

Correlation of *Dermatophagoides Pteronyssinus* with Serum Der-P Spesific Ige and Igg4

1st Hanny Siti Nuraeni^{1*}

2nd Heri Wibowo²

3rd Cytyta Putri Kwarta³

4th Iris Rengganis⁴

¹ Department of Medical Laboratory Technology, Poltekkes Kemenkes Banten

^{2,3} Departemen of Paracitology Medical Faculty, Universitas Indonesia

⁴ Division of Allergy, Dr. Cipto Mangunkusumo Hospital, Jakarta, Indonesia

*email: hanny.siti@poltekkesbanten.ac.id

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Abstract

Repeated exposure to allergens induces an increase in regulatory T cells that produce interleukin 10 (IL-10) that can lead to B cells switching to immunoglobulin G4 (IgG4). Allergen-specific IgE was used as marker of allergen exposure but was only detected in atopic subjects, not in normal subjects. Therefore, an immunological marker that could be used to scan for exposure is needed. This study was conducted to determine *Dermatophagoides pteronyssinus* (Der p) density, serum IgE and IgG4-specific Der p levels, and to determine the correlation of Der p density with specific serum levels of IgE and IgG4 Der p in normal and atopic subjects. We calculated the amount and density of Der p in house dust mites (HDM) using the Fain and Hart (1986) method and measured Der p serum levels of IgE and IgG4 using indirect ELISA. We found that the most dominant HDM species was Der p. IgE-specific Der p level in atopic asthma patients were higher than in normal patients ($p=0.002$), whereas IgG4-specific Der p level in both atopic asthma and normal patients did not exhibit significant differences ($p=0.667$). The density of Der p demonstrated a positive correlation with IgG4-specific Der p levels (Spearman $r=0.388$, $p=0.008$) compared to IgE-specific Der p. Compared to IgE, the density of Der p demonstrated a correlation with Der p-specific serum IgG4 levels, which means that Der p-specific IgG4 can be used to determine the exposure and accumulation of Der p in the residence of subjects.

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INTRODUCTION

House dust mites are potential allergens that can trigger allergic reactions, such as asthma, rhinitis, conjunctivitis, and atopic dermatitis. As many as 85% of patients with asthma are allergic to *Dermatophagoides pteronyssinus* (Der p) and *Dermatophagoides farinae* (Der f). House dust mites produce materials that are good allergens from their bodies and faeces. The material is very small and lightweight; therefore, it is easy to fly or unite with dust in the air, so that if inhaled, it can cause allergic reactions (1).

According to the World Allergy Organization (WAO), allergy is a hypersensitivity reaction initiated by an immunological mechanism that is chained by immunoglobulin E (IgE) (2). Allergies will not manifest without allergen exposure. Chances of sensitisation and the occurrence of asthma, allergic rhinitis, or atopic dermatitis will increase with more allergen exposure (3). Allergens in dust are obtained by measuring allergens in home dust samples. According to research conducted by Chew et al. in 1999. 1 g of dust can contain about 10.000 mites (4). Dust concentrations above 100 to 200 ng/g in individuals are considered sufficient to increase the risk of allergies. Dust concentrations above 10 mg/g may cause acute asthma attacks in sensitive patients. Exposure to concentrations above 10 mg/g in the early years of life increases the risk of asthma (4). In addition, house dust mite levels above 100 dust mites/g have been associated with

asthma sensitisation, and more than 500 mites/g has been associated with clinical symptoms of asthma. In addition, 10 pg/g dust level is approximately equivalent to 500 mites (5).

Allergy prevention efforts are currently aimed at identifying individuals at high risk and providing therapy to prevent chronic diseases as early as possible. Various methods have been developed in order to obtain biomarkers that can be used for diagnosis of allergens; for example, various types of cytokines and other biochemical substances produced by body cells and various surface molecules and cellular receptors. Beekeepers who are frequently exposed to bee stings will naturally be protected from allergic reactions through a mechanism involving the role of T cells that secrete IL-10 (6). In atopic dermatitis, specific IgG4 antibodies are more concentrated in repetitive stimulation. Increased regulator T cells induce an increase in IL-10 and TGF- β , thus resulting in the emergence of IgG4 antibodies (7).

If you look for or find dust and then do a microscopic examination, it could be missing, so we predict that the specific IgG4 from dust mites in house dust can be used as a predictor of the presence of house dust mites. This study aims to determine whether IgG4 can be used as a predictor of the presence of house dust mites in the home. In this experiment, allergen-specific IgE was used as a marker for allergens in atopic subjects. To avoid exposure of allergens in both atopic asthma and normal subjects, a method that can scan areas or locations suspected as the source of HDM allergies needs to be developed. Repeated exposure to the desensitization process induces an increase in regulatory T cells that produce TGF- β and or IL-10 so that it can induce IgG4 (7,8). Therefore, we are interested in determining the patterns of response to IgE and IgG4 and their correlation with Der p density.

METHODS

This research has received approval from the Medical Health Research Ethics Committee of the University of Indonesia Number 1370/UN2.F1/ETIK/2018. Subjects included in the inclusion criteria as many as 46 people underwent a skin prick test (SPT) using the allergens of house dust mites, *D. pteronyssinus*, *D. farinae*, and *B. tropicalis*. The whole world will be subject to the density of house dust mites in their homes and be tested for Der-p specific IgE and IgG4 serum levels.

Subjects with persistent bronchial asthma atopy (20 people) and normal control subjects (20 people) who are members of the asthma exercise association at Margono Hospital, Purwokerto, Central Java were used as research subjects. The research specimens were serum and house dust from atopic and normal asthma subjects. Inclusion criteria were subjects aged 30-59 years, living in Purwokerto houses for at least 3 years and having a history of moderate persistent bronchial asthma (atopic asthma subjects), or having no history of asthma and atopy (normal subjects). Exclusion criteria were not currently using antihistamines, positive SPT subjects but not showing asthma symptoms, and negative SPT subjects but showing asthma symptoms.

House dust samples were taken from the research subject's house (bedroom and living room) using a vacuum cleaner (To thv 1600) which could be directed at the end of the hose with a connecting pipe that was inserted into the tube. The cup used was a special dish with a diameter of 7 cm (ALK Laboratory), with the inside of the perforated cup closed with a Whatman filter number 41. The processing of house dust samples in this study was carried out by flotation technique.

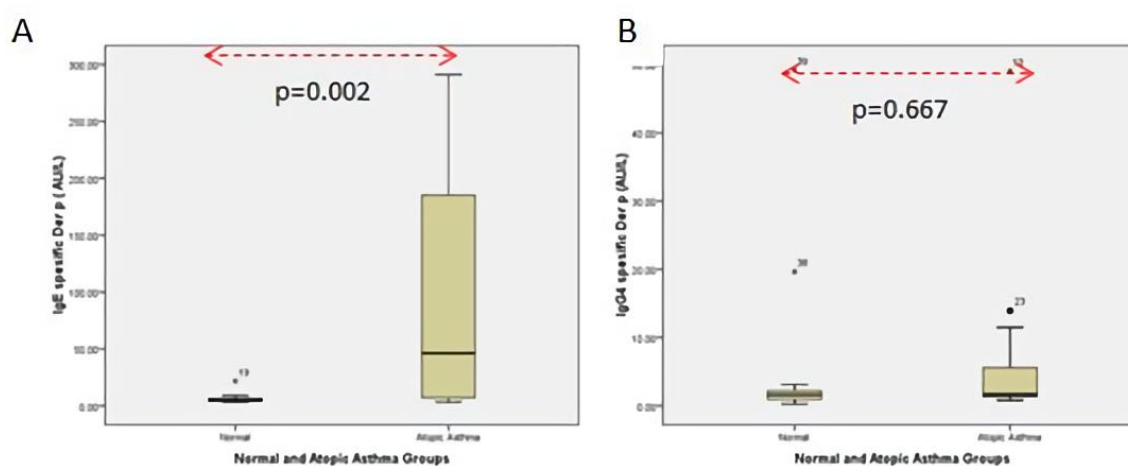
Quantitative measurement of serum levels for serum IgE and IgG4-specific-Der p was performed using the indirect ELISA method. IgE titer is expressed in AU/L. A total of 2 ug/50 μ L Der p antigen (Stalergen, France) in 0.05 M bicarbonate buffer solution pH 9.6 was coated on each well (96 nunc plate) and then incubated for 24 hours at 4°C. After incubation, the coating solution was discarded and washed 1x5 minutes using 200 μ L of washing buffer. Add 200 μ L of blocking solution (5% BSA in 0.05% PBST) and then incubate at 37°C for 2 hours. The blocking solution was discarded and washed 1x5 minutes using 200 μ L of washing buffer. Add 50 μ L of serum (1:4) and then incubate for 1

hour at 37°C. wash 3x5 minutes using 200 µL of washing buffer. Add 50 µL (1:5000) anti-human IgE (SeraCare) or anti-human IgG4 (Sigma) and incubate for 1 hour at 37°C. Wash 3x5 minutes using 200 µL washing buffer. Add 50 µL (1:3000) streptavidin peroxidase (Sago, Biotek) and incubate for 30 minutes at 37°C. Wash 7x5 minutes using 200 µL of washing buffer. Add 50 µL of ABTS substrate and incubate for 5 minutes at room temperature. Add 50 µL of stop solution (0.1 NaN₃). Read on the elisa reader at a wavelength of 405 nm.

The correlation analysis of Der-p density with IgE and IgG4 was carried out using the SPSS Spearman Correlation statistical test because the data were not normally distributed.

RESULTS AND DISCUSSION

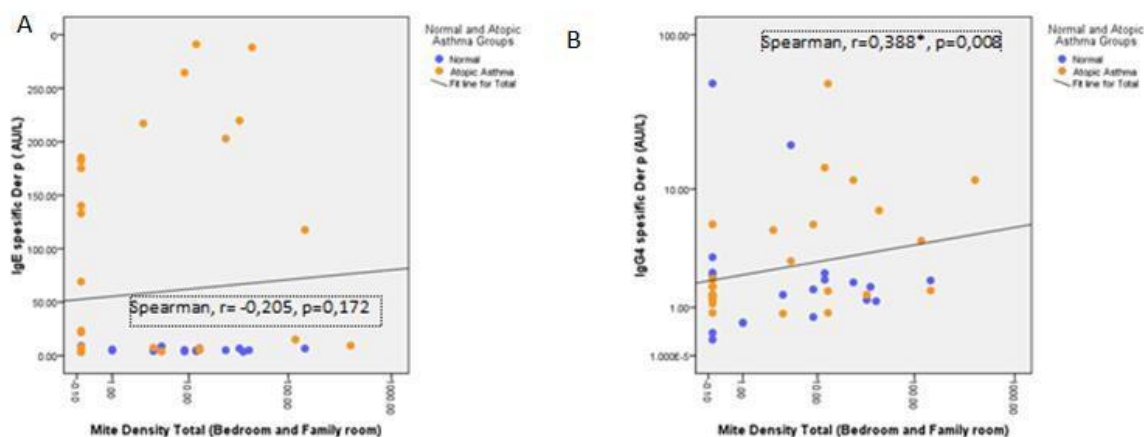
This study included 46 subjects; of the total, 25 were moderate persistent asthma patients, and their allergic SPTs were positive (atopic asthma); furthermore, 21 subjects did not suffer from asthma, and their SPT against allergens were negative (normal).



Picture 1. (A) Der p-specific serum IgE levels and (B) Der p-specific serum IgG4 Levels in the Normal and Atopic Asthma Groups.

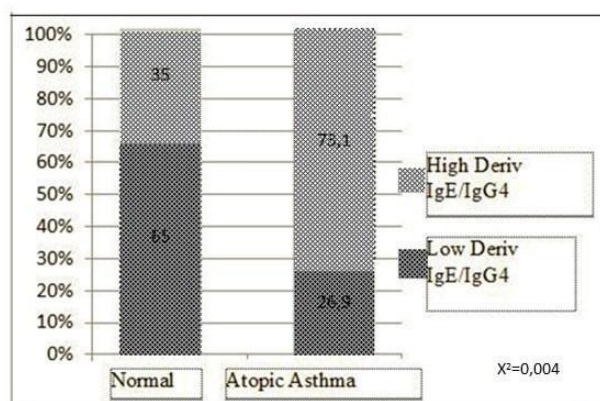
Picture 1 shows results of measurement of specific Der p levels of IgE and IgG4 in the normal and atopic asthma groups. Picture 1A reveals significant differences in terms of IgE levels between normal and atopic asthma subjects ($p = 0.002$); however, Picture 1B depicts no significant differences in IgG4 levels between normal and atopic asthma subjects ($p = 0.667$).

Results of HDM density correlation with Der p-specific IgE levels in the normal and atopic asthma groups are provided in Picture 2. From Picture 2A, it can be seen that there is no correlation between HDM density and specific Der p IgE level (Spearman $r = -0.205$, $p = 0.172$). Picture 2B reveals a positive correlation between HDM density and specific Der p IgG4 level (Spearman $r = 0.388$, $p = 0.008$).



Picture 2. HDM density correlation with (A) Specific IgE Der p Level, (B) Specific IgG4 Der p level in Normal and Atopic Asthma Groups.

Calculation of IgE/IgG4 ratio was performed to determine whether IgE tended to be present in either normal or atopic asthma group. In Picture 3, the high IgE/IgG4 ratio in the atopic asthma group is higher (73.1%) than normal group (35%), whereas the low IgE/IgG4 ratio in the normal group is higher (65%) than in the atopic asthma group (26.9%).



Picture 3. IgE/IgG4 specific Der p Ratio in Normal and Atopic Asthma Groups

The most common house dust mite species found in several villages in Purwokerto is *D. pteronyssinus* (66.7%). This study findings are in accordance with research in India that demonstrated that the most frequently acquired mites are *D. Pteronyssinus*. Research conducted by Annisa in 2018 also revealed that the HDM that dominates in most areas of Central Jakarta was *Dermatophagoides* spp. By contrast, research in China in 2015 revealed that *D. farinae* was the most widely obtained mite. Differences in findings regarding the distribution and density of these mite species are influenced by climate, species composition, microhabitat, and geographical area.

In atopic asthma subjects, specific IgE levels of Der p were higher than in normal subjects. These results are in line with Anasis's research and various other studies that concluded that serum anti-*D. pteronyssinus* IgE levels were higher in atopic subjects compared to normal ones. This high IgE level is related to the atopic subject, where the atopic subject tends to produce Der p-specific IgE in response to HDM exposure (8). In this study, IgG4 levels in both the atopic asthma group and the normal group did not differ significantly; however, it can be seen that atopic asthma subjects had small amounts of IgG4 and large amounts of IgE. Platts and Milles in their study stated that exposure to high-dose allergens leads to the production of IgG and IgG4 without IgE antibodies (7). IgG4 isotype is not dependent on IL-4

cytokines but depends on IL-10. This is also consistent with the study of Ruiter et al. in 2007 that stated that the development of tolerance in IgE-mediated allergies had been associated with higher IgG4 levels compared to IgE levels (9).

In normal patients, 65% of subjects displayed low IgE/IgG4 ratios, which suggested that the difference in levels of IgE and IgG4 was small. Low IgE/IgG4 ratio is likely related to changes from allergic Th2 predominance to Treg cell dominance. Increased regulatory T cells induce an increase in IL-10 and TGF- β , thus resulting in the appearance of IgG4 (10). IgG4 increase occurs after chronic antigen exposure and is generally associated with immune tolerance (11). IgG4 is an effective immune regulator because although it can act as an inhibiting antibody, it is not efficient in forming immune complexes. IgG4 antibodies can interact with Fc γ RI receptors, Fc γ RIIA, Fc γ RIIB, Fc γ RIIC, and Fc γ RIIIA. Receptor inhibition FcIB has low affinity for IgG1, IgG2, and IgG3 from other receptor F (FcRs), meanwhile, it has high affinity for IgG4 sehingga IgG4 who has has anti-inflammatory by inhibiting FcR by IgE.(12) IgG4 is the only IgG subclass with the same affinity to activate FcR and to inhibit FcIB receptors. If IgG4 interacts with inhibiting receptor, it will inhibit the response of effector cells.

Tsai et al. mentioned that mite-specific IgG4 antibodies could serve as an indicator in clinical results of mite allergy during immunotherapy. In atopic patients, IgE concentrations tend to be higher than normal patients, whereas, in normal patients, IgG4 concentrations are higher than in atopic patients (13). The balance between the production of IgG4 and IgE appears to influence the development of allergic hypersensitivity and immune tolerance (14).

Correlation analysis of the relationship between the density of house dust mites and Der p-specific IgE and IgG4 levels in the subject serum was conducted to determine whether Der p-specific IgE and IgG4 levels in the study patients were influenced by the density of mites obtained from the patient's house.

Based on Picture 2A, no correlation was found between Der p density and Der p-specific serum IgE levels for neither the bedroom nor the family room. This result was not in line with Anasis's research which stated that there was a relationship between IgE levels and mite density in the bedroom. This difference was probably caused by the differing habits of subjects according to the regions that they were from. In Purwokerto, West Java Indonesia, the majority of the community carried out various activities in the family room. The living room had furniture and carpets, which are ideal Der p habitats.

Based on Picture 2B, there is a positive correlation between Der p density and Der p-specific serum IgG4 levels both in the bedroom and family room. These results indicate that the higher the Der p density obtained in the bedroom and the family room of the subject, the higher Der p-specific IgG4 levels obtained in the serum of both atopic asthma and normal subjects. There was a positive correlation between Der p density and serum IgG4 levels, and there was no significant difference between atopic asthma and normal groups, therefore, IgG4 can be used as a candidate to predict the presence of Der p in dwellings. However, to determine whether or not an individual is atopic or not to Der p, Der p-specific IgE testing still needs to be performed.

CONCLUSION

The species *D. pteronyssinus* is the dominant HDM species in Purwokerto. Compared to IgE, IgG4 levels in atopic asthma and normal subjects more clearly describe exposure to Der p in the community where the subject lives. The density of Der p demonstrates a correlation with Der p-specific serum IgG4 levels, which suggests that Der p-specific IgG4 can be used as a predictor to determine the exposure and accumulation of Der p in residences.

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