Determination of Interleukin 31 (IL-31) serum levels according to severity of allergic rhinitis

Ashri NSM¹, Amin SNSM², Hamid WZWA³, Rahman AA⁴, Muhammad I⁵

Abstract

Objective: This study was conducted to compare IL-31 serum levels in allergic rhinitis (AR) patients and normal controls as well as according to severity of AR. Materials and Methods: The subjects, normal controls and AR patients, were defined by history taking. Blood were collected from subjects and patients with AR. Serum was centrifuged and analyzed for IL-31 using enzyme linked immunosorbent assay (ELISA) kits. 70 samples of normal controls and 70 samples of AR were collected respectively. Results and Discussion: The result showed that IL-31 serum level was higher in allergic rhinitis patients, mean (SD) 4107.70 (16961.51) as compared with normal controls 2195.55 (9016.57), however it was not statistically significant. There was also no significant difference of IL-31 levels between the severity of AR. Conclusions: IL-31 serum level was higher in AR patients; however it was not statistically significant. Further study which obtain the larger sample sizes should be done to get better findings.

Keywords: IL-31; serum levels; ELISA; allergic rhinitis

Introduction

Allergy refers to a tendency to immunologically respond to many common naturally occurring ingested and inhaled allergens with the continual production of immunoglobulin E (Ig E) antibodies. The immune responses are controlled by the cytokines. Interleukin 31 (IL-31) is one of the cytokines which containing four helical bundle cytokines¹ that belongs to family which includes of IL 6, IL 11 and IL 27. IL-31 appears to be an important regulator of T-helper 2 (Th2) responses. Th2 response generates depends on the infecting pathogen and triggers the response in the innate immune cells². The common manifestation of clinical diseases that are involve in environmental allergen exposure, are allergic rhinitis (AR) followed by atopic dermatitis (AD) and atopic asthma (AA)³. IL-31 serum levels are significantly higher in patients of AD than healthy people². The association between levels of IL-31 and AR is not well elaborated in literature. Thus this study was undertaken to demonstrate the level of IL-31 among the local population in Malaysia and to compare them with normal controls as well as according to severity of AR.

Materials and methods

Study subjects

Ethical approval was obtained from the Research Ethics Committee (Human), University Sains Malaysia prior to the commencement of the study and written consent was obtained from each participant before involvement in the study. A comparative cross sectional study was conducted in 2011 among patients with allergic rhinitis who met Allergic Rhinitis and its Impact on Asthma (ARIA) classification criteria for AR. They were taken from the Otorhinolaryngology-Head & Neck Surgery (ORL-HNS) clinic in Hospital University Sains Malaysia (HUSM). Normal control subjects were recruited among healthy local people in HUSM, who did not have any history or symptoms of AR. After written informed consent, clinical profiles were recorded. Five ml of blood was withdrawn into plain

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Table 1: The classification of patient’s severity of allergic rhinitis

<table>
<thead>
<tr>
<th>Classification</th>
<th>Symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild</td>
<td>Normal sleep, normal daily activities, normal work and school, no troublesome symptoms</td>
</tr>
<tr>
<td>Moderate to severe</td>
<td>Abnormal sleep, impairment of daily activities, sport and leisure, problems caused at works or school and troublesome symptoms</td>
</tr>
</tbody>
</table>

tube. The severity of AR was assessed using ARIA (Table 1).

Getting the serum
Blood was left to clot for about 1-3 hours after being withdrawn, then it was centrifuged (Centrifuge 5810 R) for 5 minutes at 2000 rpm (rotation per minute). The serum then was stored at -80°C (degree Celsius).

ELISA
IL-31 was detected by commercially available ELISA test (R&D System). The test was performed according to the manufacturer’s protocols.

Plate Preparation
A 96-wells microplate were immediately coated with 100 µL per well of the unlabelled monoclonal antibody specific for human IL-31. Then, the plate was sealed and 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 was added to each well to block the plates. The plate was incubated at room temperature for a minimum of 1 hour. The wash was repeated as in the previous washing step. Then, the plate was ready for sample addition.

Assay procedure
100 µL of sample or standards in reagent diluents was added per well. After the sample addition, the plate was incubated for 2 hours at room temperature. Then, the wash was repeated as in the previous step of plate preparation. 100 µL of biotin-labeled anti-human IL-31 detection antibody was added to each well. Then, the plate was incubated at room temperature for 2 hours and the washing process repeated. 100 µL of horseradish peroxidase (HRP) labeled anti-biotin antibody was added to each well. Then, the plate was incubated and the wash was repeated after 20 minutes of incubation process.

After that, 100 µL of tetramethylbenzidine (TMB) substrate solution was added to each well and was incubated for 20 minutes at room temperature. Then, 50 µL of stop solution was added to each well. Color development was then quenched and intensity was measured at 450 nm.

Data entry and statistical analysis
Data entry and analysis was done by using Statistical Package for Social Sciences (SPSS) version 20.0. IL-31 serum level was compared between AR patients and normal control subjects by using Independent T test. IL-31 serum level according to severity of AR was compared by using Mann-Whitney test.

Results
A total of 140 subjects (70 allergic rhinitis patients and 70 normal controls) were enrolled. Out of 70 samples of non allergic subjects, 35 of them were males (50%) and other 35 were females (50%). Besides, 30 patients were males (43%) from 70 samples of allergic rhinitis and other 40 samples were females (57%). The results showed that there was no significant difference in the IL-31 serum levels between AR patients and normal control subjects as shown in Table 3. However, the level of IL-31, mean (SD) was higher among AR patients as compared to normal

Table 2: The other sociodemographic data of subjects

<table>
<thead>
<tr>
<th></th>
<th>Allergic rhinitis, n (%)</th>
<th>Normal controls, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Race</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malay</td>
<td>61 (87)</td>
<td>68 (96)</td>
</tr>
<tr>
<td>Chinese</td>
<td>8 (11)</td>
<td>1 (2)</td>
</tr>
<tr>
<td>Indian</td>
<td>0 (0)</td>
<td>1 (2)</td>
</tr>
<tr>
<td>Others</td>
<td>1 (2)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;18</td>
<td>11 (16)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>18-35</td>
<td>21 (30)</td>
<td>43 (61)</td>
</tr>
<tr>
<td>&gt;35</td>
<td>38 (54)</td>
<td>27 (39)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>30 (43)</td>
<td>35 (50)</td>
</tr>
<tr>
<td>Female</td>
<td>40 (57)</td>
<td>35 (50)</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Mean (SD)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allergic rhinitis</td>
<td>70</td>
<td>4107.70 (16961.51)</td>
<td></td>
</tr>
<tr>
<td>Normal controls</td>
<td>70</td>
<td>2195.55 (9016.57)</td>
<td>0.406</td>
</tr>
</tbody>
</table>

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

Table 4: IL-31 serum levels according to the severity of allergic rhinitis patients

<table>
<thead>
<tr>
<th>Severity of AR</th>
<th>n</th>
<th>Median (IQR) IL-31 levels</th>
<th>Z statistic</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild</td>
<td>24</td>
<td>168.08 (637.24)</td>
<td>-1.163</td>
<td>0.245</td>
</tr>
<tr>
<td>Moderate-severe</td>
<td>46</td>
<td>264.10 (1089.96)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

Out of 70 subjects from AR patients, 24 were diagnosed as having mild AR while the other 46 were diagnosed as moderate-severe subjects. The results also shown, no significant difference in IL-31 serum levels according to severity of AR. Median (IQR) was shown higher in moderate-severe AR than mild AR as shown in Table 4.

**Discussion**

Allergy is defined as one’s propensity to produce IgE antibodies and sensitization in response to environmental triggers. In this study, the result showed no significant differences for IL-31 levels for AR and normal controls but, we observed that serum from AR patients induce higher levels of IL-31 than normal controls. The finding from this study similar with the study done before in AD patients as compared in terms of IL-31 levels, which demonstrated higher levels of IL-31 in allergic patients rather than healthy controls. However, this showed no statistically significant of IL-31 for AR and controls. The severity of AR patients also varied which was classified in different severity of mild, and moderate-severe. However, the result showed no significant difference between IL-31 levels according to severity of AR. This situation happened might be due to the subjects not normally distributed instead of variety as well as according to their limited sample sizes.

Majority of patients in the study sample were female. Gender is important determinants of AR occurrence and hospitalization. The effect of sex on AR varies with age. However, it is not clear if the sex difference stays similar in adults across ages. In the previous study of AR in Malaysia, majority of the patients were diagnosed as having moderate-severe AR according to ARIA classification. Similar findings were noted in our study. The patients were in treatment progress which might be healed as taking continuity remedy that might be going to mild and cured from this disease. The design of this study was a cross sectional study with randomized sampling method as also similar with the previous study done before in Malaysia.

Although many studies reported the presence of IL-31 in atopic dermatitis (AD) however the relationship between IL-31 and AR is still remain unclear. IL-31 was closely related to dermatitis, AD and non-atopic dermatitis as shown in vivo animal. IL-31 serum levels were found to be significantly higher in patients with AD compared to healthy subjects. IL-31 is also higher in allergic asthmatic patients. Anticipating similar findings, our study demonstrated that IL-31 presence in AR patients, although not statistically significant, IL-31 serum level was shown to be higher as compared with control group. This non significant higher serum level of IL-31 may be seen as in line with other authors that concluded IL-31 displays a unique and independent role in the pathophysiology of allergic rhinitis.

**Conclusion**

Our study suggested that, IL-31 serum level was higher in AR patients; however it was not statistically significant. There was also no significant difference of IL-31 levels between the severity of AR. This might be due to small sample sizes. Future study need to be done with larger sample sizes need to be collected in order to get better findings.

**Acknowledgement**

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**Conflict of interests**

The authors declare that there is no conflict of interests regarding the publication of this paper.
Interleukin 31 levels in allergic rhinitis

References


