ORIGINAL ARTICLE

ASSOCIATION BETWEEN IL-31 SERUM LEVELS AND OTHER PREDISPOSING FACTORS WITH ALLERGIC DISEASES IN HRPZ II AND HUSM, KELANTAN, MALAYSIA

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ABSTRACT

Interleukin 31 (IL-31)is one of the cytokines which appears to be an important regulator of Th2 responses. Previous study has been done to determine IL-31 serums levels in atopic dermatitis (AD). However, the serum levels of IL-31 in allergic rhinitis (AR) and atopic asthma (AA) is not many reported and still unclear. The objective of this cross sectional study is to determine an association between IL-31 and other predisposing factors with allergic diseases in HRPZ II (Hospital Raja PerempuanZainab II) and HUSM (Hospital UniversitiSains), Kelantan, Malaysia. This study involved 70 patients of AD, 70 patients of AR, 70 patients of AA and 70 healthy controls from staffs and people in HUSM.Five milliliters of blood were withdrawn and centrifuged for 5 minutes at 2000 rpm to obtain the serum and analyzed for IL-31 levels by using enzyme-linked immunosorbent (ELISA) kits (Human IL 31 Duoset, R&D System). Simple and multiple logistic regressions were used to analyze the association between IL-31 levels and predisposing factors among allergic diseases. The levels of IL-31 and other predisposing factors showed significant associations in smoking status, occupational exposure and area of living for AD and AR, however in AA, the significant association only found in smoking status and occupational exposure. In conclusion, we found that there were associations between IL-31 serum levels and other predisposing factors with AD, AR and AA. The findings can be the pilot study to determine IL-31 levels in allergic diseases in Malaysia.

Keywords:*IL-31; serum levels; predisposing factors; allergic diseases; Kelantan*

INTRODUCTION

Atopy usually related to the implication to an allergic disease due to IgE response to one or several common environmental allergens[1]. The most common allergens in Malaysia that trigger the symptoms of allergic diseases are house dust mites which are, *Dermatophagoidespteronyssinus* and*Dermatophagoides farinae*[2]. Allergy refers to the reaction of protective immunity and hypersensitivity[3]. Atopic march concept is described to begin with AD which progress into AR and AA. This fact had been described to have close relationship between this triad of diseases. AD is a relapsing, chronic and inflammatory skin disease that can be characterized by pruritic, eczematoid skin lesions[4].

Allergic rhinitis (AR) is described as IgE-mediated inflammation of nasal mucosa, characterized by one or multiple of the major symptoms like nasal obstruction, rhinorrhea,sneezing and nose itchiness[5]. Classically, simple rating diagnostic of severity were recorded which included of mild AR and moderate to severe AR[6].
Atopic asthma (AA) is a condition where occurrence of shortness of breaths due to airway inflammation and wheezing. Asthma is also linked with the immune system just like the other allergic reactions[7].

The cytokines are the chemicals produced by the cells in form of soluble molecules that act on other cells to stimulate their function. They are the regulator of the immune system in T_H2 responses[8]. AD was resulted from dysregulated T_H2 biased immune responses to environment stimuli[8]. The studies done before in other cytokine suggested that, IL-31 levels increased in allergic patients than normal person[9,10]. The predisposing factors like occupational exposure, area of living and also smoking status will triggerhigher levels of cytokine production in human body[11].The development of allergic diseases in an individual has both genetic and environmental components. The diagnosis of triad diseases (AD, AR and AA) are usually based on

clinical history and physical examination. The study on association between IL-31 and other predisposing factors in Kelantan is not unclear yet. Although the power of this study not highly enough to show a statistical significance, this study was done to determine the association between IL-31 serum levels and other predisposing factors with allergic diseases in HRPZ II and HUSM, Kelantan, Malaysia in order to initiate the pilot study of interleukin and predisposing factors in allergic diseases in Malaysia.

METHODOLOGY

Population

This was a comparative cross sectional study conducted among allergic patients and normal controls.This study was conducted at Outpatient Specialist Clinics, Immunology laboratory in Hospital UniversitiSains Malaysia (HUSM), Kelantan, Malaysia and Outpatient Specialist Clinic in Hospital Raja PerempuanZainab II (HRPZ II), Kelantan, Malaysia. Healthy people (individuals do not suffer from allergic disease and chronic disease) for controls were obtained from people (staff and students) in HUSM. This study was approved by the Research and Ethics Committee, School of Medical Sciences of UniversitiSains Malaysia (USM), Health Campus and Medical Research and Ethics Committee, Ministry of Health Malaysia.

Sampling and sample

Simple random sampling method was used to get both cases and control.Detailed explanation regarding the study protocol and a written consent form was given to all participants or their guardian before participated in the study. This study involved 70 patients of AD, 70 patients of AR, 70 patients of AA from and 70 healthy controls. Blood samples (5 ml) were collected from the peripheral vein of the subjects (AD, AR, AA and controls) were left to clot in the plain tube about 1-3 hours after blood withdrawn. Serum was separated soon after being centrifuged at 2000 rpm for 5 minutes. The collected serum was kept in -80^{o}C prior to testing by ELISA assay.ELISA tests were carried out to quantify antibodies against IL-31. IL-31 was detected by commercially available ELISA kits (Human IL-33 Duoset) supplied by R&D System (USA). The test was performed according to the manufacturer's protocols which involving of plate preparation, three times of washing buffer process, termination process by the addition of 50 µl of stop solution and measuring the absorbance by ELISA plate reader supplied by *µ*Bio Quant (USA) at a wavelength of 450 nm.

Data Collection

The allergic patients were recruited from the medical clinic of HUSM, KubangKerian, Kelantan, Malaysia and HRPZ II, Kota Bharu, Kelantan, Malaysia. The healthy controls were recruited among staff and students of USM. Subject recruitment was done based on the strict inclusion and exclusion criteria. AD patients were classified by using objective SCORAD (Scoring of Atopic dermatitis), Allergic rhinitis and Its Impact on Asthma (ARIA) classification was using to classify AR patients and AA patients were classified by using Global Initiative for Asthma (GINA) classification.

Data analysis

Data entry and analysis was done by using Statistical Package for Social Sciences (SPSS) version 20.0. The associations between IL-31 levels and predisposing factors among allergic diseases were determine by using simple logistic regression and multiple logistic Regression. The results obtained the statistical tests were considered to be significant as *p* value less than 0.05 ($p<0.05$).

Operational definition

- Occupational exposure: A personworking in an environmentwithone or moreriskfactorspresent.
- Family history: History of a condition in at leastone of thefollowingfamilymembers such asparent,sibling,grandparent,great-grandparent,aunt,uncle,nephew,niece or cousin.
- History of allergy: History of common symptoms appear in our body that contribute to allergic reaction.

RESULTS

Demographic data of atopic dermatitis, allergic rhinitis and atopic asthma

The demographic data of subjects including control, AD, AR and AA included in this study were shown in Table 1. The mean (SD) age of AD was 31.46 (20.38), AR was 36.16 (18.22), AA 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 accounted for 60.0%. For AA, majority of patients were over than 35 years old (70.0%). For patients more than 50.0% were females as recorded in Table 1. Majority of racial distribution were Malays for patients and controls with percentage range 87.1% - 97.0%.

Predisposing factors among controls, atopic dermatitis, allergic rhinitis and atopic asthma

The predisposing factors of subjects including control (C), AD, AR and AA are shown in Table 2. Most of the subjects were non-smokers compared

to smokers with the ratio of 69:1 for controls, 3:1 for AD, 7:1 for AR and 3:1 for AA. All the smokers were male. The results showed more than half (>50.0%) of the patients were occupationally expose, however in AR and AA, the ratio between occupationally exposed and non-exposed person were slightly similar with the ratio 1:1. Patients of AD and AR showed domination of no family history with similar disease. On the contrary, AA was recorded higher percentage in family history. Most of the subjects showed they were from urban area. The data showed majority of patients had history of allergic triggers.

Association between IL-31 serum levels and other predisposing factors with atopic dermatitis

From the results, independent t-test revealed that there was no significant difference in mean levels of IL-31 between AD and controls, however mean (SD) of IL-31 was higher in AD as compared to controls group. Multiple logistic regressions revealed that there was no significant association between IL-31 and AD after adjusted for smoking status, occupational exposure and area of living. Pearson Chi square and multiple logistic regressions revealed that there were significant associations between smoking status and occupational exposure with AD. The results showed that the smokers were 31.22 at odds of having AD as compared to non-smokers while those without occupational exposure were less likely to have AD as compared to those with occupational exposure with adjusted odds ratio of 0.15. There was no significant association between area of living and AD, howeverpatients live in urban area were more likely to have AD symptoms (Table 3).

Association between IL-31 serum levels and other predisposing factors with allergic rhinitis

From the results, independent t-test revealed that there was no significant difference in mean levels of IL-31 between AR and controls, however mean (SD) of IL-31 was higher in AR as compared to controls group. Multiple logistic regressions revealed that there was no significant association between IL-31 and AR after adjusted for smoking status, occupational exposure and area of living. Pearson Chi square and multiple logistic revealed that there were significant associations between smoking status and occupational exposure with AR. The results showed the smokers were 15.44 at odds of having AR as compared to non-smokers while those without occupational exposure were less likely to have AR as compared to those with occupational exposure with adjusted odds ratio of 0.23. Pearson Chi square shows no significant association between area of living and AR. However multiple logistic regressions revealed that area of living was significantly associated with AR. The results showed those living in urban area were 3.48 at odds of having AR as compared to those living in rural area (Table 4).

Association between IL-31 serum levels and other predisposing factors with atopic asthma

From the results, independent t-test revealed that there was no significant difference in mean levels of IL-31 between AA and controls, however mean (SD) of IL-31 was higher in AA as compared to controls group. Multiple logistic regressions revealed that there was no significant association between IL-31 and AA after adjusted for smoking status and occupational exposure. The 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 5).

Table 1- Demographic data of controls, atopic dermatitis, allergic rhinitis and atopic asthma

		Controls, n (%)	AD, n (%)	AR, n (%)	AA, n (%)
Age< 18		0 (0.0)	19 (27.1)	11 (15.7)	9 (12.9)
18-35		43 (61.3)	22 (31.4)	21 (30.0)	12 (17.1)
> 35		27 (38.6)	29 (41.5	38 (54.3)	49 (70.0)
Sex Male		35 (50.0)	25 (35.7)	30 (42.9)	24 (34.3)
Female		35 (50.0)	45 (64.3)	40 (57.1)	46 (65.7)
Race	**Malay**	68 (97.0)	66 (94.3)	61 (87.1)	65 (92.9)
	Chinese	1 (1.5)	4 (5.7)	8 (11.4)	5 (7.1)
	Indian	1 (1.5)	0 (0.0)	0 (0.0)	0 (0.0)
	Others	0 (0.0)	0 (0.0)	1 (1.5)	0 (0.0)

Table 2- Predisposing factors among control, atopic dermatitis, allergic rhinitis and atopic asthma

Predisposing factors	C, n(%)	AD, n(%)	AR, n(%)	AA, n(%)
Smoking status				
Non-smokers	69 (98.6)	50 (71.4)	61 (87.1)	50 (71.4)
Smokers	1 (1.4)	20 (28.6)	9 (12.9)	20 (28.6)
Occupational exposure				
Exposed	19 (27.1)	49 (70.0)	39 (55.7)	37 (52.9)
Non-exposed	51 (72.9)	21 (30.0)	31 (44.3)	33 (47.1)
Family history				
No	70 (100.0)	70 (100.0)	68 (97.1)	22 (31.4)
Yes	0 (0.0)	0 (0.0)	2 (2.9)	48 (68.6)
Area				
Urban	64 (91.4)	59 (84.3)	57 (81.4)	64 (91.4)
Rural	6 (8.6)	11 (15.7)	13 (18.6)	6 (8.6)
History of allergy				
No	70 (100.0)	4 (5.7)	17 (24.3)	3 (4.3)
Yes	0 (0.0)	66 (94.3)	53 (75.7)	67 (95.7)

Table 3- Association between IL-31 serum levels and other predisposing factors with atopic dermatitis

	AD n (%)	Controls n (%)	Crude OR (95% CI)	*p* value	Adjusted OR (95% CI)	*p* value [d]
IL-31	8102.62 [a] (38170.25)	2195.55 [a] (9016.57)		0.211 [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						
Urban						
Rural	59 (48.0)	64 (52.0)	1.99 (0.69, 5.72)	0.196 [c]	2.58 (0.74, 9.00)	0.137
	11 (64.7)	6 (35.3)	1.00		1.00	

[a]Mean (SD), [b] Independent t-test, [c] Pearson Chi-square, [d] Wald statistic (multiple logistic regression), OR : odd ratio, CI: confidence interval

Table 4- Association between IL-31 serum levels and other predisposing factors with allergic rhinitis

	AR n (%)	Controls n (%)	Crude OR (95% CI)	*p* value	Adjusted OR (95% CI)	*p* value [d]
IL-31	4107.70[a] (16961.51)	2195.55 [a] (9016.57)		0.406[b]	1.00 (1.00, 1.00)	0.805
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						
Urban	57 (47.1)	64 (52.9)	2.43 (0.87, 6.82)	0.084[c]	3.48 (1.14, 10.63)	0.029
Rural	13 (68.4)	6 (31.6)	1.00		1.00	

[a]Mean (SD), [b] Independent t-test, [c] Pearson Chi-square, [d] Wald statistic (multiple logistic regression), OR : odd ratio, CI: confidence interval

Table 5- Association between IL-31 serum levels and other predisposing factors with atopic asthma

	AA n (%)	Controls n (%)	Crude OR (95% CI)	*p* value	Adjusted OR (95% CI)	*p* value [d]
IL-31	2936.04[a] (8814.03)	2195.55 [a] (9016.57)		0.624[b]	1.00 (1.00, 1.00)	0.544
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	

[a]Mean (SD), [b] Independent t-test, [c] Pearson Chi-square, [d] Wald statistic (multiple logistic regression), OR : odd ratio, CI: confidence interval

DISCUSSION

Form this study, the mean age of AD patients was 31.46 with a minimum age of 2 years old and the maximum age was 77 years old. The mean age of AR patients was 36.16 with a minimum age of 10 years old and the maximum age was 77 years old. The mean age of AA patients was 40.93 with a minimum age of 9 years old and the maximum age was 76 years old. The mean age of control subjects was 32.49 with a minimum age of 18 years old and the maximum age was 54 years old. This study was slightly similar with the study done before which showed the mean age of subjects was 32.30 with the age range of 18 to 64 years old[12].

More than half of subjects for AD, AR and AA patients were female. This figure may reflect the

predomination of females in our country. In contrary, some studies in general, the prevalence of AR is equal between men and women[13]. Gender and age are important determinants of allergy occurrence and hospitalization. The effect of sex on allergy varies with age. Greater prevalence, incidence and hospitalizationof allergy have been found in female than in male. The finding was also similar to this study which female was greater than male. However, it is not clear if the sex difference stays similar in adults across ages[13]. In this study, Malay was higher compared to the other races for allergic diseases in this study. This might be happened due to most of the population in Kelantan were Malay, followed by Chinese and Indian. Both Chinese and Indian contribute only a small proportion of the population.

Most of the subjects were non-smokers. The previous study found that both atopy and smoking are associated with IgE levels[14]. This study 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 suggested that allergy is reflected to the environmental and stimulation of the bacteria[11].There is an evident need to learn more about the association between environmental and behavioural factors as well as the allergen in working places in order to clarify the situation.

In this study, AA was recorded higher in getting the diseases from their family rather than AR and AD. If there was somebody in the family suffered from AA, the tendency of their generation to get asthma was increased. This finding was in accordance with previous study which was reported that genetic factors put a person at considerable risk of developing asthma in childhood[15].

In contrast, the relationship between a family history of allergy and asthma in older persons is debated[16,17]. 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[12]. Nguyen and Close (2005)[13] also suggested that children of individuals with allergies have been shown to have higher incidence of allergies than that of other children. If both parents have allergies, their child has 80.0% chance of having the same problem[18].

From the results, most of the allergic diseases had history of allergy. AD patients had history of allergy which account for 94.3%, AR (75.7%) and AA (95.7%). This finding was regarding to sensitization towards the allergen, area of living and other factors. Nguyen and Close (2005)[13] found that allergic history is not only important in identifying the allergic triggers but also in guiding the treatment plan.

Majority of the subjects in this study live in urban area. This finding also in accordance with the study done by Guiote-Dominguez *et al*. (2008)[19] that reported the diagnosis of allergic diseases was higher in the city rather than on the coast.This result reflects that 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[20]. Most of the patients in this study had history of allergy. This study suggested that patients should aware with allergen avoidance.

The results showed, the smokers were more likely to have allergic diseases in AD, AR and AA as compared to controls group. There is an association between allergic diseases and smoking behaviour. This finding was also in accordance with the study done by Sherill*et al*. (1994)[14]. This finding might be explained by pathophysiologic itself. The smokers had higher total IgE levels that can increase the risk of sensitisation towards the allergen that might trigger worsen symptoms of getting allergic diseases[21].

From the results, patients with non-occupational exposed were less likely to have allergic diseases than occupationally exposed patients. This situation might be due to the location or geographical factors that triggered the allergic symptoms towards the patients. Holt (2000)[22] stated that allergen avoidance was the most prevention from getting the allergy as also supported with the hygiene hypothesis.
In this study, most of the patients came from urban area. This might also be the reasons of increasing number of people getting allergic problems from urban area rather than rural areas as also supported with the study done by Sousa *et al*[23]. Rural areas had very high O_3 (ozone) concentrations in upper level ozone and very low concentrations of other pollutants[23]. Ozone is a major component of smog which is visible. Therefore, this area must be the suitable place for asthmatic patients to survive.

Air quality regulation was significantly associated with allergic diseases especially in asthmatic patients in terms of healing[24]. Most of the pollutants were found in urban areas 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.

Strength and limitation

The total number of patients and controls in this study should be more to have an ideal statistical power to obtain the better results. This is a study which obtains the allergic patients those attended HUSM and HRPZ II, Kelantan for treatment. It does not represent all the allergic patients in Malaysia. The findings can be the pilot study to determine IL-31 levels in allergic diseases.

CONCLUSIONS

The levels of IL-31 and other predisposing factors showed significant associations in smoking status, occupational exposure and area of living for AD and AR, however in AA, the significant association only found in smoking status and occupational exposure. In conclusion, we found that there were associations between IL-31 serum levels and other predisposing factors with AD, AR and AA.

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