 years old. This study was slightly similar with the previous study which showed the mean age of subjects was 32.30 with the age range of 18 to 64 years old. More than half of subjects for AA patients were females.

This figure may reflect the domination of females in our country. In contrary, some studies in general, the prevalence of allergic patients is equal between men and women [2].Gender and age are important determinants of allergy occurrence and hospitalization. The effect of sex on allergy varies with age. However, it is not clear if the sex difference stays similar in adults across ages.

Malay was recorded higher compared to other races. This might happened due to most of the population in Kelantan were Malays, followed by Chinese and Indian. In this study, subjects were taken from people around HUSM with the random sampling method due to time constraint during sample's collecting.

Table 2. Demographic data of controls and atopic asthma patients

	Controls, n (%)	AA, n (%)
Age		
< 18	0 (0.0)	9 (12.9)
18-35	43 (61.3)	12 (17.1)
> 35	27 (38.6)	49 (70.0)
Sex		
Male	35 (50.0)	24 (34.3)
Female	35 (50.0)	46 (65.7)
Race		
Malay	68 (97.0)	65 (92.9)
Chinese	1 (1.5)	5 (7.1)
Indian	1 (1.5)	0 (0.0)
Others	0 (0.0)	0 (0.0)

3.2. IL-33 in Normal Controls and Atopic Asthma Patients

The patients were taken from clinic with the random sampling method. Table 3 showed the mean (SD) IL-33 serum level was higher for AA patients rather than the normal control subjects. However, there was no significant difference in the IL-33 serum levels between AA patients and healthy control subjects.

Table 3.IL-33 levels in normal control subjects and atopic asthma patients

Subjects	Frequency, n	Mean (SD)/µg/ml	*p-Value
Atopic asthma	70	1875.27 (11075.83)	0.218
Normal controls	70	233.02 (670.34)	

*Result was significant if p-value < 0.05 by independent-t test

IL-33 level was higher in AA patients rather than normal controls group although the result showed no significant differences for both group. The finding also similar with other study which demonstrated that higher expression of IL-33 was found in allergic patients like AD [9]. Interleukin-33 is considered to be linked to the development of several allergic diseases such as asthma and atopic dermatitis. Other study also reported that IL-33 levels are elevated in allergic diseases patients compared to healthy individuals [10-12].

Of the 70 AA patients, 7 were diagnosed with controlled AA, 56 were diagnosed with partly controlled AA while the other 7 were diagnosed with uncontrolled AA. No significant difference in the IL-33 serum levels according to AA severity was found. The levels of IL-33 according to the severity of AA are shown in Table 4. The median (IQR) IL-33 serum level was higher in the controlled AA subjects than in the other two groups.

Table 4.IL-33 levels according to the severity of atopic asthma patients

Severity of AA	Frequency, n	Median (IQR) IL-33 Levels/µg/ml	Z Statistic	* p-Value
Controlled	7	128.82 (184.26)	1.853	0.396
Partly controlled	56	28.23 (101.51)	(2)	
Uncontrolled	7	14.07 (2657.08)		

*Result was significant if p-value < 0.05 by Kruskal Wallis test

3.3. Predisposing Factors of Atopic Asthma

In this study, AA was recorded higher in getting the diseases from their family rather than other allergic diseases. If there was somebody in the family suffered from AA, the tendency of their generation to get asthma was slightly increased. This finding was in accordance with the previous study which was reported that genetic factors put a person at considerable risk of developing asthma in childhood [9].

In contrast, the relationship between a family history of allergy and asthma in older persons is still debated. A review of current literature on the high prevalence of asthma, particularly in adolescents makes clear that the increase cannot be attributed to genetic factors alone. If both parents have allergic, their child has 80.0% chance of having the same problem.

Most of the allergic diseases had history of allergy. This finding was closely related to sensitization towards the allergen, area of living and the other factors. Other study also found that allergic history is not only important in identifying allergic triggers but also in guiding the treatment plan.The conceptual distinction between atopic and non-atopic asthma and bronchitis in children including the parental history of atopy, clinical picture, lung function and immunological status remarkable difference as revealed by the present study is the significantly higher prevalence of fathers and lower prevalence of brothers with a history of asthma and allergic diseases in non-atopic asthmatics when compared with the atopics. Mothers' history of

asthma and atopy dominate in children with atopic asthma as the majority of studies revealed. Majority of the subjects in this study live in urban area. This finding also in accordance with the previous study that reported the diagnosis of allergic diseases was higher in the city rather than on the coast [2]. This result reflect those living in urban area had tendency to get allergic symptoms which have close relationship with certain lifestyle, thus making it to contribute serious problem. The predisposing factors of subjects between normal controls and AA patients were shown in Table 5. Most of the subjects were non-smokers compared to smokers with ratio of 69:1 for the controls and 3:1 for AA. All smokers were male.

Table 5. Predisposing factors between normal controls and atopic asthma patients

Predisposing Factors	Controls, n (%)	AA, n (%)
Smoking status		
Non-smokers	69 (98.6)	50 (71.4)
Smokers	1 (1.4)	20 (28.6)
Occupational exposure		
Exposed	19 (27.1)	37 (52.9)
Non-exposed	51 (72.9)	33 (47.1)
Family history		
No	70 (100.0)	22 (31.4)
Yes	0 (0.0)	48 (68.6)
Area		
Urban	64 (91.4)	64 (91.4)
Rural	6 (8.6)	6 (8.6)
History of allergy		
No	70 (100.0)	3 (4.3)
Yes	0 (0.0)	67 (95.7)

The results showed that more than half (> 50.0%) of the patients were occupationally exposed. However, in AA patients, the ratio between occupationally exposed and non-exposed person were slightly similar with the ratio 1:1.

3.4. Association between IL-33 Levels and Predisposing Factors

From the results, independent t-test revealed that there was no significant difference in mean levels of IL-33 between AA and controls. However, mean (SD) of IL-33 was higher in AA as compared to controls group. Multiple logistic regressions revealed that there was no significant association between IL-33 and AA after adjusted for smoking and occupational exposure.

The association between IL-33 serum levels in AA with area of living cannot be performed by using multiple logistic regressions as there was no association between area of living and AA when tested with simple logistic regression at first step.

Pearson chi square and multiple logistic regressions revealed that there were significant associations between smoking status and occupational exposure with AA. The results showed the smokers were 29.84 at odds of having AA as compared to non-smokers, while those without occupational exposure were less likely to have AA as compared to those with occupational exposure with adjusted odds ratio 0.31 (Table 6). There is also comparison between AA and the other allergic diseases such as atopic dermatitis (AD) and allergic rhinitis (AR) as shown in Table 7 and Table 8. Most of the subjects were non-smokers. All of them were female. The previous study found that both atopy and smoking status are associated with IgE levels. It showed that non-smokers were less likely to have allergic diseases.

More than half of the allergic diseases patients were occupationally exposed to the allergen in their working places. This study also supported with the previous study which stated that allergy is reflected to the environmental and stimulation of the bacteria [9].

IL-33 was shown to activate and directly induce degranulation of IgE-sensitized mast cells [9]. This non-significant higher serum level of IL-33 may be seen in line with other study that conclude IL-31 as in the same group of IL-33 displays a unique and independent role in the pathophysiology of allergic response to the body [13]. The high associations between AA and other allergic diseases, including AD also recorded [14-15]. In some animal study, the mast cell activation has trigger IL-33 to induce allergic bronchoconstriction in mice. The enlargement of IL-33 expression may increase of tryptophan hydroxylase 1, the synthesis of serotonin hormone and storage. Thus, this reaction is resulting in airway obstruction in asthma.

The results of IL-33 showed no significant difference might be due to the outliers occurred during statistical analysis in this study. Allergic diseases also related to the genetics, family history, lifestyle and food intake itself. Thus, food intake, safety and other allergic avoidance should be emphasized in order to prevent an occurrence of allergic diseases.

Other study also suggested that food safety and the implementation of quality system in food should be emphasized in order to vitalize our lifestyle. The overproduction levels of IL-33 also can be reduced due to allergic prevention and education on allergic avoidance. The results showed that the smokers were more likely to have allergic diseases rather than the controls group. There is an association between allergic diseases and smoking behaviour. This finding might be clarified by pathophysiologic itself. The smokers had elevated total IgE levels that can increase the risk of sensitization towards the allergen. This factors might be triggered worsen symptoms of getting allergic diseases towards the smokers [13].

In this study, patients with non-occupational exposed were less likely to have allergic diseases than occupationally exposed. This situation might be due to the location or geographical factors as well as the surrounding environment that triggered the allergic symptoms towards the patients. The allergen avoidance was the most prevention from getting the allergy sensitization [14].

The results in this study showed most of the patients came from urban area. This might also be the reasons of increasing number of people getting allergic problems. Rural areas had very high O_3 (ozone) concentrations in upper level ozone and very low concentrations of other pollutants. Ozone is visible and is a major component of smog. Therefore, this area must be suitable place for asthmatic patients to survive with the tranquility of air quality.

Air quality regulation was significantly associated with allergic diseases especially in asthmatic patients in terms of healing. Most of the pollutants were found in urban area which nearer to ground-level ozone. It is an air pollutant linked with many harmful effects on respiratory health at levels commonly found in urban areas throughout the world.

Exercise and respiratory infections can be linked together as an asthmatic attack. The exposure to environmental factors like allergens, tobacco smoke, air pollution and other factors are also contributed to asthmatic attacks. However, it can be reduced by avoiding the exposure to known triggers and taking medications for healing process.

The diagnosis of AA include complete medical history, physical examinations and test for diagnosis and monitoring which include measurements of lung function, measurement of airway responsiveness and measurements of allergic status.

Peak expiratory flow (PEF) measurement was made by using a peak flow meter. The values are obtained in different peak flow meters, which vary in the range of predicted values.

For patients with normal lung function, but consistent with asthma, measurements of airway responsiveness to methacholine, histamine, mannitol, adenosine monophosphate or exercise challenge can be help in healing process of asthma. The measurements of allergic status can be done as the strong association present between asthma and other allergic diseases like AR and AD.

Skin testing or measurements of specific IgE in serum can be done to detect allergic diseases. All these things were done to detect asthmatic problem in individuals. The general health care of the patient also can be obtained through the quality of life score. The remedial process for AA can be controlled in different ways such as inhaled medication, orally treatment or injection proceedings.

The most currently effective anti-inflammatory medication for asthma is inhaled glucocorticosteroids. This type of treatment can reduce asthmatic symptoms, make better lung function and get improvement in quality of life. The airway inflammation and remodelling after certain period of therapy for mild or controlled asthmatic patients can be reduce through an anti-inflammatory therapy with fluticasone propionate as stated in other study [5].

Leukotriene modifiers also can lower the risk of asthmatic problems. Montelukast, pranlukast, zafirlukast and zileuton are reflected to the type of cysteinyl-leukotriene 1 receptor antagonists that functioned in reducing airway inflammation as well as improve lung function. The effect on anti-neutrophilic and LABA (long acting beta agonists) like salmeterol and formoterol in mild or controlled asthma patients also have been mentioned in the few studies before [6].

Therefore, practitioners would have chances in rational for combining inhaled steroids with other type of medication for their patients. Inhaled steroids can decrease airway inflammations. However, the remodelling is less evident due to its effect. Theophylline is one type of bronchodilator (anti-inflammatory properties) when given in low dosage to

asthmatic patients, it works as a first line controller. The patients might be controlled after the usage of Theophylline or under control before getting the second line of rescue.

The severe AA will be controlled with Anti-IgE such as omalizumab. Anticholinergics (inhaled ipratropium bromide) are a less effective controller in asthma [1].

The cooperation between asthmatic patients and physicians should be improved in order to develop an effective management of asthma. Patients also must avoid an exposure towards the risk factors of asthma [5].

Table 6. Association between IL-33 serum levels and predisposing factors with atopic asthma patients

	AA, n (%)	**Controls, n (%)**	**Crude OR (95% CI)**	*** p-Value**	**Adjusted OR (95% CI)**	*** p-Value [d]**
IL-33	245.62 [a] (705.34)	233.02 [a] (670.34)		0.914 [b]	1.00 (1.00, 1.00)	0.967
Smoking status						
Smokers	20 (95.2)	1 (4.8)	27.60 (3.59, 212.49)	< 0.001 [c]	29.84 (3.80, 234.44)	**0.001**
Non-smokers	50 (42.0)	69 (58.0)	1.00		1.00	
Occupational exposure						
Non-exposed	33 (39.3)	51 (60.7)	0.33 (0.16, 0.67)	0.002 [c]	0.31 (0.15, 0.68)	**0.003**
Exposed	37 (66.1)	19 (33.9)	1.00		1.00	

Table 7. Association between IL-33 serum levels and predisposing factors with atopic dermatitis patients

	AD, n (%)	**Controls, n (%)**	**Crude OR (95% CI)**	*** p-Value**	**Adjusted OR (95% CI)**	*** p-Value [d]**
IL-33	1875.27 [a] (11075.83)	233.02 [a] (670.34)		0.218 [b]	1.00 (1.00, 1.00)	0.550
Smoking status						
Smokers	20 (95.2)	1 (4.8)	27.60 (3.59, 212.49)	< 0.001 [c]	31.22 (3.77, 258.44)	**0.001**
Non-smokers	50 (42.0)	69 (58.0)	1.00		1.00	
Occupational exposure						
Non-exposed	21 (29.2)	51 (70.8)	0.16 (0.08, 0.33)	< 0.001 [c]	0.15 (0.07, 0.35)	**< 0.001**
Exposed	49 (72.1)	19 (27.9)	1.00		1.00	
Area of living	59 (48.0)	64 (52.0)	1.99 (0.69, 5.72)	0.196 [c]	2.58 (0.74, 9.00)	0.137
Urban	11 (64.7)	6 (35.3)	1.00		1.00	
Rural						

Table 8. Association between IL-33 serum levels and predisposing factors with allergic rhinitis patients

	AR, n (%)	Controls, n (%)	Crude OR (95% CI)	* p-Value	Adjusted OR (95% CI)	* p-Value [d]
IL-33	250.12 [a] (1061.38)	233.02 [a] (670.34)		0.909 [b]	1.00 (1.00, 1.00)	0.954
Smoking status						
Smokers	9 (90.0)	1 (10.0)	10.18 (1.25, 82.68)	0.009 [c]	15.44 (1.80, 132.16)	**0.012**
Non-smokers	61 (46.9)	69 (53.1)	1.00		1.00	
Occupational exposure						
Non-exposed	31 (29.2)	51 (70.8)	0.30 (0.08, 0.33)	< 0.001 [c]	0.23 (0.11, 0.50)	**< 0.001**
Exposed	39 (67.2)	19 (32.8)	1.00		1.00	
Area of living	57 (47.1)	64 (52.9)	2.43 (0.87, 6.82)	0.084 [c]	3.48 (1.14, 10.63)	**0.029**
Urban	13 (68.4)	6 (31.6)	1.00		1.00	
Rural						

3.5. Enzyme-Linked Immunosorbent Assay

An enzyme-linked immunosorbent assay also called ELISA is a test that measures antibodies in the blood. ELISA's principle is a combination of antibodies specificity with the sensitivity of simple enzyme assays. ELISA can provide a useful measurement of antigen or antibody concentration. ELISA will provide two main variations on this method which are detection on the presence of antigens that are recognized by an antibody and it can be used to test for antibodies that recognize an antigen. This test can be used to determine antibodies related to certain infectious conditions. In this study, ELISA was used to determine the levels of IL-33.

The result from measurement will indicate the value that associate to the disease from the patients. ELISA screening for IL-33 can provide a guideline for future medication in the medical treatment of allergic response.

4. CONCLUSION

Interleukin-33 is a unique cytokine that plays an essential role in regulating IgE and immune responses in allergic diseases. The roles of IL-33 in diseases associated with allergic diseases are demonstrated in the IL-33/ST2 pathway as a novel therapeutic target for understanding the implementation process. However, the mechanisms of the releasing process, expression, processing and regulation of IL-33 in allergic diseases are still unclear yet. This may be crucial for the development of future therapeutic targets.

Future studies are essential to recognize the biological and clinical significance of IL-33 in allergic diseases. We found that IL-33 levels were higher in the serum of AA patients than in healthy controls. However, this difference was not statistically significant. This might be due to the small sample size of the study. The association between IL-33 serum levels in AA with area of living cannot be performed by using multiple logistic regressions as there was no association between area of living and AA when tested with simple logistic regression at the first step.

Pearson chi square and multiple logistic regressions revealed that there were significant associations between smoking status and occupational exposure with AA. The results showed the smokers were 29.84 at odds of having AA as compared to non-smokers while those without occupational exposure were less likely to have AA as compared to those with occupational exposure with adjusted odds ratio 0.31. Future studies with larger sample sizes should be conducted.

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