The Differences in Serum Quantitative Specific IgE Levels Induced by *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae* and *Blomia tropicalis* Sensitization in Intermittent and Persistent Allergic Asthma

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ABSTRACT

**Latar belakang:** tungau debu rumah (TDR) merupakan alergen hirup yang penting pada asma alergik. Namun, penelitian diagnostik molekular menggunakan Imunoglobulin E (IgE) spesifik akibat sensitisi asal TDR dihubungkan dengan derajat keparahan asma alergik belum pernah dilakukan di Indonesia. Penelitian ini bertujuan mengetahui perbedaan kadar IgE spesifik serum kuantitatif akibat sensitisi alergen *Dermatophagoides (D.) pteronyssinus*, *D. farinae* dan *Blomia (B.) tropicalis* pada asma alergik intermiten dan persisten. **Metode:** desain penelitian potong lintang pada pasien asma alergik dewasa yang diundang untuk pemeriksaan IgE spesifik serum dan merupakan bagian dari penelitian payung di Divisi Alergi dan Imunologi Klinik, RS Cipto Mangunkusumo. Derajat keparahan asma ditentukan berdasarkan kriteria Global Initiative on Asthma (GINA) 2015 dan dikelompokkan menjadi intermiten dan persisten. Pemeriksaan IgE spesifik serum kuantitatif menggunakan metode multiple allergosorbent test (Polycheck Allergy, Biocheck GmbH, Munster, Germany). Alergen TDR yang diperiksa adalah D. pteronyssinus, D. farinae, dan B. tropicalis. Perbedaan antara dua kelompok dianalisis dengan uji Mann-Whitney. **Hasil:** sebanyak 87 subjek dilibatkan dalam penelitian ini; 69 (79,3%) subjek adalah perempuan. Rerata usia pasien adalah 40,2 tahun. Enam puluh tiga (72,4%) pasien menderita asma dan rinitis alergik. Sebanyak 58 (66,7%) pasien asma persisten. Gambaran sensitisi alergen TDR adalah 62,1% D. farinae; 51,7% D. pteronyssinus dan 48,3% B. tropicalis. Median kadar IgE spesifik secara bermakna lebih tinggi pada asma persisten dibandingkan asma intermiten untuk alergen D. farinae (1,30 vs. 0,0 kU/L; p=0,024) dan B. tropicalis (0,57 vs. 0,0 kU/L; p=0,015). Kadar IgE spesifik D. pteronyssinus lebih tinggi pada asma persisten dibandingkan intermiten (0,67 vs. 0,00 kU/L; p=0,066). **Kesimpulan:** gambaran sensitisi alergen secara berurutan didapatkan D. farinae 62,1%, D. pteronyssinus 51,7% dan B. tropicalis 48,3%. Kadar IgE spesifik akibat sensitisi D. farinae dan B. tropicalis lebih tinggi secara bermakna pada pasien asma persisten dibandingkan asma intermiten. Kadar IgE spesifik akibat sensitisi D. pteronyssinus lebih tinggi pada pasien asma persisten dibandingkan asma intermiten, tetapi secara statistik tidak bermakna.

**Kata kunci:** asma alergik, asma intermiten dan persisten, IgE spesifik, sensitisi asal, tungau debu rumah.
INTRODUCTION

Worldwide prevalence of allergic asthma is projected to increase in the near future. By 2025, an estimated 400 million people will suffer from allergic asthma and nearly 500 million people will suffer from allergic rhinitis.1 Allergic asthma is a type of asthma mediated by immunologic mechanisms. The immunoglobulin E (IgE) is often associated with allergic asthma symptoms. Based on the symptoms and severity of airway obstruction, asthma is classified into two major groups, intermittent and persistent asthma.2

There are hundreds of different allergens that can cause clinical symptoms of asthma and it is hard to identify which allergen has the most potential to cause clinical symptoms of asthma.3 For this reason, molecular diagnosis of allergic diseases is developed to help us understand the sensitization of a specific allergen by using IgE levels measurement. Using the allergen-specific IgE, we have a stronger basis to establish allergic asthma diagnosis, which is the second largest phenotype of asthma. This procedure has been recommended in the guidelines on respiratory allergy disease management.4

There are three methods of the allergen-specific IgE test: the qualitative method, which result is presented in a positive/negative form, the semi-quantitative method which result is presented in a unit/class form (class 0–6), and the quantitative method which result is presented in a kKU/L form which also serves as a standard reference recognized by WHO. Quantitative detection of allergen-specific IgE is important in allergy diagnosis.5 The study about serum quantitative specific IgE levels and its correlation with allergic asthma is still lacking, there are only two known studies on the subject with different allergen profile. House dust mite (HDM) is the most common type of allergen causing asthma in China6, while cat and dog dander is the most common type of allergen causing asthma in Sweden.7

In tropical countries, HDM exposure is the most important factor in the development

ABSTRACT

Background: house dust mites (HDM) are an important inhalant allergen in allergic asthma. However, molecular diagnostic study of specific IgE to HDM allergens has not been done in Indonesia. In addition, the association of quantitative specific IgE measurement with asthma severity has not been investigated. This study aimed to investigate the difference of serum quantitative specific IgE levels induced by Dermatophagoides (D.) pteronyssinus, D. farinae and Blomia tropicalis sensitization in intermittent and persistent allergic asthma. Methods: this was a cross-sectional study on adult allergic asthma patients who were invited for serum specific IgE testing. This study was a part of a larger study within the Division of Allergy and Immunology, Cipto Mangunkusumo Hospital. Asthma severity was defined based on Global Initiative on Asthma (GINA) 2015 criteria and were grouped as intermittent or persistent. Quantitative specific IgE testing was done on blood serum using a multiple allergosorbent test (Polycheck Allergy, Biocheck GmbH, Munster, Germany). The HDM allergens tested were D. pteronyssinus, D. farinae, and Blomia tropicalis. Difference between two groups were analyze using Mann-Whitney test. Results: a total of 87 subjects were enrolled in this study; 69 (79.3%) were women. Mean patients’ age was 40, 2 years. Sixty-three (72.4%) subjects had asthma and allergic rhinitis. Fifty-eight (66.7%) subjects were classified as persistent asthma. The prevalence of sensitization was 62.1% for D. farinae, 51.7% for D. pteronyssinus, and 48.3% for Blomia tropicalis. The median of specific IgE levels were significantly higher in persistent asthma compares to intermittent asthma induced by D. farinae (median 1.30 vs. 0.0 kU/L; p=0.024) and B. tropicalis (median 0.57 vs. 0.0 kU/L; p=0.015) sensitization. Level of Specific IgE D. pteronyssinus was also to be higher in persistent asthma than the level measured in intermittent asthma (0.67 vs. 0.00 kU/L; p=0.066). Conclusion: Sensitization of HDM allergens was shown to be highest for D. farinae 62.1%, followed by D. pteronyssinus 51.7% and Blomia tropicalis 48.3%. Specific IgE level induced by D. farinae and Blomia tropicalis sensitization were significantly higher in patients with persistent asthma compared to intermittent asthma, whereas specific IgE level induced by D. pteronyssinus sensitization was higher in persistent asthma although not statistically significant.

Key words: allergic asthma intermittent and persistent, specific IgE, allergen sensitization, house dust mites.
of allergy diseases, especially respiratory allergy. Among all type of house dust mites, *D. pteronyssinus*, *D. farinae* and *Blomia tropicalis* are the main sources of HDM allergens in tropical countries. In Jakarta, HDM is the most important indoor allergen that can cause allergic reaction, including allergic asthma.

The study on quantitative serum specific IgE levels induced by *D. pteronyssinus*, *D. farinae*, and *Blomia tropicalis* associated with allergic asthma in Indonesia has never been published until this study is conducted. Besides that, the HDM allergen profile in Indonesia is still unknown, as well as its application in determining the severity of allergic asthma. Considering the limitation of in vivo study using skin prick test and the difficulty of finding the allergen used in skin prick test, an in vitro study using quantitative measurement of specific IgE levels is needed. This in vitro study is very sensitive in measuring the IgE levels, compared to skin prick test.

**METHODS**

This was a cross-sectional study on adult allergic asthma patients who were invited for serum specific IgE testing. This study was a part of a larger study within the Division of Allergy and Immunology of Cipto Mangunkusumo Hospital. This study was conducted on December 2016 until February 2017.

The samples used in this study were allergic asthma patients, with age ranging from 19 – 59 years old. Patients with non-allergic asthma; those who were not willing to be a participant of this study; patients with contraindications for spirometry testing, such as patients with increased intracranial pressure, patients with space occupying lesion of brain, retinal ablation, or acute exacerbation of asthma; patients who were receiving systemic steroid therapy, and patients with contraindications for skin prick test were excluded from this study.

**Asthma Severity Classification**

Every subject went through these steps: history taking, physical examination, spirometry test, skin prick test, quantitative serum specific IgE test, and asthma severity classification based on Global Initiative on Asthma (GINA) 2015 criteria (i.e. intermittent and persistent). The data collected from each subject were then processed and analyzed. Intermittent asthma was defined as a degree of asthma where the symptoms occur <2 days per week; nocturnal asthma occurs <2 days per month; the use of β-2 agonist to relieve the symptoms is <2 times per week; with no limitation of normal activities; the lung function predicted FEV1 >80% and normal FEV1/FVC. Persistent asthma was defined as an asthma degree where the symptoms occur >2 days per week but not daily; nocturnal asthma occurs <3 – 4 times a month; the use of β-2 agonist to control symptoms is >2 times per week but it is not used daily, and it is not used more than twice a day; minor limitation in normal activities; lung function predicted FEV1 >80% and normal FEV1/FVC.

Skin prick test was considered positive if the diameter of the wheal is >3 mm. The specific IgE test was considered significant if the specific IgE levels is >0.35 kU/L (Class 1). The cut-off value of specific IgE testing is 0.35 - 101 kU/L. In this analytic statistic calculation, data valued <0.15 kU/L was considered as 0 KU/L, while data valued >100 kU/L was considered as 101 KU/L. The commercial cassette allergen kit from Polycheck Allergy, Biocheck GmbH, Munster, Germany was used to test the specific IgE levels.

**Statistical Analysis**

The data was then processed and analyzed using SPSS software version 16.0 for Windows. The result of specific IgE test was presented as numeric and categorical data. The numeric data shows mean levels of IgE and confidence interval (CI) of 95%. Specific IgE levels was also presented in categorical (class) form, ranging from class 0 – 6 according to the calibration curve of WHO. The difference of specific IgE levels in intermittent asthma group and persistent asthma group was then analyzed using the Mann-Whitney test. P-value of 0.05 or less was considered statistically significant.

Spoken and written explanations about the aim, use, and procedure of this study were given to every subject. This study had obtained the ethical clearance number 53/UN2.F1/ETIK/2017 from FK UI Ethical Committee.

**RESULTS**

This study was conducted on December 2016 – February 2017 at the outpatient clinic of
Allergy and Clinical Immunology Division of Cipto Mangunkusumo Hospital in Jakarta with the sample size of 88 subjects. One patient failed to undergo the specific IgE test, thus the sample size was decreased to 87 subjects.

The majority (79.3%) of the patients were women. The average age of the subjects was 40.2 years old and ranged from 19 to 59 years of age. Most of these patients suffer from allergic asthma or allergic rhinitis and the majority of them were classified into the persistent-asthma group. The highest allergen sensitization was caused by *D. farinae* (Table 1).

### Table 1. Characteristics of subjects (n = 87)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (male), n (%)</td>
<td>18 (20.7)</td>
</tr>
<tr>
<td>Age (years), mean (SD)</td>
<td>40.2 (12.04)</td>
</tr>
<tr>
<td>Age group (y.o), n (%)</td>
<td></td>
</tr>
<tr>
<td>- &lt;20</td>
<td>1 (1.1)</td>
</tr>
<tr>
<td>- 20-29</td>
<td>21 (24.1)</td>
</tr>
<tr>
<td>- 30-39</td>
<td>18 (20.7)</td>
</tr>
<tr>
<td>- 40-49</td>
<td>24 (27.6)</td>
</tr>
<tr>
<td>- 50-59</td>
<td>23 (26.4)</td>
</tr>
<tr>
<td>Diagnosis, n (%)</td>
<td></td>
</tr>
<tr>
<td>- Allergic asthma</td>
<td>24 (27.6)</td>
</tr>
<tr>
<td>- Allergic asthma + rhinitis</td>
<td>63 (72.4)</td>
</tr>
<tr>
<td>FEV1/FVC, mean (SD)</td>
<td>91.5 (9.0)</td>
</tr>
<tr>
<td>FEV1 (%), mean (SD)</td>
<td>69.3 (19.6)</td>
</tr>
<tr>
<td>ACT score, n (%)</td>
<td></td>
</tr>
<tr>
<td>- Fully controlled</td>
<td>16 (18.4)</td>
</tr>
<tr>
<td>- Controlled</td>
<td>20 (23.0)</td>
</tr>
<tr>
<td>- Poorly controlled</td>
<td>51 (58.6)</td>
</tr>
<tr>
<td>Asthma severity, n (%)</td>
<td></td>
</tr>
<tr>
<td>- Intermittent</td>
<td>29 (33.3)</td>
</tr>
<tr>
<td>- Persistent</td>
<td>58 (66.7)</td>
</tr>
<tr>
<td>Sensitization prevalence, n (%)</td>
<td></td>
</tr>
<tr>
<td>- <em>D. pteronyssinus</em></td>
<td>45 (51.7)</td>
</tr>
<tr>
<td>- <em>D. farinae</em></td>
<td>54 (62.1)</td>
</tr>
<tr>
<td>- <em>Blomia tropicalis</em></td>
<td>42 (48.3)</td>
</tr>
</tbody>
</table>

Specific IgE serum levels test resulted in quantitative data ranged from 0.15 – 100 kU/L. In this descriptive and analytic statistic calculation, data valued <0.15 kU/L was considered as 0 KU/L, while data valued >100 kU/L was considered as 101 KU/L. All of the mites-specific IgE serum levels tested in this research showed abnormal data distributions. It was resulted from the absence of HDM-specific IgE in some patients (Table 2). Thus, data is presented in median and IQR form.

The data of specific IgE serum levels also presented in categorical (class) form, ranged from class 0 – 6 according to calibration curve of WHO. It appeared that subjects’ specific IgE levels varied widely. The majority of sensitized patients belong to class 2 compared to other classes. Nevertheless, an equal proportion of class 6 was shown by each group, which was the class with the highest levels of IgE beyond the machine’s detection range (Figure 1).

### Table 2. Quantitative specific IgE serum level profile

<table>
<thead>
<tr>
<th></th>
<th>Median (IQR) - (kU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>D. pteronyssinus</em></td>
<td>0.46 (12.0)</td>
</tr>
<tr>
<td><em>D. farinae</em></td>
<td>0.80 (14.0)</td>
</tr>
<tr>
<td><em>Blomia tropicalis</em></td>
<td>0.32 (3.1)</td>
</tr>
</tbody>
</table>

Analysis of the difference of specific IgE serum levels was done by comparing the median value and by using the Mann-Whitney test because the data distribution was abnormal. The analysis showed that persistent-asthma patients have higher levels of specific IgE serum compared to intermittent-asthma patients, although the statistic significance was only achieved in *D. farinae* and *B. tropicalis* allergen (Table 3).

### DISCUSSION

The majority of the subjects were women (79.3%). On the contrary, another research stated that most of allergic asthma patients were men (with a ratio of 1:2), while women were more prone to non-allergic asthma. A study based in India which was conducted on 20 people with allergic asthma and/or allergic rhinitis showed that 16 out of 20 subjects (80%) were men. According to the literature, it was stated that most of asthmatic children were boys, but when they reach puberty the ratio switched; the prevalence of asthma becomes higher in girls. However, the exact effect of estrogen on asthma is still unknown. It is suspected that the effect of estrogen is mediated by its receptors, estrogen receptor alpha (ER-α), which are expressed by mast cell. Estrogen may trigger mast cell
The differences in serum quantitative specific IgE levels

degranulation and increase IgE-mediated immune reactions.\textsuperscript{14}

The average age of the subjects in this study was around 40 years old. It is higher than the result of a study conducted in India with a sample size of 20 subjects, which stated that the average age of allergic asthma patients was 34.3 years old.\textsuperscript{12} Patients aged $>$60 years old were excluded from this study, thus the maximal age of the subjects is 59 years old.

Allergic rhinitis was found in the majority of subjects. A study in India (stated above) showed that 65% bronchial asthma patients also suffer from allergic rhinitis.\textsuperscript{12} Around 50% of respiratory allergy patients either suffer from allergic asthma or allergic rhinitis.\textsuperscript{15} Around 80% of all allergic asthma patients are suspected to suffer from allergic rhinitis as well. This finding soon became the basis of “one airway, one disease” concept found in respiratory allergy treatment.\textsuperscript{2}

From the result of this study, the highest HDM exposure came from $D$. farinae (62.1%), followed by $D$. pteronyssinus and Blomia tropicalis. A study using immunoassay method (ImmunoCAP) found that the number of $D$. farinae sensitization in allergic rhinitis patients in the United States is around 37.2%, this number is comparable to $D$. pteronyssinus (38.2%). Up until now, ImmunoCAP still becomes the gold standard of in vitro specific IgE levels testing, because the result from this method is consistent in a variety of allergen extract.\textsuperscript{17} ImmunoCAP testing kit is still unavailable in Indonesia and this procedure costs more compared to other technique.

Nevertheless, the result of this study is different with the epidemiological profile of HDM allergen in Indonesia which were tested using skin prick test. A study based in Jakarta conducted on 107 subjects with respiratory allergy found the allergen with the highest sensitization prevalence was $D$. pteronyssinus (77.5%) and Blomia tropicalis (72.0%), but $D$. farinae was not inspected in that study.\textsuperscript{18} Another study in Jakarta showed that $D$. pteronyssinus allergen prevalence is as high as 77.3%, followed by $D$. farinae (69.6%) and cockroaches (44.9%).\textsuperscript{19}

Compared to previous studies which used skin prick test, the HDM allergen prevalence checked

\begin{table}[h]
\centering
\begin{tabular}{|l|c|c|c|}
\hline
Allergen & Intermittent asthma (kU/L) & Persistent asthma (kU/L) & p value* \\
\hline
$D$. pteronyssinus & 0.00 & 0.67 & 0.066 \\
$D$. farinae & 0.00 & 1.30 & 0.024 \\
Blomia tropicalis & 0.00 & 0.57 & 0.015 \\
\hline
\end{tabular}
\caption{Median value difference of specific IgE serum level based on allergen type}
\end{table}

Figure 1. Distribution of specific IgE serum level class

Table 3. Median value difference of specific IgE serum level based on allergen type
using in vitro technique appear to be lower. The median value of specific IgE levels in subjects ranged from 0.3 – 0.8 kU/L. The highest median value in this study was caused by *D. farinae* allergen sensitization. This number is considered low, considering negative or class 0 patients were also taken into account in statistical analysis. On the other hand, the highest number that can be measured by the machine is limited to 100 kU/L. Thus the median value of specific IgE levels in this study did not represent the actual IgE levels in the subjects.

In this study, the subjects were classified into 2 groups based on the severity of asthma. To further compare the IgE levels in mild, moderate, and severe intermittent asthma, a bigger sample size is needed. Moreover, the measurement of specific IgE levels could not be done with absolute number. Patients with IgE levels below the detection limit (<0.15 kU/L) were considered 0 kU/L, while the IgE levels above the detection limit (>100 kU/L) were calculated by inputting the number 101 kU/L.

A study conducted in Spain found that bronchial asthma patients showed increased specific IgE concentration caused by *D. pteronyssinus* when compared with non-asthmatic patients (median 40.2 vs 11.7 kU/L; p<0.001). That study used the immunoassay technique (ImmunoCAP-250 system, Thermo Fisher Scientific) with the highest detection limit of 100 kU/L. Similar to our study, to calculate the median value, researcher established 100 kU/L as the value for patients with specific IgE levels above the detection limit.

The result of this study showed that specific IgE serum levels is significantly higher in persistent asthma patients sensitized to *D. farinae* and *B. tropicalis* allergen compared to intermittent asthma, but the increasing levels was not significant in *D. pteronyssinus* allergen sensitization. An epidemiological survey that took place in China and conducted on 6304 allergic asthma and rhinitis found that the percentage of *D. pteronyssinus* and *D. farinae* sensitization are higher in more severe asthma cases. Although in that survey, the absolute levels was not analyzed. On the contrary, a study on 772 asthmatic patients in Belgium found that specific IgE levels caused by HDM sensitization in severe persistent asthma patients were comparable to IgE levels found in mild-moderate persistent asthma patients (median value of 0.47 vs 0.65 kU/L, p>0.05). In that study, specific IgE serum levels (unknown species) was tested using RAST method (Phadia, Belgium) with positive detection limit >0.35 kU/L.

This observed differences of IgE reactivity in Asian and European population may be caused by HDM sensitization difference. Even though HDMs are the most important indoor allergen in respiratory allergy worldwide, there is a geographical difference in allergen distribution. For example, a study in Sweden on asthmatic patients using immunoassay method (ImmunoCAP) found that dog (60.3%) and cat (56.1%) dander as the main allergen causing allergic asthma, while the prevalence of HDM allergen is far below, 27.0% for *D. pteronyssinus* and 24.8% for *D. farinae*. Meanwhile, in China, the prevalence of sensitization to *D. pteronyssinus* allergen in mild- intermittent asthma patients is around 22.7% and it reached 79.1% in severe-persistent asthma patients; the prevalence of sensitization to *D. farinae* is around 21.0% in mild-intermittent asthma patients and 77.2% in severe-persistent asthma. Another study on asthmatic patients in Sweden and New Zealand showed that increased specific IgE levels in Sweden mainly was caused by cat allergen, while in New Zealand it was mainly caused by HDM allergen.

In Indonesia, the prevalence of HDM allergen checked by skin prick test is higher than 70%, showing the importance of HDM allergen as the trigger of respiratory allergy symptoms. However, the result of this study showed that there is no statistically significant difference in specific IgE levels caused by *D. pteronyssinus*. It was probably due to the high number of *D. pteronyssinus* sensitization in society because of the high prevalence of *D. pteronyssinus*. Among all the HDM allergens, *D. pteronyssinus* has the biggest number of species in the world. Nevertheless, *D. pteronyssinus* sensitization not necessarily trigger allergic symptoms or asthma attack.

The strength of this study are: first, to
researchers’ knowledge, this is the first study about the correlation of quantitative HDM specific IgE serum levels testing and allergic asthma in Indonesia. Secondly, the measurement of specific IgE serum levels in this study used a quantitative method, while other studies commonly used semi-quantitative method. Thirdly, the amount of drop out patients in this study was <2%. The limitations of this study are: first, the subjects in this study are patients that have been diagnosed with allergic asthma and were voluntarily willing to contribute in this study. Thus, the demographic profile of the subjects is not necessarily portray the actual demographic profile of allergic asthma patients in a bigger population. Secondly, the measurement of specific IgE serum levels in this study used a commercial kit available in Indonesia and its diagnostic performance, especially its sensitivity and specificity in detecting specific IgE in respiratory allergy patients, have never been investigated. Thirdly, the specific IgE serum levels tested in this study was only triggered by three type of HDM allergens, while the severity of allergic asthma may be caused by the sensitization of another type of allergen.

The clinical application of our study is that quantitative serum of IgE levels are recommended to be performed routinely in patients with persistent allergic asthma, especially those who are difficult to stop therapies and to control their medicine consumption. Further studies can be developed to determine the prognosis of allergic asthma patients with the consecutive sampling methods and the larger samples number for each group of asthma.

CONCLUSION

Specific IgE serum levels induced by Dermapthagoideaes farinae and Blomia tropicalis sensitization are significantly higher in persistent asthma patients compared to intermittent asthma patients. Specific IgE serum levels induced by Dermatophagoides pteronyssinus sensitization is higher in persistent asthma patients compared to intermittent asthma patients, but the difference was not statistically significant.

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