

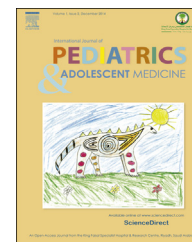
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ORIGINAL RESEARCH ARTICLE

Determination of interleukin 31 (IL-31) serum levels in allergic rhinitis patients



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KEYWORDS

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Abstract *Background and objectives:* IL-31 appears to be an important regulator of Th2 responses and was found to be present in patients with atopic dermatitis. This study was conducted to compare IL-31 serum levels in allergic rhinitis (AR) patients and normal controls and to determine IL-31 serum levels according to AR severity.

Patients and methods: A cross-sectional study was conducted with AR patients who met the Allergic Rhinitis and its Impact on Asthma (ARIA) classification criteria for AR. Normal control subjects were recruited from local healthy people in HUSM. The severity of AR was assessed using ARIA. Five ml of blood were drawn from each subject. The serum samples were analyzed for IL-31 using ELISA kits.

Results: The results showed that IL-31 serum level was higher in allergic rhinitis patients (mean (SD) 4107.70 (16961.51)) than in normal controls (2195.55 (9016.57)); however, this difference was not statistically significant, with a *p* value of 0.406 determined by an independent-T test. The results also showed no significant difference in IL-31 levels according to AR severity, with a *p* value of 0.245 determined by a Mann–Whitney test.

Conclusion: There was no significant difference in IL-31 serum levels between normal controls and AR patients or between patients with different AR severities. Future studies with larger sample sizes should be performed.

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1. Introduction

An allergy is a tendency to immunologically respond to a common, naturally occurring ingested or inhaled allergen by continually producing immunoglobulin E (Ig E) antibodies. Immune responses are controlled by cytokines. Interleukin 31 (IL-31) is a cytokine that contains four helical bundle cytokines [1] and belongs to family that includes IL-6, IL-11 and IL-27. IL-31 appears to be an important regulator of T-helper 2 (Th2) responses.

IL-31 stimulates the secretion of proinflammatory cytokines, chemokines and matrix metalloproteinases (MMPs) [2,3]. IL-31 may function as a proinflammatory cytokine in the recruitment of polymorphonuclear cells, monocytes and T cells.

The most common clinical disease involving environmental exposure to allergens is allergic rhinitis (AR) followed by atopic dermatitis (AD) and atopic asthma (AA) [2]. IL-31 serum levels are significantly higher in AD patients than in healthy individuals [4]. The association between IL-31 levels and AR is not well described in the literature. Thus, this study was conducted to demonstrate the levels of IL-31 in a local population in Malaysia. We compared IL-31 levels between AR patients and normal controls and between patients with AR of differing severity.

2. Patients and methods

2.1. Study subjects

A comparative cross-sectional study was conducted in 2011 and included patients with allergic rhinitis who met the Allergic Rhinitis and its Impact on Asthma (ARIA) classification criteria for AR. The patients were enrolled from the Otorhinolaryngology-Head & Neck Surgery (ORL-HNS) clinic at the Hospital Universiti Sains Malaysia (HUSM). To be included, the patients could not have received any systemic treatment for two months prior to the study. Normal control subjects were recruited from local healthy people at the HUSM who did not have any history or symptoms of AR. The sample size was calculated using PS software, Dupport and Plummer, 1997. Based on the *t*-test, 70 patients with AR and 70 normal control subjects were included in this study. After written informed consent was obtained, clinical profiles were recorded. Five ml of blood were drawn from each subject into plain tubes. The severity of AR was assessed using the ARIA criteria (Table 1).

2.2. Obtaining the serum

The blood samples were left to clot for 1–3 h after being drawn. Then they were centrifuged (Centrifuge 5810 R) for 5 min at 2000 rpm (rotation per minute). The serum then was stored at -80°C (degree Celsius).

2.3. ELISA

IL-31 was detected using a commercially available ELISA kit (R&D System). The test was performed according to the

manufacturer's protocol. Based on our standard curves, the detection limit was approximately 50 pg/ml.

2.4. Plate preparation

Each well of a 96-well microplate was immediately coated with 100 μL of the unlabeled monoclonal antibody specific for human IL-31. Then, the plate was sealed, and it was incubated overnight at room temperature. Each well was aspirated and washed with wash buffer. The process was repeated two times for a total of three washes. Then, 300 μL of reagent diluents were added to each well to block the plates. The plate was incubated at room temperature for a minimum of 1 h. The wash was repeated as in the previous washing step, after which the plate was ready for sample addition.

2.5. Assay procedure

One hundred microliters of a sample or a standard in reagent diluents was added per well. After sample addition, the plate was incubated for 2 h at room temperature. Then, the wash was repeated as during plate preparation. One hundred microliters of biotin-labeled anti-human IL-31 detection antibody was added to each well. Then, the plate was incubated at room temperature for 2 h, and the washing process was repeated. One hundred microliters of horseradish peroxidase (HRP) labeled 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 μL of tetramethylbenzidine (TMB) substrate solution was added to each well, and the plate was incubated for 20 min at room temperature. Then, 50 μL of stop solution was added to each well. Then, the color development was quenched, and the intensity was measured at 450 nm.

2.6. Data entry and statistical analysis

The data entry and analysis were performed using Statistical Package for Social Sciences (SPSS) version 20.0. IL-31 serum levels of AR patients and normal control subjects were compared using the Independent-T test. IL-31 serum levels according to severity of AR were compared using the Mann–Whitney test.

Table 1 Classification of patients' allergic rhinitis severity.

Classification	Symptoms
Mild	Normal sleep, normal daily activities, normal work and school activities, no troublesome symptoms
Moderate to severe	Abnormal sleep; impairment of daily activities, sports and leisure; problems at work or school and troublesome symptoms

Table 2 Sociodemographic characteristics of respondents.

		Allergic rhinitis, n (%)	Normal controls, n (%)
Race	Malay	61 (87)	68 (96)
	Chinese	8 (11)	1 (2)
	Indian	0 (0)	1 (2)
	Others	1 (2)	0 (0)
Age (years)	<18	11 (16)	0 (0)
	18–35	21 (30)	43 (61)
	>35	38 (54)	27 (39)
Sex	Male	30 (43)	35 (50)
	Female	40 (57)	35 (50)

3. Results

A total of 140 subjects (70 allergic rhinitis patients and 70 normal controls) were enrolled in the study. Of the 70 normal control subjects, 35 were male (50%) and 35 were female (50%). Of the 70 allergic rhinitis subjects, 30 were male (43%) and 40 were female (57%). The other socio-demographic data of the subjects are shown in Table 2.

As shown in Table 3, there was no significant difference in the IL-31 serum levels between AR patients and normal control subjects. However, the mean (SD) IL-31 serum level was higher for the AR patients than for the normal control subjects.

Out of 70 AR patients, 24 were diagnosed with mild AR, while the other 46 were diagnosed with moderate–severe AR. No significant difference in the IL-31 serum levels according to AR severity was found. The median (IQR) IL-31 serum level was higher in the moderate–severe AR subjects than in the mild AR subjects, as shown in Table 4.

4. Discussion

Atopy is one's propensity to produce IgE antibodies and develop sensitization to environmental triggers [5]. In this study, the results showed no significant differences in IL-31 levels between AR and normal control subjects; however, we observed that the serum samples from the AR patients had higher levels of IL-31 than those of the normal controls. The findings of this study are similar to those of a previous study in AD patients; that study demonstrated higher levels of IL-31 in allergic patients than in healthy controls [3]. However, the difference was not statistically significant.

The AR patients in a study had AR of mild or moderate–severe severity. However, the results showed no

Table 3 IL-31 serum levels in normal control subjects and allergic rhinitis patients.

Group	n	Mean (SD) pg/ml	p value
Allergic rhinitis	70	4107.70 (16961.51)	0.406
Normal controls	70	2195.55 (9016.57)	

Result was significant if p value < 0.05 by independent-T test.

Table 4 IL-31 serum levels according to allergic rhinitis severity.

Severity of AR	n	Median (IQR) IL-31 levels (pg/ml)	Z statistic	p value
Mild	24	168.08 (637.24)	–1.163	0.245
Moderate–severe	46	264.10 (1089.96)		

Result was significant if p value < 0.05 by Mann–Whitney test.

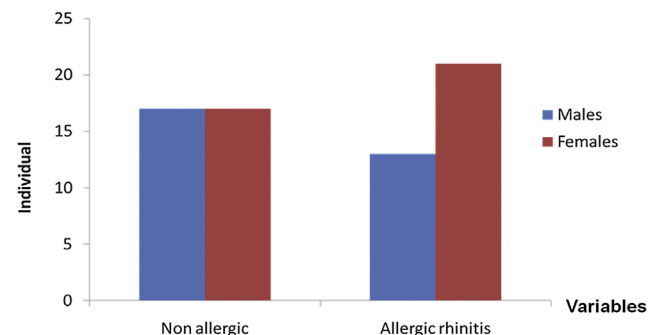
significant difference between IL-31 levels according to AR severity. This may be due to the subjects being non-normally distributed and to the limited sample size of this study.

The majority of patients in the study sample were female (Fig. 1). Gender is an important determinant of AR occurrence and hospitalization. The effect of sex on AR varies with age. However, it is not clear if the difference in AR occurrence by sex remains similar throughout adulthood. In a previous study of AR in Malaysia, the majority of the patients were diagnosed with moderate–severe AR according to the ARIA classification criteria [6]. Similar findings were noted in our study.

Although many studies reported the presence of IL-31 in atopic dermatitis (AD) patients, the relationship between IL-31 and AR is still unclear [2,7,8]. In vivo animal studies have shown that IL-31 is closely related to dermatitis, AD and non-atopic dermatitis [9]. IL-31 serum levels were found to be significantly higher in patients with AD compared to healthy subjects [10–12]. IL-31 is also higher in allergic asthmatic patients than in healthy subjects [13].

Similarly, our study demonstrated that IL-31 was higher in AR patients than in healthy control subjects, although the difference was not statistically significant. This non-significant difference in IL-31 serum level may be considered to be similar to results reported by other authors who concluded that IL-31 has a unique and independent role in the pathophysiology of allergic rhinitis [14].

Previous studies found that IL-31 is higher in AD patients compared to normal controls; thus, a major weakness of this study was that patients with AD were not excluded. This is because of the high association between AR and other allergic diseases, including AD (60–80%). Additionally, although the patients did not receive any systemic treatment for AR for at least two months prior to study

**Figure 1** The gender involve in IL-31 measurement between non allergic subjects and allergic rhinitis patients.

enrollment, they were still permitted to use topical treatments to reduce their AR symptoms; this is another weakness of this study.

5. Conclusion

We found that IL-31 was higher in the serum of AR patients than in healthy controls; however, this difference was not statistically significant. This might be due to the small sample size of the study. Future studies with larger sample sizes should be performed.

Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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