

**SERO-PREVALENCE OF HOUSE DUST MITES
ALLERGEN AMONG ALLERGIC PATIENTS IN HUSM**

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Dissertation submitted in partial fulfillment for the
Degree of Bachelor of Health Science (Biomedicine)

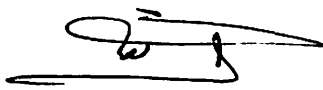
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CERTIFICATE


This is certify that the dissertation entitled
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Abbreviation

HDM : House Dust Mites

ELISA : Enzym linked immunosorbent Assay

SPT : Skin Prick Test

HUSM : Hospital University Science Malaysia

IgE : Immunoglobulin E

Abstract

This final year research project is about determination of dust mites prevalence among allergic patients in HUSM. There are many type of allergic and my hypothesis is over half of the allergic patients is due to dust mites. From twenty samples that been tested with ELISA method, only one samples showed positive result. And from chi-square test, it has been determined that there is significant association between difference IgE level and the age, sex and race. Several factor regarding validity of the sample, collecting and processing have been written down as reason how such results have been achieved.

Abstrak

Projek penyelidikan tahun akhir ini adalah mengenai pengenalpastian prevalen kutu debu antara pesakit alergi di HUSM. Terdapat banyak jenis alergi dan hipotesis saya adalah, separuh daripada pesakit tersebut disebabkan kutu debu. Daripada dua puluh sampel yang diuji menggunakan kaedah ELISA, hanya terdapat 1 sampel yang menunjukkan keputusan positif. Dan daripada pengiraan chi-square, dapat dipastikan terdapat hubungkait perbezaan tahap IgE antara umur, jantina dan bangsa pesakit. Beberapa faktor yang melibatkan keberkesanan sampel, pengumpulan dan pemprosesan telah dinyatakan untuk menunjukkan bagaimana keputusan yang sedemikian diperolehi.

1. Introduction

This dissertation is prepared as a partial fulfillment for the Bachelor of Health Science in Biomedicine. This project concerns about prevalence of house dust mite allergy among HUSM allergic patients. Research on association of house dust mites and its contribution to the disease had been done by many researchers and published in countless journals around the world. However there is still no prevalence study specifically on allergic patients in HUSM, Kubang Kerian, Kelantan, especially to house dust mites.

There are 2 surveillance study using questionnaire done by researchers of Pediatric Unit HUSM to measure the prevalence of asthma, eczema and allergic rhinitis among school children in Kota Bharu, Malaysia. It was done two times over 6 years from 1995 to 2001 and they found that there is no major changes in the prevalence rates of the diseases. (Quah, 2005)

However, other source of study state that the prevalence of allergic is greatly increase in last 30 years and the triggers have been determined (Sporik *et al*,1992). Researchers also found that allergic disease is associated with significant gene and inherited among family members. So, the focus of this study is to measure the prevalence and association of HDM among patients in various ways.

1.1. Allergic

The definition given by Dorland's Medical Dictionary, 2001 W. B. Saunders Company; allergy is a hypersensitivity state acquired through exposure to particular allergen, re-exposure bringing to light an altered capacity to react. Whereas allergen is an antigenic substance capable of producing immediate hypersensitivity (*allergy*). In medical practical clinical, allergy can be classified into immune mediated injury hypersensitivity type 1.

Allergy to house dust mite is accepted as genetically predisposed (Young *et al*, 1992). There are also many type of allergy and its triggers.

1.1.1. Allergic Conjunctivitis

Conjunctivitis is one of the most common and treatable eye conditions in children and adults. It can be determined by a few symptoms such as redness in the white of the eye or inner eyelid, increased amount of tears, itchy eyes, blurred vision and swelling of the eyelid. When the inflammation occur, the tissue that lines the inside of the eyelid helps keep the eyelid and eyeball moist.

1.1.2 Anaphylaxis

'Anaphylaxis is a serious, potentially life-threatening allergic response that is marked by swelling, hives, lowered blood pressure and dilated blood vessels. Anaphylaxis may begin with severe itching of the eyes or face and progress to more serious symptoms. It is anaphylactic shock; a manifestation of immediate hypersensitivity in which exposure of a sensitized individual to a specific antigen or hapten results in life-threatening respiratory distress, usually followed by vascular collapse and shock and accompanied by urticaria, pruritus and angioedema' (Dorland's,2003). In severe cases, blood pressure will drop severely and swelling occurs in the bronchial tissues of the lungs, causing the person to choke and lose consciousness. Anaphylactic shock can be fatal if not treated immediately.

This condition occurs when the immune system creates specific antibodies, IgE toward a substance that is normally harmless, such as food and mites. Upon first exposure to the substance, the body does not react, but it does produce the antibodies. When exposed to the substance again, the antibodies will go into action, releasing large amounts of a protein called histamine that causes the symptoms described above.

1.1.3 Drug Allergy

Many drugs can cause adverse side effects, and certain medicines can trigger allergic reactions. When a drug first enters the body, the immune system mistakenly responds by creating specific IgE antibodies, that recognize the drug as a foreign substance. When the drug is taken again, these antibodies will release large amounts of histamine in an attempt to expel the drug from the body.

Drug allergy symptoms can range from mild to life-threatening. Many drugs can cause irritation, such as an upset stomach. During an allergic reaction, the release of histamine can cause symptoms like hives, skin rash, itchy skin or eyes, congestion, and swelling in the mouth and throat. In severe reaction people can have a difficulty of breathing, blueness of the skin, dizziness, fainting, anxiety, confusion, rapid pulse, nausea, diarrhea, and abdominal problems.

1.1.4 Eczema

Eczema is term for a group of medical conditions that cause the skin to become inflamed or irritated. The most common type of eczema is known as atopic dermatitis, or atopic eczema. Atopic refers to a group of diseases with an often inherited tendency to develop other allergic conditions, such as asthma and

hay fever. In Malaysia, the prevalence of atopy in children with history of eczema is 90% (Mujahid,2004).

'The most obvious example of acute eczematous dermatitis is an acute contact reaction to poison ivy or other contact antigen, such as laundry detergent. Such lesions are characterized by itchy, edematous, oozing plaques, often containing small and large blisters' (Robbin *et al*,2003)

Affected areas usually appear very dry, thickened or scaly. In fair-skinned people, these areas may initially appear reddish and then turn brown. Among darker-skinned people, eczema can affect pigmentation, making the affected area lighter or darker.

1.1.5 Food Allergies

A food allergy occurs when immune system responds defensively to a specific food protein that is not harmful to the body.

Symptoms may appear almost immediately, or up to two hours after ate the food. Symptoms include a tingling sensation of the mouth, swelling of the tongue and throat, hives, skin rashes, vomiting, abdominal cramps, difficulty breathing, diarrhea, a drop in blood pressure, or even a loss of consciousness. Severe reactions called anaphylaxis can result in death.

There are six foods that cause over 90% of food allergies in children - milk, eggs, peanuts, wheat, soy, and tree nuts. In adults, 90% of food allergies are caused by peanuts, tree nuts, fish and shellfish.

The incidence of HDM and food allergy especially crabs and prawns, is significantly greater in Malaysian children with rhinitis symptoms (Mujahid,2004).

1.1.6 Latex Allergy

Latex, also known as rubber or natural latex, is derived from the milky sap of the rubber tree. Latex allergy is an allergic reaction to substances in natural latex. Rubber gloves are the main source of allergic reactions.

When people have a close contact with any latex substances it will induce certain reaction. There are three types of latex reactions; irritant contact dermatitis, allergic contact dermatitis and immediate allergic reaction.

Irritant contact dermatitis is the least threatening type of latex reaction, classified as a non-allergenic skin reaction. It usually occurs as a result of repeated exposure to chemicals in latex gloves and results in dryness, itching, burning, scaling and lesions of the skin.

Allergic contact dermatitis, a delayed reaction to additives used in latex processing, which results in the same type of reactions as irritant contact

dermatitis (dryness, itching, burning, scaling and lesions of the skin), but the reaction is more severe, spreads to more parts of the body and lasts longer.

Immediate allergic reaction is the most serious reaction to latex. It can show up as rhinitis with hay fever-like symptoms, conjunctivitis, cramps, hives and severe itching. It is rare, but symptoms may progress to include rapid heartbeat, tremors, chest pain, difficulty breathing, low blood pressure, anaphylactic shock or potentially, death.

1.1.7 Diagnosis

Until now there is no cure for allergy, but it can be treated and controlled. There are numerous of tests to diagnose asthma and allergy. Firstly, doctor need to review the medical history of the patients, symptoms and general health.

Test can be done using skin prick test and blood test. It is also the most favorable method by researcher to evaluate the common allergens that contribute to allergy as well as asthma.

The skin prick test is the most common and reliable test for most allergies. The procedure is fairly painless. A small needle is used to lightly prick or scratch back or forearm with a tiny amount of allergen. After 15-20 minutes, doctor will be able to interpret the results by examining each spot where allergens were scratched or pricked into skin. The spots where are allergic will become red and swollen, and the others will remain normal.

Other alternative test is intradermal test. It is done when the skin prick test results are unclear. It is similar to the SPT, but involves injecting a small amount of allergen under the skin using a needle. Reactions to skin testing should clear up quickly because skin testing involves the injection of allergens under the skin. Skin test involve few risk of anaphylaxis. So, it should only be performed in a medical setting, with access to emergency treatment.

The blood test measures the levels of allergy antibody, IgE, produced when blood serum is mixed with a series of allergens in a laboratory, perform using ELISA method. It will show positive result if the IgE levels increase above the threshold. The blood test may be used if patients have skin problems like eczema or history of anaphylaxis to avoid any risk.

ELISA is used to detect and quantify specific serum antibodies (serum is blood plasma without the clotting factors). In ELISA, serum to be tested is exposed to specific antigens. Serum antibodies that combine with their antigens are detected by treating the test system with a conjugate, or another antibody linked to an enzyme. This antibody enzyme complex serves as a marker and attaches only to a specific substrate. When a substrate for the enzyme is added to the assay, a reaction between the substrate and the conjugate is usually indicated by a color change. Using calorimetric methods, the density of the solution can be determined and the concentration of IgE is proportional with the density.

1.2. Objectives

Primary objective :

- Prevalence study of house dust mites allergen among HUSM patients.

Secondary objectives :

- Comparisons between healthy and allergy patients
- Statistical analysis between parameter of sex, race and age.

2. Review of literatures

2.1 House Dust Mites

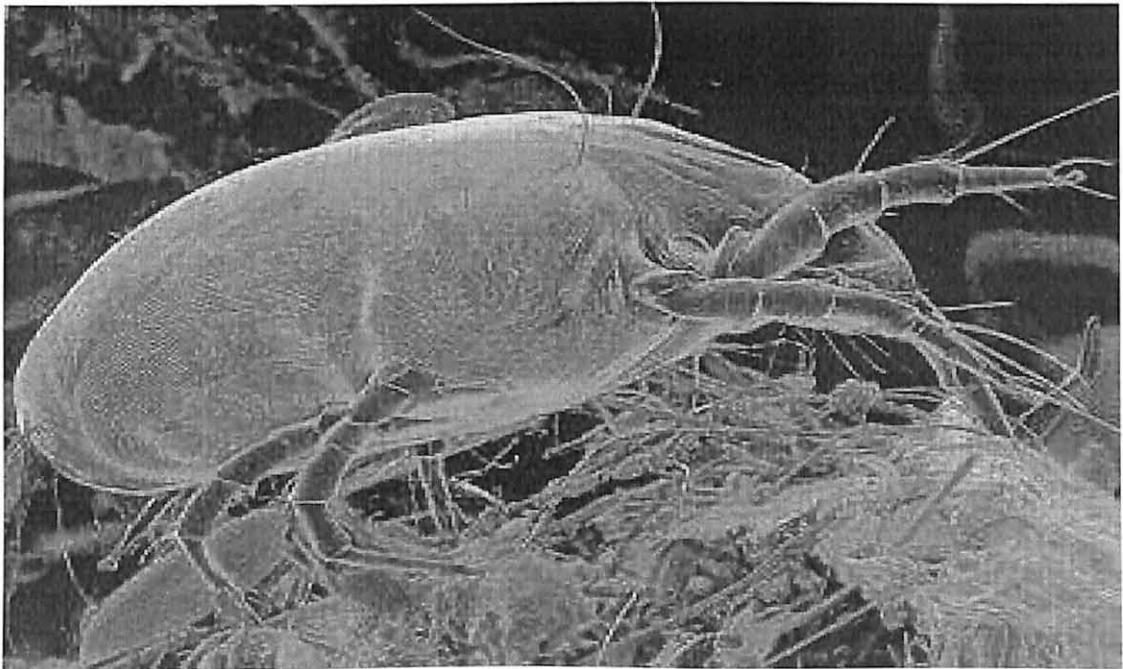


Figure 2.1 House dust mite views under electron microscope

(Reproduce from : <http://www.alerchek.com/mite3.jpg>)

As can be seen in figure 2.1, house dust mites, due to their very small size (250 to 300 microns in length) and translucent bodies, are not visible to the unaided eye. "The adult mite's cuticle (covering) has simple striations that can be seen from both the dorsal (top) view and from the ventral (bottom) view. The ventral view of the house dust mite reveals long setae (hairs) extending from the

outer margins of the body and shorter setae on the rest of the body. Through the microscope, one will see many oval-shaped mites scuttling around and over one another. There are eight hairy legs, no eyes, no antennae, a mouthpart group in front of the body (resembles head) and a tough and translucent shell (Mollet and Robinson,1996)

Its scientific classification is shown below:

- Kingdom: Animalia
- Phylum: Arthropoda
- Class: Arachnida
- Order: Acarina
- Family: Pyroglyphidae
- Genus: Dermatophagoidea

Binomial name : *Dermatophagoidea pteronyssinus* (figure 2.1)

House dust mites need certain conditions to live and survive. The conditions needed are warm, dark and moisture. Optimal growth for a mite colony occurs at a temperature of 25°C. They are unable to regulate their body temperature, have no eyes, never drink and have no organized respiratory systems and their body weight is up to 75% water. The house dust mite has eight legs, each with a sucker and hooks. This ensures easy travel on clothing,

blankets, soft toys and old furniture to colonise and infest suitable nest sites. Male can live from 19-30 days while a female up to two months, laying eggs for the last 30 days of her life. From eggs to adult the mite will pass through six stages of life (Chapman *et al.*1997)

House dust mite is one of the major allergens responsible for asthma. This study has been made by Sporik *et al* in 1992. There are 16 known allergens of the house dust mite. Other researcher his research said that, at least four of the *Dermatophagoides pteronyssinus* house dust mite allergens are proteinases. 'Der p1 is a cysteine proteinase and Der p3, 6 and 9 are serine proteinases. These proteins thus fulfil a criteria of both allergens and proallergic adjuvants' (Hewitt *et al*, 1995)

The several studies that have been made show the prevalence of asthma connected with allergy and HDM exposure. The excretion of the mites contains a number of protein substances. When these are inhaled or touch the reparatory mucosa and skin, the body produces antibodies. These antibodies cause the release of a chemical called histamine that leads to swelling and irritation of the upper respiratory passages cause typical asthma and hay fever symptoms.

2.2 Case Study

Survey of literature revealed that, there are many studies have been made on prevalence of house dust mites allergy specifically among the allergic patients around the world. Still, in Malaysia there are also few study reveal the association between allergic and asthma by making test through the common allergen that contribute the disease.

Using the specific test such as skin prick test and serum IgE level, (allergen-specific test results, table 2.1) many researcher are able to show that some specific allergens had stronger associations with asthma.

<i>Skin prick tests*</i>	<i>% Non asthmatics atopic (n =399)</i>	<i>% Asthmatics atopic (n =315)</i>	<i>Relative risk</i>	<i>% of cases attributable to atopy</i>
No. of positive tests				
1+	35.1	57.1	2.5	34
2+	16.5	40.6	3.5	29
3+	7.3	27.3	4.8	22
4+	2.0	14.3	8.1	13
5+	1.0	9.8	10.8	9
6+	0.5	7.0	14.9	7
7+	0.3	2.9	11.7	3
Specific skin prick tests				
Any positive test	35.1	57.1	2.5	34
Rye grass	23.4	44.4	2.6	27
House dust mite	19.4	43.7	3.2	30
Cat	5.5	23.2	5.2	19
<i>Alternaria</i>	4.5	8.6	2.0	4
Dog	2.2	9.9	4.9	8
Horse	1.8	10.2	6.3	9
<i>Cladosporium</i>	1.3	5.7	4.7	4
Kapok	1.5	4.1	2.8	3
<i>Aspergillus fumigatus</i>	0.5	4.8	9.7	4
Wool	0.8	4.4	6.0	4
<i>Penicillium</i>	2.0	2.5	1.3	1

Table 2.1. Percentage of asthma cases attributable to atopy based on number of positive skin prick tests and specific skin prick tests (Sears *et al*,1989)

In Asian region, many research was carried out. Example of it is the research made by Abdulrahman *et al* in 1997. It was done in Saudi Arabia region using skin prick test method to evaluate the allergen. SPT results of 462 patients tested with *D. pteronyssinus* and *D. farinae* show that up to 25.1% and 19.1% positive reactions were obtained by *D. pteronyssinus* and *D. farinae* respectively in asthmatic children in the mountainous region, while 56.3% positive reactions were obtained by *D. farinae* in coastal areas. In agriculture and dry regions, the figures were 7.6% and 12.6% respectively for *D. pteronyssinus* and *D. farinae*. However, 31% positive reactions to HDM reveal sensitization of individuals (or those already sensitized) in the dry region as well.

In Singapore, a study has been carried by Kidon *et al* in 2005 to define the prevalence, clinical characteristics and risk factors of species-specific mite sensitization in paediatric allergic rhinitis (AR) patients. One hundred and seventy-five patients were included, 119 (68%) males, 142(81%) Chinese, age mean 7.9 years (range 2 - 16). Sixty-eight patients (39%) reported a concomitant diagnosis and/or clinical complaints of bronchial asthma and 84 (48%) of atopic dermatitis. Skin prick test results were positive for traditional house dust mites (*Dermatophagoides pteronyssinus*. and *D. farinae* mix) in 85% of patients and for *B. tropicalis* in 62%. Overall mite sensitization was 98%, household pets 10%, moulds 9% and food proteins 12%. By far the single most significant factor associated with *Dermatophagoides* sensitization in the group studies was the presence of allergic eczema (odds ratio (OR) 31.8%, 95%

confidence interval (CI) 3.6 - 285, $P=0.002$). Allergic eczema was negatively associated with *B. tropicalis* sensitization (OR 0.26%, 95% CI 0.14 - 0.5).

A group of researchers have made a study to evaluate 16 foods common to the Malaysian diet and 4 common aeroallergens and measure the percentage of each allergen. This study was made by Mujahid *et al* in 2004. In this research, one hundred forty one children (up to 12 years) were skin prick tested to evaluate 16 foods common to the Malaysian diet and 4 common aeroallergens. Eighty-five percent of patients had positive SPT reactivity. The most commonly implicated aeroallergen and food allergen was house dust mite (HDM) and Prawn. Seventy percent had positive SPT reactivity results to HDM and 24.8% to prawns. Fifty five percent were positive to more than one allergen and 17.7% positive to single aeroallergen. The prevalence of atopy in children with history of eczema was 90%. They concluded that the incidence of HDM and food allergy, is significantly greater in Malaysian Children with rhinitis symptoms.

Since it is accepted that HDM is the major allergen that contribute to asthma, previous study was carried out to evaluate the dust mite fauna in the Klang Valley. Dust samples were collected from 20 houses from March 1994 to February 1995. Thirty-three dust samples from mattresses were examined monthly for the occurrence of HDM. A total of 22 species in 9 families of HDM was identified. The most common and densely populated species was *Blomia tropicalis* with an average density of 8,934 mites/g of dust. *Dermatophagoides*

pteronyssinus was the next in abundance, followed by *Malayoglyphus intermedius* (Mariana et al, 2000).

While other research was done to determine the contribution of HDM to asthma, Vervloet in 1999 has went further to study whether HDM level of exposure is related to the development of asthma in HDM sensitive individuals in France. The cumulative prevalence of asthma was evaluated in a group of 157 schoolchildren, aged 10 and 11 yrs, who were allergic to HDM allergen, and compared it with HDM allergen exposure and atopic status, using univariate and multivariate analysis. HDM allergen levels were measured in mattress dust using ELISA method. From the mattress dust samples, 94% had an HDM allergen level >2 mg.g dust-1. In conclusion, the risk of the occurrence of asthma in sensitized individuals depends on the degree of atopy (number of positive skin tests). Mite allergen exposure was not predictive of the occurrence of asthma. The hypothesis is, house dust mite-exposure is so high in the area studied that virtually all genetically-predisposed individuals will become more sensitive to mites.

3. Materials and Methods

3.1 Materials

3.1.1 Samples

40 Serum samples

- 20 serum samples from allergic patients
- 20 serum samples from healthy individuals

3.1.2 Reagent and Kit

ALerCHEK's Allergen Specific Human IgE (dust mites)

- Allergen coated microwell strips 12x8 with plastic frame
- HRP conjugated anti-human IgE - 2 X 12mL
- TMB/peroxide substrate color developer II – 30ml
- Sulfuric acid termination reagent (0.5N) –12mL

3.1.3 Solution and Buffer

15 X Wash buffer concentrate – 60mL, Saline

3.1.4 Equipment

Pipette 100uL, Volumetric Flask.