

Original Article

Air-conditioner filters enriching dust mites allergen

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Abstract: We detected the concentration of dust mites allergen (Der f1 & Der p1) in the air of different places before and after the starting of air-conditioners in Wuhu City, Anhui, China, and to discuss the relation between the dust mites allergen in air-conditioner filters and the asthma attack. The dust samples were collected from the air-conditioner filters in dining rooms, shopping malls, hotels and households respectively. Concentrations of dust mites major group allergen 1 (Der f 1, Der p1) were detected with enzyme linked immunosorbent assay (ELISA), and the dust mite immune activities were determined by dot-ELISA. The concentration of Der f1 in dining rooms, shopping malls, hotels and households was 1.52 µg/g, 1.24 µg/g, 1.31 µg/g and 1.46 µg/g respectively, and the concentration of Der p1 in above-mentioned places was 1.23 µg/g, 1.12 µg/g, 1.16 µg/g and 1.18 µg/g respectively. The concentration of Der f1 & Der p1 in air was higher after the air-conditioners starting one hours later, and the difference was significant ($P < 0.05$, respectively). Additionally, dot-ELISA findings revealed that the allergen extracted from the dust was capable of reacting with IgE from the sera of asthma mice allergic to dust mites. The study concludes that air-conditioner filters can enrich dust mites major group allergen, and the allergens can induce asthma. The air-conditioner filters shall be cleaned or replaced regularly to prevent or reduce accumulation of the dust mites and its allergens.

Keywords: Acaridia, Astigmatina, dust mite, major group allergen 1, air-conditioner filters

Introduction

In recent decades, the incidence of asthma and other allergic diseases increases significantly all over the world [1-3]. In patients with asthma and allergic disease, a large part of people are very sensitive to indoor allergens such as dust mites, animal dander, cockroaches, and mold spores, etc. The dust mite's allergens are considered to be induced and worsen asthma symptoms of asthma risk factors [4-7]. Various kinds of allergic disease in clinically, allergic disease resulted in mites accounted for about 70%~80%. Therefore, it is particularly important to study the propagation of dust mites in indoor and its spread way of mite allergen for exploring the mechanism of asthma. Wuhu City is located in the middle and lower reaches of the Yangtze River, with a subtropical monsoon climate, rich rainfall and four distinctive seasons. Local residents all have the habit of using air-conditioner in summer and winter, but dust mites in air-conditioner filters is easy

to breed. Body shell, feces and so on of dust mite all have very strong allergen and it seriously damages to human health [8, 9]. While air conditioner is running, the enrichment dust mite's allergens from the filters of air-conditioning system extremely likely spread in the interior. To investigate the relationship between dust mite allergen in air-conditioner filters and asthma, we detected the concentrations of the dust mites major group allergen 1 (Der f1, Der p1) in air-conditioner filters and the concentrations in air before and after starting air-conditioner from different places in Wuhu City. We hope that the conclusion can prompt residents how to scientific and reasonable use of air conditioner, and prevention by using air conditioner mite allergy.

Materials and methods

Dust samples collecting

Dust samples were collected from the air conditioner filters in different places, such as dining

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rooms, shopping malls, hotels, and households in Wuhu City and the air-conditioner filters of above places were not cleaned at least nearly one month. Selected ten sets of air-conditioners each place, and set up air dust sampler (TQC-1500, Jiangsu electronics) near to air-conditioners at 1 meter spacing, and set air flow rate to 20 L/min. Continuous taking sample two hours before and after the starting one hour respectively. After the sampling, then take down the air-conditioner filters immediately, and swept gently to collect dust in a plate with brush, then load dust samples into a plastic bags, and record the related information, tack these back to the lab for later using.

Preparation for mice serum of dust mite's allergic asthma

We adopted the method of Secor *et al.* [10] and slightly improved to prepare the model mice of specificity dust mites allergic asthma, as described below. The dust mites allergens (Der f1 and Der p1) were blended by equal ratio, and diluted into concentration of 100 µg/ml, The BALB/c mice (SFP, Center of comparative medicine, Yangzhou University) were sensitized by intraperitoneal injection. Take 30 mice with typical performance of dust mite's allergic asthma; take blood from orbital, centrifugal separation for serum, place to 40°C for later use.

Extraction of dust mite's main allergen in air-conditioner filters

The large particles of dust impurities were removed by 60 targets screening. The dust was dissolved in phosphate buffer PBST containing 0.05% Tween-20 according to w/v (mg/ml). After ultrasonic grinding (200 V) 5 min, the solution was set in a air bath temperature oscillators (4°C, 100 r/min) for 24 hours, and then the solution was centrifuge at 3000g and 4°C for 15 min. At last, the supernatant were filtrated with 0.22 µm microporous membrane, and placed to -20°C for later use.

Extraction of dust mites main allergen in air surrounding the air-conditioner

The glass fiber membrane in dust sampler were removed and cut into pieces. The pieces were put in a syringe (5 ml) containing 2 ml PBST, then the syringe was placed in a air bath temperature oscillators (4°C, 100 r/min) for 24 hours. The solution was centrifuge at 3000 g

and 4°C for 15 min also. At last, the supernatant were filtrated with 0.22 µm microporous membrane, and placed to -20°C for later use.

Test of allergen

Concentrations of dust mite's main allergen (Der f 1, Der p1) in extract were detected with double antibody sandwich ELISA method. The serum of dust mites allergic asthma mice (the experimental group, n=30) and PBST (the control group, n=30) were join the ELISA plate hole respectively, package these at 4°C for overnight. The plates were washed with TBST buffer (50 mmol/L Tris-Cl, Ph 7.5; 1 ml/L Tween-20) for five times, and then the plates were enclosed one hour at 37°C with 200 µL PBST + 10 g/L BSA buffer. The mice serum were diluted with PBST and 10 g/L BSA for 1:5 proportion, and joined to the plate for 100 µL /hole, hatched at 37°C for 1 hour. The plates were washed with TBST buffer for 5 times, and added HRP labeled sheep-anti-rat IgE antibody 100 µL /hole (using PBST + 10 g/LBSA buffer to diluted to 1:1000), hatched at 37°C for 2 hours. The plates were washed with TBST buffer 5 times again, and added the TMB solution for reacting 20 min in 37°C. At last, his reaction was terminated by termination liquid (50 µL/hole). The OD value was obtained by microplate reader at wavelength of 450 nm.

Statistical analysis

Measurement data is expressed "mean + standard deviation". The SPSS19.0 statistical software was used for statistical analysis. The concentrations of dust mites main allergen in different places were compared with F test; The concentrations of IgE in experimental group and control group were compared with two samples t test; The concentrations of dust mites allergens before and after starting were compared with primarily matching t test, to say have statistical significance when $P < 0.05$.

Results

Concentrations of dust mite's main allergen in air-conditioner filters

The concentrations of dust mites main allergen (Der f 1) in of air-conditioner filters of dining rooms (n=10), shopping malls (n=10), hotels (n=10) and residents (n=10) in Wuhu City is 1.208 ± 0.720 µg/g, 1.046 ± 0.611 µg/g, $1.127 \pm$

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Table 1. Concentration of Der f1 in the air of the different places before and after starting of air-conditioner

Group	n	Before starting	After starting	$\bar{d} \pm sd$	Paired t value	P value
dining rooms	10	5.874±2.491	8.275±2.708	2.401±2.289	3.317	<0.01
shopping malls	10	4.109±1.558	6.771±1.967	2.662±1.953	4.310	<0.01
hotels	10	4.352±1.879	7.245±2.220	2.893±2.292	3.991	<0.01
residents	10	4.481±2.116	7.630±2.341	3.149±3.443	2.892	<0.05

Table 2. Concentration of Der p1 in the air of the different places before and after starting of air-conditioner

Group	n	Before starting	After starting	$\bar{d} \pm sd$	Paired t value	P value
dining room	10	6.943±1.649	9.366±2.081	2.423±2.899	2.643	<0.05
shopping malls	10	4.485±1.510	6.676±1.684	2.191±2.778	2.494	<0.05
hotels	10	5.246±1.453	7.224±1.690	1.978±2.702	2.315	<0.05
residents	10	5.878±1.438	8.022±1.888	2.144±2.538	2.672	<0.05

0.555 µg/g and 1.318±0.754 µg/g respectively, $F=0.305$, $P>0.05$, the difference had no statistically significant. The results show that the concentration of dust mites allergens Der f1 in air-conditioner filters of different places has no difference. The concentrations of dust mites main allergen (Der p1) is 1.251±0.767 µg/g, 0.941±0.536 µg/g, 1.174±0.541 µg/g and 1.358±0.686 µg/g respectively, $F=0.764$, $P>0.05$, the difference had no statistically significant also. The results show that the concentration of dust mite's allergens Der p1 in air-conditioner filters of different places also has no difference.

Concentration of dust mite's main allergen in the air nearby air-conditioner before and after the starting

The concentration of Der f 1 in the air nearby air-conditioner in dining rooms, shopping malls, hotels, and residents has statistical significance ($P<0.01$ or $P<0.05$) before and after the starting, tested by paired t test (**Table 1**). The concentration of Der p1 in the air of above 4 places also has statistical significance ($P<0.05$) compared with paired t test (**Table 2**). Therefore, running of air-conditioner had the affect on the concentration of Der f1 and Der p1 in the air, and the concentration increased obviously after starting.

Concentration of serum specific IgE in mice

The concentration of serum specific IgE of experimental group (n=30) is 64.444±1.369

IU/mL, and that of control group (n=30) is 27.095±1.206 IU/mL, the concentration between the two groups has statistically significant difference compared with two samples t test ($t=112.104$, $P<0.01$). The results show that the concentration of serum specific IgE of the experimental group is obviously higher than that of control group.

Discussion

In recent years, incidence and prevalence of asthma has increased significantly in many countries, asthma has become a global public health and social problems. According to the world health organization estimates, there are about 300 million people worldwide suffer from asthma [1], thus caused by the social burden and medical and health care costs has made many governments, families and individuals less weight. Our country for the first time issued a national asthma disease and related risk factors investigation results. According to the survey, asthma prevalence rising significantly in recent years in our country, the total prevalence of asthma is 1.24% in mainland of China, and there are about 30 million asthma sufferer [11]. But the exact causes of the rise in the incidence and prevalence remain unclear. To do this, scientists around the world are looking for a global rise in allergic disease causes and countermeasures. Research data show that the main reason for the rise in asthma incidence and prevalence may be related to the change of the indoor environment and the suction allergens

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in the air, especially dust mites allergens suspended in indoor. It may lead to sensitization when the concentration of dust mite allergen in indoor environment reach to 2 µg/mL, and the concentration >10 g/mL can bring about asthma attacks [7]. Result of study by Arroyave et al. [12] reveals that dust mites such as *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* are the most common inhaled allergens indoor, can cause systemic allergic diseases including asthma, allergic rhinitis, allergic conjunctivitis, atopic eczema, and urticaria, etc. Voorhorst & Spieksma [13] study reported that the dust mites is the major allergen in house dust, the dust mites has been attached importance to by scholars of Western Europe, the United States, Japan and other countries, together in their respective works proof dust mites (mainly to *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*) are widely distributed and very strong inhaled allergens. Asthma due to dust mite belong to exogenous I type of allergy, allergens I specific IgE antibody. The activity of Der p1 and Der f1 in dust mites fecal pellet is similar to thiol protease in physicochemical property, binding rate is 80%~100% with allergies serum IgE [12, 14-16].

There are more than 150 kinds of mites were discovered in room environment globally so far, the dust mites belong to Superorder Acariforms, Order Sarcoptiformes, Suborder Oribatida, Cohorte Astigmatina, Superfamily Analgoidea, Family Pyroglyphidae [17]. The Family Pyroglyphidae currently has reported 19 genera and about 50 species around the world, dust mites on the rooms main belong to 6 genera and 14 species, *Dermatophagoides* is the most common genera, including *D. pteronyssinus* (Trouessart, 1897), *D. farinae* (Hughes, 1961) and *D. microceras* (Griffiths & Cunnington, 1971), etc [18]. Due to dust mites allergens mainly distributed in the indoor mattress, sofa, wardrobe, ground dust, when cleaning flap clothes, bed sheets, etc it can be lifted and suspended in the air, and can be adsorbed on air-conditioner filters. Using air-conditioner after a period of time, a lot of dust particles and microbial adsorption can be adsorbed on the filters, there is likely to dust mites breeding and antigen of dust mites and molds. When people open the air-conditioner again, dust mites and its antigen (mite excretion, excreta, and death degradation products) on air-conditioner filters are blown into the indoor air, may lead to the occur-

rence of dust mites allergic disease [19]. Secondly, air-conditioner filters accumulated a lot of dust, the body falls off scurfy, cotton fiber, fungal spores, etc, combined with the temperature and humidity of the indoor relatively stable, thus create a comfortable environment for the mites breeding. Dust mites breed in the warm, humid, concealment and rich food environment [5, 20]. So air-conditioner filters constitute plants for concentration, processing and modification of dust mite's antigen. Urban residents spent more than 80% of the time in a variety of indoor environment every day, air-conditioners in use process continuously spread dust mites and mite allergen which deposited in indoor in dynamic way, this way may do more hazards to the human body than that of static way with do not using air conditioners. People are likely to be sensitized by inhaled the allergen. This may be an indoor sensitization path which is unknown to people but exists universally. In recent years, the widespread use of household appliances such as air conditioner and humidifier, living environment has become increasingly closed, which lead to dust mites allergic disease incidence increased year by year [7]. Patients with allergic disease allergic to dust mites has reached 60%~80%, in some areas even more than 80% in the children or youth patients with asthma yielded positive results for dust mites [21-23]. The detection of dust mite allergen in air-conditioner filter has been reported by scholars [24], but ours results show that the concentrations of two major allergen (Der f1 & Der p1) in the air of different places were significantly increased after starting air-conditioner, which declared that the air-conditioner have played an important role in gathering dust mites allergens, this should cause the attention of peoples who living or working in indoor with air-conditioner working for long time, and develop a good living habits of regular cleaning room and opening the window for ventilating. At the same time people should pay attention to cleaning the air-conditioner filters regularly to prevent or reduce the propagation of dust mites. This can reduce the concentration of dust mite allergen indoor to a certain extent, and lower the morbidity of dust mite's asthma.

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Disclosure of conflict of interest

None.

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