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Original Article

Acaroid mite allergens from the filters of air-conditioning system in China

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Abstract: Accumulation of acaroid mites in the filters of air-conditioners is harmful to human health. It is important to clarify the allergen components of mites from the filters of local air-conditioning system. The present study was to detect the allergen types in the filters of air-conditioners and assesse their allergenicity by asthmatic models. Sixty aliquots of dust samples were collected from air conditioning filters in civil houses in Wuhu area. Total protein was extracted from the dust samples using PBS and quantified by Bradford method. Allergens I and II were also detected by Western blot using primary antibody (anti-*Der f*1/2, *Der p*1/*Der f*2/*Der p*2, respectively). Ten aliquots of the positive samples were randomly selected for homogenization and sensitized the mice for developing asthmatic animal models. Total serum IgE level and IFN-γ, IL-4 and IL-5 in the bronchoalveolar lavage fluid (BALF). The allergenicity of the extraction was assessed using pathological sections developed from the mouse pulmonary tissues. The concentration of extract from the 60 samples was ranged from 4.37 μg/ml to 30.76 μg/ml. After analyzing with Western blot, 31 of 60 samples were positive for 4 allergens of acaroid mites, and yet 16 were negative. The levels of total IgE from serum IL-4 and IL-5 from the BALF in the experimental group were apparently higher than that of negative control and PBS group ($P < 0.01$), but there were no statistical difference compared to OVA group ($P > 0.05$). However, the IFN-γ level in BALF was lower compared with the negative control and PBS group ($P < 0.05$) but with the OVA group ($P > 0.05$). The pathological changes were evidently emerged in pulmonary tissues, which were similar to those of OVA group, compared with the PBS ground and negative controls. The air-conditioner filters in human dwellings of Wuhu area potentially contain the major group allergen 1 and 2 from *D. farinae* and *D. pteronyssinus*, which may be associated with seasonal prevalence of allergic disorders in this area.

Keywords: Acaroid mite allergen, asthma, air condition filters, China

Introduction

Allergic asthma is a frequent clinical entity, which is sharply increase by its prevalence and morbidity [1-3]. However, the pathogenesis of allergic asthma is so intricate due to the production of specific IgE as a result of the active response of body immune system to allergen exposure of the individual in his/her living surroundings, chronic airway inflammation through eosinophil and neutrophil recruitment, and variable airflow obstruction with airway hyper responsiveness [4] as well as the association of the un-equivalent presence of Th1 and Th2 resulted from type 2 cytokine domination [5]. Individual attack of asthma is primarily associated with exposure to certain allergens. However, acaroid mite allergen is one of the most important allergens leadings to asthma or allergic disorders [6, 7].

Wuhu (119°21'E/31°20'N) lies in the southeast of Anhui Province, China. It is characterized by pleasant temperature, rich rainfall and distinct seasons and superior to breed acaroid mites. The air-conditioning equipments used widely are helpful to accumulate the feces and remains of acaroid mites, which are harmful to human health [8-10]. In this study, we investigated the types of allergens in the filters of air-conditioners and analyzed their allergenicity. 60 samples were collected from the filters of the air-conditioning system in civil houses in Wuhu area, the allergens from major group 1 and/or 2 of acaroid mites were detected with Western blot, and determined tentatively the

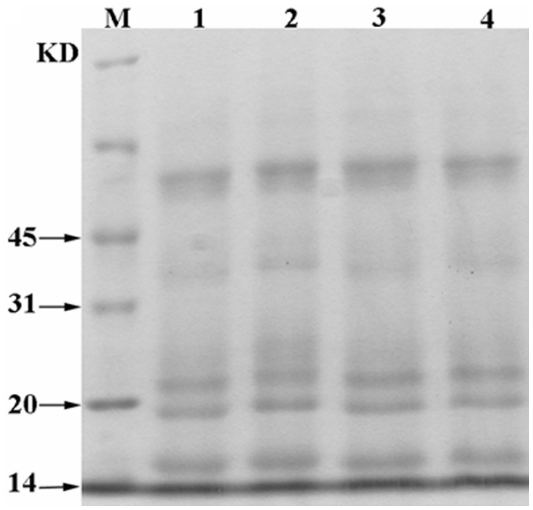

Figure 1. Treatment of the protein from the positive sample extract with SDS-PAGE. M: Protein marker. 1-4: Indicating the extract concentration at 5.0 μg/ml, 10.0 μg/ml, 20.0 μg/ml and 30.0 μg/ml, respectively.

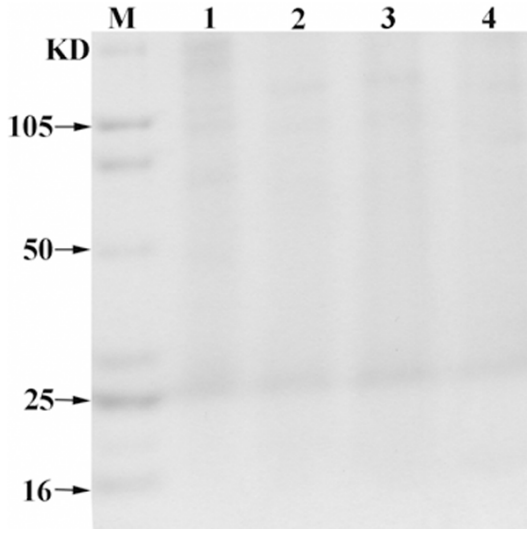

Figure 2. Treatment of the protein from the negative sample extract with SDS-PAGE. M: Protein marker. 1-4: The negative sample extract selected at random.

allergenicity by asthmatic mouse models to supply basis for prevention and therapy of the allergic disorders.

Materials and methods

Animals

Female BALB/c mice (6 weeks of age) were purchased from the Center for Comparative Medicine, Yangzhou University (License No.: SCXK 2007-0001) and provided with food and water *ad libitum* under specific-pathogens free conditions. All procedures were approved by the Research Ethics Board of Wannan Medical College.

Sample collection: Sixty samples were collected randomly from the air-conditioner filters in living rooms or bedrooms of the civil houses in Wuhu City between June and August of 2012, which were consent by the owners. The dust samples were treated as follows.

Allergen extraction and concentration determination: Ten gram samples from air-filters were dissolved in PBS solution at a ratio of 1:30 (W/V). The mixture was treated with ultrasonic smash (200 V) for 5 min and gas bath thermostats oscillator at 4°C by 50 r/min for 48 h. The extraction was centrifuged at 3000 g for 10 min, and the supernatant was filtered through 0.22 μm microporous membrane filter. Protein concentration was determined with Bradford method (595 nm) at -80°C for further use.

SDS-PAGE: Equal volumes (about 20 μg of total soluble proteins) of clarified extract of each treatment were analyzed on a 12.5% polyacrylamide gel according to Laemmli's method [11] in a Mini-PROTEAN 3 system (Bio-Rad, Berkeley, CA, USA) and stained with Coomassie blue R-250 (Sigma-Aldrich® Co. LLC. St Louis, MO, USA) to visualize the proteins.

Western blotting

For Western blot analysis, different concentrations of samples were analyzed on a 12.5% SDS-PAGE gel according to Laemmli's method [11] in a Mini-PROTEAN 3 system (Bio-Rad) and transferred onto an Immobilon-P membrane (EMD Millipore, Billerica, MA, USA). Membranes were incubated in blocking buffer (5% dried milk, 0.5% Tween-20 in PBS, pH 7.2) for at least 30 min. Afterward, the membranes were incubated for 2 h in blocking buffer containing Der f1 (*Der P1/Der f2/Der P2*)-specific rabbit polyclonal antiserum (obtained after immunization with the relevant purified native protein, respectively) mixed with PBS (pH 7.2) at a ratio of 1:10000. A horseradish peroxidase-conjugated goat anti-rabbit IgG (Sigma-Aldrich® Co. LLC.) mixed with PBS at a ratio of 1:10000 was used

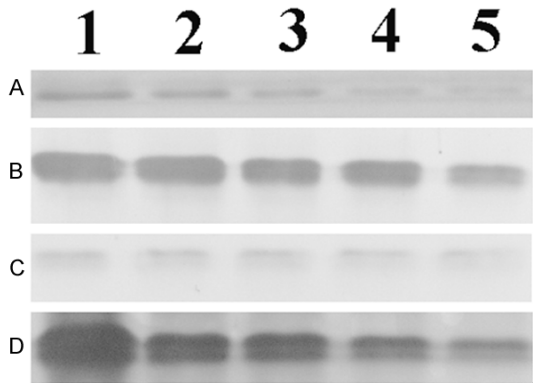

Figure 3. Western blot assay of the sample extract by dissimilar primary antibody incubation of the *Der f1, Der p1, Der f2* or *Der p2*. A-D: The rabbit serum sensitized with *Der f1, Der p1, Der f2* or *Der p2* as primary antibody; 1-5: The sample concentration at 30.0 μg/ml, 20.0 μg/ml, 10.0 μg/ml, 5.0 μg/ml and 1.0 μg/ml, respectively.

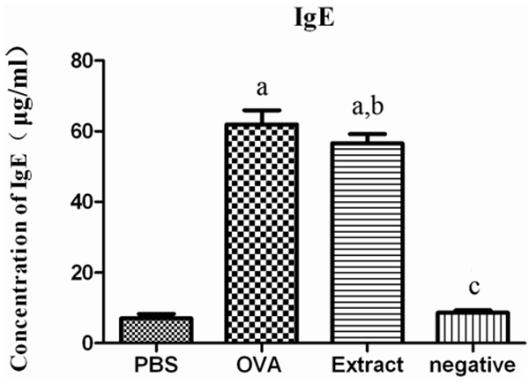

Figure 4. Serum IgE level for mice in each group. A: Compared with PBS group and negative controls, the statistics is different ($P < 0.01$); B: There is no statistical difference as compared with OVA group ($P = 0.85$); C: No statistical difference is found compared to PBS group ($P = 0.87$).

as secondary antibody, followed by three washes in blocking buffer (20 min each). Transferred proteins were visualized using DAB Horseradish Peroxidase Color Development Kit (Sangon Biotech, Shanghai, China) in PBS (pH 7.2) according to the manufacturer's instructions.

Development of mouse models with asthma

Forty BALB/c mice were randomly assigned to 4 groups ($n = 10$ for each), i.e., PBS group, OVA group, extract group (referred to the samples containing 4 allergens of acaroid mites), and negative group (referred to the samples not containing the allergens above). On days 0, 7 and 14, mice were intraperitoneally injected with 10 μg relevant allergen, respectively, which was dissolved in 100 μl PBS containing 2% (W/V) Al $(OH)_3$ suspension. The PBS group received PBS injection instead. At day 21, the animals were caged in the airway challenge apparatus, and challenged by nebulized inhalation of total protein suspension (20 μg/ml) for 30 min on 7 successive days. The concentration of OVA was 10 μg/ml. The PBS group was challenged by PBS instead.

Detection of cytokines in BALF and antibodies in sera

Twenty-four hours after the final aerosol challenge, the mice were anesthetized with intraperitoneal injection of 100 μl 0.5% pentobarbital sodium. After the trachea of each mouse was cannulated, a syringe with 19-gauge needle was used to infuse 0.3 ml of sterilized PBS and withdraw bronchoalveolar lavage fluid (BALF). This was repeated 2 more times, and a total of 0.9 ml BALF was obtained per mouse. Subsequently, BALF was centrifuged at 3000 × g for 5 min at 4°C, and the supernatant was collected and stored at -80°C. The blood samples were also collected via orbital cavity, centrifuged by 4000 × g at 4°C for 5 min and stored at -80°C. ELISA was performed to detect the levels of IFN-γ, IL-4, and IL-5 in BALF, as well as serum antibodies of IgE, according to the manufacturer's protocol.

Preparation of pathological sections from pulmonary tissue

The pulmonary sections were obtained from the other side of the lung free of lavage, fixed in 10% formalin overnight, embedded in paraffin, sliced conventionally and stained with hematoxylin and eosin (HE). The inflammatory changes were examined microscopically and assessed based on the extent of eosinophils infiltration, epithelia damage, and edema in the lung, according to the scoring protocol described by Underwood [12].

Statistical analysis

Statistical analysis was carried out using SPSS for Windows, version 16.0 (SPSS, Chicago, IL, USA), and the statistical data for each group

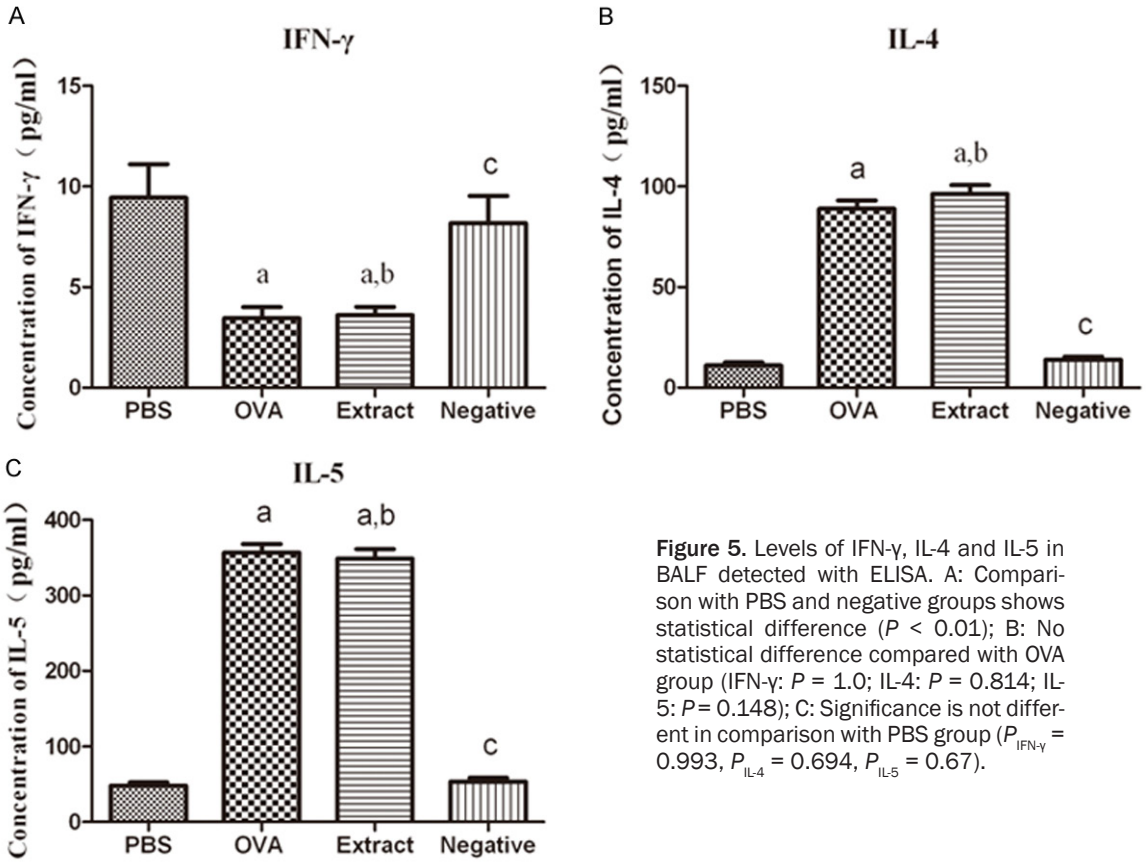

Figure 5. Levels of IFN-γ, IL-4 and IL-5 in BALF detected with ELISA. A: Comparison with PBS and negative groups shows statistical difference ($P < 0.01$); B: No statistical difference compared with OVA group (IFN-γ: $P = 1.0$; IL-4: $P = 0.814$; IL-5: $P = 0.148$); C: Significance is not different in comparison with PBS group ($P_{IFN-\gamma} = 0.993$, $P_{IL-4} = 0.694$, $P_{IL-5} = 0.67$).

were expressed in ($\bar{x} \pm s$) in terms of one-factor analysis of variance. The group comparisons were performed using least significant difference-*t* (LSD-*t*) and Thamhane's T_2 method. A *p*-value ≤ 0.05 was accepted as significant.

Results

SDS-PAGE and Western blot

The concentrations of total proteins varied from 4.37 μg/ml to 30.76 μg/ml among 60 samples after quantification. The major strips revealed between 14KD and 50KD by SDS-PAGE (**Figure 1**). The extracts with different concentrations were further assayed using different primary antibodies by Western blot, the results showed that positive samples revealed characteristic footprints, and there was no significant imprinting for the extract from negative samples (**Figure 3**). Contrarily, the samples confirmed by Western blot with negative mite allergens failed to present distinct strips (**Figure 2**).

Serum IgE concentration

The level of total sera IgE was not significantly different between PBS group (7.07 ± 3.86 μg/ml) and negative group (8.61 ± 2.14 μg/ml) ($P = 0.87$). However, compared with PBS and negative groups, the level of IgE in extract group (56.65 ± 8.12 μg/ml) was significantly different ($P < 0.01$, **Figure 4**), and there was no statistical difference between extract group and OVA group (61.99 ± 12.49 μg/ml) ($P = 0.85$) (**Figure 4**).

Determination of the levels for IFN-γ, IL-4 and IL-5 in BALF

IFN-γ levels in the extract group (3.61 ± 1.29 pg/ml) showed no statistical difference with OVA group (3.46 ± 1.74 pg/ml) ($P = 1.0$), but were lower than PBS group (9.45 ± 5.23) pg/ml and negative group (8.19 ± 4.25 pg/ml) ($P < 0.01$, respectively), and PBS group remained similar with negative group ($P = 0.993$) (**Figure 5A**). IL-4 levels in the extract group (96.27 ± 14.08 pg/ml) were significantly higher than

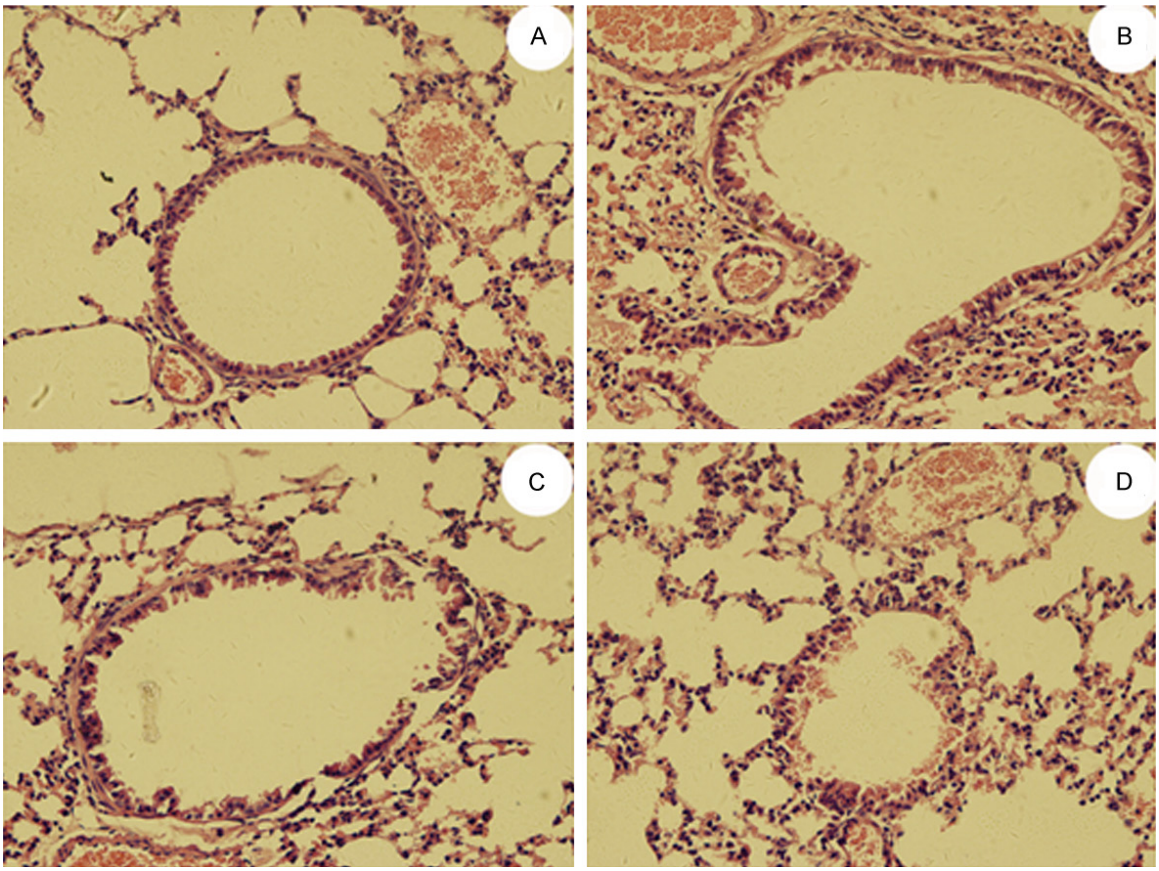

Figure 6. Pathological sections for mice in each group (× 400). A: PBS group; B: Negative controls; C: OVA group; D: Positive extract sensitized group.

those in groups of PBS (11.09 ± 4.65 pg/ml) and negative control (13.98 ± 4.58 pg/ml) ($P < 0.01$, respectively), yet indicated no statistical difference with OVA group (88.91 ± 13.25 pg/ml) ($P = 0.814$). PBS group was not as different as the negative controls in statistics ($P = 0.694$) (**Figure 5B**). Higher levels of IL-5 were seen in the extract group (338.67 ± 36.47 pg/ml) as compared with PBS group (48.11 ± 13.83 pg/ml) and negative controls (53.42 ± 16.03 pg/ml) ($P < 0.01$, respectively), whereas the levels had no significant difference with OVA group (356.95 ± 35.68 pg/ml) ($P = 0.148$), and PBS group also not different from the negative controls ($P = 0.67$) (**Figure 5C**).

Histopathological examination of the lung tissues

On histopathological examination of the lung tissues from each group of mice, slightly infiltrated inflammatory cells and minor effusion were found in PBS group, yet the bronchial wall remained intact (**Figure 6A**). The negative controls presented partially collapsed bronchial wall with more inflammatory exudates and eosinophil infiltration than PBS group (**Figure 6B**). The sections from OVA and positive extract sensitized groups exposed significant inflammatory cell infiltration into the pulmonary tissues and mucosal layers of the bronchi, with major eosinophils and massive inflammatory effusion in interstitium, and the bronchial wall was affected to different degrees, including damaged cilia of columnar epithelial cells, shed epithelial cells, spasm of smooth muscle and thickened bronchial walls with visible edema (**Figure 6C** and **6D**).

Discussion

Major group allergens 1 and 2 are greatly responsible for asthma and allergic disorders of variety [13-15]. The filters in air-conditioning

systems may supply favorable breeding grounds for the acaroid mites, thus resulting in cumulative air-borne allergens and onset of asthmatic symptoms. Our SDS-PAGE findings suggested that the total protein extracted from some of the samples was distributed at between 14KD and 50KD. Still, specific immunoblotting by Western blot assay was seen under diverse primary antibody incubation, this further confirmed that the total protein from previous extract contained major allergens 1 and 2, which are consistent with the previous reports, such as the findings on the indoor allergens investigated by Liu et al [16].

Allergic asthma attack is generally recognized as the results of so excessive proliferation of Th2 cells that leads to imbalanced presence of Th1/Th2, reduced cytokine IFN-γ secreted by Th1 cells, but increased IL-4 and IL-5 secretion from Th2 cells, eventually causing allergen-specific IgE antibody production and onset of asthmatic symptoms. In current study, the mice were sensitized with the positive extract from the dust samples, and the results determined by ELISA suggested no significant difference with animals treated with OVA regarding the levels of IgE, IFN-γ, IL-4 and IL-5, whereas that the difference was significant as compared with PBS group and negative controls. Pathological changes to a certain degree were seen in pulmonary sections for each group of mice, yet the lesion were prominent in animals these findings demonstrated that the dusts from the air-conditioner screens are highly allergenic effect to induce allergic response, including asthma. No significant statistical difference found for the animals treated with negative extract and PBS may further explain that the pathological changes in the previous lungs tissues are associated with major group allergens 1 and 2. Lian et al [17] detected the *Der p1*, *Der f1* and *Der 2* in the dust samples with monoclonal antibody-based ELISA, and concluded that mite allergens exist in the filters of air conditioner and are important source of indoor allergens and cause of the increasing prevalence of allergic asthma, which is in consistence with our findings.

Dust accumulation at the screens of air-conditioning system tends to increase the risks of mite breeding and allergen concentration, which are causative effects on human health. Regular cleaning of the filters and indoor desiccation by operating the air-conditioner can effectively reduce the humidity and control the mite breeding in a house [18]. This will availably bring down the acaroid mite allergen in living space and prevent from asthma attack as well as its prevalence [19-21]. The special geographical environment of Wuhu city is likely to supply the acaroid mites with favorable breeding conditions, and inappropriate operation of the air-conditioning system may otherwise make air-borne allergens accumulative in the house [22]. In our investigation on the sixteen negative samples, we found that those residents had habit of frequent cleansing their air filters in air-conditioning system. Whereas, the dwellers whose samples were positive reported irritation of upper respiratory tract symptom to a certain degree and even asthma attack soon after the air-conditioner was operating. Therefore, we recommend that the air filters in an air-conditioner should be cleansed regularly at least one to twice a week, which may be sound and practical approaches to improving the health of individuals with asthma by effectively getting rid of or reducing the indoor mite allergen concentration [23].

In summary, by application of molecular biology identification technique to detect the mite allergens in the air filters of air-conditioning system, we found that such allergens are almost present in the samples, with major group 1 and 2 predominant. These allergens are highly allergenic effects that shall call our public attention. These findings may supply with scientific and theoretical basis for appropriate operation of the air-conditioning system to effectively control the generation of the acaroid mite allergens in living environment as well as prevention of the allergic disorders due to sensitization to house acaroid mites.

Acknowledgements

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Disclosure of conflict of interest

None.

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References

[1] Beasley R. The burden of asthma with specific reference to the United States. J Allergy Clin Immunol 2002; 109: S482-489.

[2] Asher MI, Montefort S, Bjorksten B, Lai CK, Strachan DP, Weiland SK and Williams H. Worldwide time trends in the prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and eczema in childhood: ISAAC Phases One and Three repeat multicountry cross-sectional surveys. Lancet 2006; 368: 733-743.

[3] Lewkowich IP, Lajoie S, Clark JR, Herman NS, Sproles AA and Wills-Karp M. Allergen uptake, activation, and IL-23 production by pulmonary myeloid DCs drives airway hyperresponsiveness in asthma-susceptible mice. PLoS One 2008; 3: e3879.

[4] Galli SJ, Tsai M and Piliponsky AM. The development of allergic inflammation. Nature 2008; 454: 445-454.

[5] Prescott SL. Maternal allergen exposure as a risk factor for childhood asthma. Curr Allergy Asthma Rep 2006; 6: 75-80.

[6] Vichyanond P, Pensrichon R and Kurasirikul S. Progress in the management of childhood asthma. Asia Pac Allergy 2012; 2: 15-25.

[7] Zheng YW, Li J, Lai XX, Zhao DY, Liu XF, Lin XP, Gjesing B, Palazzo P, Mari A, Zhong NS and Spangfort MD. Allergen micro-array detection of specific IgE-reactivity in Chinese allergy patients. Chin Med J (Engl) 2011; 124: 4350-4354.

[8] Arlian LG. Dust mites: update on their allergens and control. Curr Allergy Asthma Rep 2001; 1: 581-586.

[9] Caplin J, Capriles-Hulett A, Iraola V, Pinto H, Sanchez-Borges M, de los Santos G and Fernandez-Caldas E. Allergic sensitization to domestic mites in Corpus Christi, Texas. Allergy Asthma Proc 2009; 30: 166-170.

[10] Sih T and Mion O. Allergic rhinitis in the child and associated comorbidities. Pediatr Allergy Immunol 2010; 21: e107-113.

[11] Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 1970; 227: 680-685.

[12] Underwood S, Foster M, Raeburn D, Bottoms S and Karlsson JA. Time-course of antigen-induced airway inflammation in the guinea-pig and its relationship to airway hyperresponsiveness. Eur Respir J 1995; 8: 2104-2113.

[13] Fasce L, Tosca MA, Olcese R, Milanese M, Erba D and Ciprandi G. The natural history of allergy: the development of new sensitizations in asthmatic children. Immunol Lett 2004; 93: 45-50.

[14] Hales BJ, Martin AC, Pearce LJ, Laing IA, Hayden CM, Goldblatt J, Le Souef PN and Thomas WR. IgE and IgG anti-house dust mite specificities in allergic disease. J Allergy Clin Immunol 2006; 118: 361-367.

[15] Pittner G, Vrtala S, Thomas WR, Weghofer M, Kundi M, Horak F, Kraft D and Valenta R. Component-resolved diagnosis of house-dust mite allergy with purified natural and recombinant mite allergens. Clin Exp Allergy 2004; 34: 597-603.

[16] Liu Z, Bai Y, Ji K, Liu X, Cai C, Yu H, Li M, Bao Y, Lian Y and Gao B. Detection of Dermatophagoides farinae in the dust of air conditioning filters. Int Arch Allergy Immunol 2007; 144: 85-90.

[17] Lian YY, Liu ZG, Wang HY, Chai CY and Liu XY. [Detection of mite allergens in the dust of filternet and air of air-conditioned room]. Zhongguo Ji Sheng Chong Xue Yu Ji Sheng Chong Bing Za Zhi 2007; 25: 325-327, 332.

[18] Arlian LG, Neal JS, Morgan MS, Vyszenski-Moher DL, Rapp CM and Alexander AK. Reducing relative humidity is a practical way to control dust mites and their allergens in homes in temperate climates. J Allergy Clin Immunol 2001; 107: 99-104.

[19] Lintner TJ and Brame KA. The effects of season, climate, and air-conditioning on the prevalence of Dermatophagoides mite allergens in household dust. J Allergy Clin Immunol 1993; 91: 862-867.

[20] van Strien RT, Gehring U, Belanger K, Triche E, Gent J, Bracken MB and Leaderer BP. The influence of air conditioning, humidity, temperature and other household characteristics on mite allergen concentrations in the northeastern United States. Allergy 2004; 59: 645-652.

[21] Kidon MI, See Y, Goh A, Chay OM and Balakrishnan A. Aeroallergen sensitization in pediatric allergic rhinitis in Singapore: is air-conditioning a factor in the tropics? Pediatr Allergy Immunol 2004; 15: 340-343.

[22] Shah S and Bapat MM. Improper window air-conditioning of home and occurrence of house dust mite allergen infestation in Mumbai City women. Indian J Med Sci 2006; 60: 472-474.

[23] Diette GB, McCormack MC, Hansel NN, Breysse PN and Matsui EC. Environmental issues in managing asthma. Respir Care 2008; 53: 602-615; discussion 616-607.