PREVALENCE OF Fel d 1 CAT ALLERGEN IN HOMES IN SAUDI ARABIA

SYED M. HASNAIN1*, HALIMA A. ALSINI1, FUTWAN A. AL-MOHANNA1, ABDULRAHMAN S. AL-FRAYH2, HUSNI AL-RAYES3, HAZIM S. MUNSHIE3 AND SULTAN T. AL-SEDAIRY1

1King Faisal Specialist Hospital and Research Centre, P.O.Box 3354, Riyadh 11211, MBC: 03, KSA.
2Department of Pediatric, College of Medicine, King Saud University, P.O.Box 2925 (39), Riyadh 11461, KSA.
3Department of Pediatrics, Maternity and Children Hospital, P.O.Box 4134, Makkah 21955, KSA.

AUTHORS’ CONTRIBUTIONS
This work was carried out in collaboration between all authors. Author SMH designed the study, wrote the protocol, edited and completed the final version of the manuscript. Author HAA performed the ELISA, data analysis and wrote the first draft of the manuscript. Author FAAM obtained the fund approval of the project, discussion and overall supervision. Author ASAF managed the analyses of the study and sample collection. Author HAR contributed in sample collection and site selection and review. Author HSM participated in sample collection and review. Author STAS advised and guidance of the project. All authors read and approved the final manuscript.

Received: 23rd May 2017
Accepted: 4th July 2017
Published: 12th July 2017

ABSTRACT

Aims: Cat allergen levels in settled house dust and their determinants in Saudi Arabia are unknown. The aim of this study was to quantify the levels of the major cat allergen in house dust samples of Saudi homes.

Study Design: Random collection of house dust samples from patients and control homes were quantitatively analyzed for Fel d 1 cat allergen levels using immunoassay techniques.

Place and Duration of Study: The study was carried out in three major cities in Saudi Arabia, viz. Riyadh, Makkah and Jeddah between August 2014 and October 2016.

Methods: A total of 428 house dust samples were randomly collected from allergic patients (n=155) and control homes (n=273). Sieved dust samples were extracted in PBS and, using mAb ELISA, analyzed for Fel d 1 cat allergen. Cat allergen concentration levels were expressed in microgram per gram of dust.

Results: Our results showed that Fel d 1 was present in 86% of homes. The range of values of cat allergen was 0.001-1.938 μg/g dust. Though, the Fel d 1 concentration levels in house dust samples were generally low, clinically significant levels of Fel d 1 (1-8 μg/g dust) were found in 7% of homes. The majority of homes (both control and patients) with higher levels had no cats in their premises. The levels in Makkah samples (12.7%) were significantly higher compared to Riyadh (4.4%).

Conclusion: The study revealed that a large number of homes, of cat free homes, contained the major allergen Fel d1 and some homes reaching clinical threshold. Therefore, for in vivo and in vitro diagnostic screenings, it is advisable to include cat allergen even if the patients not exposed to cat directly.

*Corresponding author: Email: hasnain@kfshrc.edu.sa;
Email: HAAlsin@kfshrc.edu.sa;
Keywords: Allergy; indoor allergens; cat allergen; asthma; ELISA.

1. INTRODUCTION

The indoor environment has been recognized as a common source of exposure to many allergens. Inhalant allergens play a major role in the pathogenesis of allergic asthma and allergic rhinitis. Indoor allergens from house dust mites, cat, cockroaches, and fungi are of particular importance [1-3]. Dust found inside the home was the most relevant environmental factor related to positive cases of IgE sensitization in children [4]. The quantity of danders that is distributed by cats, dogs, or humans is sufficient to supply food for dust mites. Cat allergen, Fel d 1 (and Dog allergen, Can f 1) can be found in microgram quantities in dust samples [5,6]. Exposure to sources of allergens such as cats, house-dust mites, fungi, and insects play a significant role in patients with allergic rhinitis and asthma [7]. The identification of the major allergens has led to methods that can quantitate exposure, e.g., immunoassays for Fel d 1 in settled dust samples [8]. Increasing exposure and increasing sensitivity to indoor allergens represent a progressively higher risk factor for the development of asthma [9,10]. The development of sensitivity to indoor allergens and the symptoms and severity of asthma in later childhood are directly related to the exposure to allergens in infancy.

Cat allergens are among the most important indoor allergens and a common cause of IgE mediated allergic disease world-wide [11,12]. Sensitization to individual cat and dog allergenic molecules can contribute differently to the development of allergy to these animals.

Studies have shown that sensitization to cat is a strong risk factor for asthma, and in order to prevent respiratory allergic disease, early detection of sensitization is useful [13].

Efforts to characterize allergenic molecules from cat dander and cat hair have so far resulted in a number of identified major and minor allergens. The major cat allergen is Fel d 1 (an uteroglobin, originally termed ‘Cat 1’), responsible for 60-90% of all IgE reactivity to cat dander [13]. Among the minor cat allergens are the serum albumin Fel d 2 (67Kda), the lipocalins Fel d 4 (20 Kda) and Fel d 7 (18Kda), and the latherin-like Fel d 8 (24 Kda) [14]. In addition, Fel d 3 (20 Kda, cystatin protease inhibitor allergen), Fel d 5 (400 Kda) and Fel d 6 (800-1000 Kda) have been suggested as allergens [15,16]. Fel d 1 is produced by the skin and by salivary and lacrimal glands of the cat. Fel d 1 is transferred to the fur by licking and grooming. Dried salivary protein and dandruff are actively released and spread into the surrounding environment. Inhalation of these protein particles by susceptible individuals cause sensitization (production of specific IgE antibodies) [17]. Diagnosis of cat allergy using crude cat dander extracts are well established [18-20]. These extracts contain a variety of allergenic and non-allergenic components [21].

As mentioned above, characterization of cat dander extract has so far identified 8 allergenic molecules but the majority of patients, 80–95%, have IgE antibodies directed towards Fel d 1. The dominance of Fel d 1 is also emphasized by the finding that more than 60% of all IgE antibodies induced by cat dander are directed to this particular allergen [22]. Cat allergy is unique among allergies to mammals as the major Cat allergen, Fel d 1, is an uteroglobin-like protein (with anti-inflammatory and immuno-modulatory properties) [23] and not a lipocalin [24,25], generally found in mammals. In Europe, absence of cat in home is associated with substantially lower Fel d 1 concentration, but does not protect against high Fel d 1 exposure in communities where cat ownership is common [26].

Cat allergen levels in house dust and their determinant in Saudi Arabia is unknown. There appear to be no such studies carried out in the country. Therefore the main aim of this study was to detect immunochemically and quantify the levels of most potent Fel d 1 cat allergen in house dust samples in Saudi homes.

2. MATERIALS AND METHODS

2.1 Dust Collection

House dust samples were randomly collected from allergic patients (individuals suffering from allergic symptoms such as bronchial asthma, allergic rhinitis, and conjunctivitis and attending allergy clinics. All samples came through their clinics) and control homes in three major cities of the Kingdom. Viz. Riyadh, Makkah, and Jeddah. In majority of cases, samples were provided by either patients or controls in sterilized plastic bags. Patients delivered samples to the allergy clinics of the collaborators.

They were provided with guidelines in local language how to collect the dust sample.

Out of 536 house dust samples collected 108 did not contain enough dust for extraction and analysis and thus discarded. A total of 428 samples from 155
patient homes and 273 control homes were accepted for analysis.

Samples were collected in sterile plastic ziploc bag by vacuuming carpet in the bedroom, living room, mattresses, sofas and other non-synthetic furniture. In some cases, a holder attachment device or connector (MODEL ALK) especially designed were used for this purpose. Individuals using their own vacuum cleaners were advised to use a new vacuuming bag and transfer the dust in a plastic bag (provided to them). Collected samples on the filter dishes were sealed, labeled and transported safely to the laboratory. The purpose of collection with or without attachment connector is to get reasonable sample. The sample collected by holder attachment was done by our staff to avoid contamination from house to house, where samples were obtained directly from the house occupants, it was from their own vacuum cleaners requiring no holder attachment.

2.2 Dust Extraction

All accepted dust samples (428) were extracted. A 100±5 mg of sieved dust was extracted with 2 mL of phosphate-buffer saline with Tween20 (PBS-T). Phosphate buffer (8.0 g NaCl, 0.2 g KCl, 1.15 g Na2HPO4, 0.20 g KH2PO4, Thimerosal 0.10 g in 1 L distilled water, pH 7.4) contained 0.05% Tween 20 [27]. Thimerosal was added as preservative in the PBS-T. Extraction was performed at room temperature for 2 h, with constant shaking. Dust extracts was centrifuged for 10 min at 3000 rpm. Supernatants were stored at -20°C until analyzed for allergen content.

2.3 Reagents

Allergen levels (Fel d 1) in the dust were measured using reagents for the ELISA assay purchased from Indoor Biotechnologies Ltd (Cardiff, UK). Kit was supplied with double monoclonal antibodies (mAbs) and standard (Capture antibody 6F9). The assay used biotinylated monoclonal antibody 3E4 as detecting antibody. Assay was standardized against reference standards defined by the World Health Organization/International Union of Immunological Societies (WHO/IUIS). These standards were declared to contain 1000 ng / ml of allergen. They were further diluted with 1% bovine serum albumin (BSA) PBS-T (Sigma, USA) to obtain working solution in the concentration range of (0.2-100) ng/ml to construct the calibrating curve. Streptavidin-horseradish peroxidase (HRP) was used as detecting reagent, and a solution mixture of ABTS (2,2-azino-diethylbenzthiazoline sulfonic acid) and peroxide were used as substrate (Sigma). The reagents were kept at 4°C. The ELISA assay was performed at room temperature. All reagents were added to the microtiter wells in the volume of 0.1 ml.

2.4 ELISA Protocol

Microtiter plates (NUNC Maxisorp. Cert- Thermo scientific) were coated with anti Fel d 1 monoclonal antibody (10 μL per 10 mL of 50 mmol L-1 sodium carbonate buffer, pH 9.6), covered and incubated at 4°C overnight. Capture antibody was diluted immediately before use. After washing with PBS-T (three times), the plates were blocked with 1% BSA-PBS-T (100 μL) for 30 min and washed. The plates were incubated with diluted samples and standards for 1 h. Then the wells were washed (three times) with PBS-T and treated with biotinylated antibody (10 μL per 10 mL of BSA-PBS-T) for 1 h and washed. All wells were then incubated with Streptavidin -HRP for 30 min and washed. A substrate solution of ABTS/peroxide was added and colour (green) developed within 15 min. The optical density was read after 10 min at 405 nm on BioTek ELISA microplate reader (Gen5). Following the protocol of the kit controls were added to the respective wells. Measurements were done semi-automatically.

Computer-based curve-fitting statistical software (B.E.N version 2) was used to calculate concentrations of cat allergen from the calibrating curve prepared by dilution of standard stock solution. Results were calculated as microgram Fel d 1 per gram of dust (μg/g).

2.5 Scale Classification

Based on Fel d 1 levels, the results were divided into four categories:

ND: below the limit of detection of the assay (not detected), the lower limit of detection was 4 ng/g dust for Fel d 1.

Very low [(< 0.5) μg/g],
Low [(> 0.5) μg/g],
High [1-8 μg/g].

This is similar to the classification proposed by the authors of reference [28]:

Fel d 1 cat allergen levels with risk for sensitization:

*Low [(< 0.5) μg/g],
*Moderate [(8-20) μg/g], and
*High (1-8 μg/g),
* The allergen exposure sensitization thresholds. The results are based on two studies that observed individuals who were frequently exposed to high levels of Fel d 1 and Can f 1, developed a tolerance to these allergens which resulted in mild allergic symptoms when exposed to 8-20 μg/g dust. Individuals with less frequent exposure to high levels of Fel d 1 and Can f 1, 1-8 μg/g dust, may experience more severe allergic symptoms because their immune system has not developed a tolerance [28].

3. RESULTS

High level of Fel d 1 ranged from (1.94 - 1 μg/gm).
Low level of Fel d 1 ranged (0.001-0.446 μg/gm).

Our results showed that Fel d 1 was present in the majority of the homes in KSA (Fig. 1A).
Only about 14% of homes had no detectable levels of Fel d 1.

About 71.5% of homes (control and patients) had Fel d 1 within the low range of (< 0.5) μg/g (Fig. 1B).

Fig. 1A. House dust samples with Cat allergen Fel d 1 concentration in all samples (n=428)
(ND: Not detected, Low: <0.5 μg/g)

Fig. 1B. Pie representation showing levels of cat allergen Fel d 1 in house dust samples from different cities in KSA
Fel d 1 levels in control homes:

Fel d 1 was detected in 85.35% of control homes (n=273).

Low levels (< 0.5 μg/g) of Fel d 1 were detected in 72.53% of control homes, levels > 0.5 μg/g in 6.6% of homes, and while high levels ≥ 1.0 μg/g were found in 6.23% (Fig. 2).

Fel d 1 levels in patients’ homes:

Fel d 1 was detected in 87.1% of patient homes (n=155).

Low levels were found in 69.68% of patients home, levels > 0.5 μg/g were detected in 9%, and high levels ≥ 1.0 μg/g in 8.39% of patient homes (Fig. 3).

In this study, clinically significant Fel d 1 levels (1-8 μg/g) were found in 7% of the samples.

Homes where low levels of Fel d 1 were detected had no cat. Homes with high level of Fel d 1, about 0.7% had cat.

Fel d 1 levels were detected in 91% of samples in Riyadh city (n=268), in 96% of samples in Makkah city (n=110), and was detected in 90% of samples in Jeddah (n=50) (Fig. 4).
4. DISCUSSION

This is one of the rare studies conducted on cat allergens concentration levels in house dust samples from different regions of Saudi Arabia. Cat (*Felis domesticus*) contains several antigens in their hair and saliva. Fel d 1 is the most common and found in salivary proteins of cat. When salivary proteins dry on hair it can easily become airborne or actively released and can enter any place through the exchange of air [22].

Our data revealed that majority of homes had no cats in the premises but up to 86% samples were detected having Fel d 1 allergen. Out of this, 7% had levels considered clinically, [28] that can either elicit allergic symptoms or can induce new IgE sensitization. The data revealed that low level of Fel d 1 (<0.5 µg/g) was marginally higher in control homes (72.5%) compared to patients’ home (69.6%). Nevertheless, the level of Fel d 1 (>0.5 µg/g and ≥1.0 µg/g) were marginally higher (9% and 8.4%) respectively, in patients’ home (Table 1).

There was some inequality in the number of samples collected (Patients #155, and controls #273), not by choice but because of logistic reasons. It was therefore, not possible to have an even number for comparison (Table 1). Yet, within the given number of samples analyzed, Makkah samples resulted with higher levels of Fel d 1 (12.7%) compared to Riyadh (4.4%) and Jeddah (8%) (Table 2).

As clinically significant levels (≥ 1.0 µg/g) [28] were found in 7% homes, these homes were identified having no cat, explains aerodynamic ability of Fel d 1 to remain suspended in the air and enter all possible indoor environment [17].

Cat allergen has been incriminated in asthma, allergic rhinitis and allergic conjunctivitis [29], and 10-15% sensitivity to cat has been reported [30]. According to recent publication there is no association among individuals exposed to concentrations higher than 8 µg/g. However, exposure to medium cat allergen concentrations (0.24-0.63 µg/g) was positively associated with symptoms when near the cats [30]. It

![Fig. 4. Detection of cat allergens (Fel d 1) in three major cities](image)

Table 1. Percent of patients and control samples, detected & not detected and levels of Fel d 1

<table>
<thead>
<tr>
<th></th>
<th>Patients No.</th>
<th>%</th>
<th>Controls No.</th>
<th>%</th>
<th>Total No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Samples</td>
<td>155</td>
<td>36</td>
<td>273</td>
<td>64</td>
<td>428</td>
<td>100</td>
</tr>
<tr>
<td>ND</td>
<td>20</td>
<td>13</td>
<td>40</td>
<td>14.7</td>
<td>60</td>
<td>14</td>
</tr>
<tr>
<td>D</td>
<td>135</td>
<td>87</td>
<td>233</td>
<td>85.3</td>
<td>368</td>
<td>86</td>
</tr>
<tr>
<td>&lt; 0.5 µg/g</td>
<td>108</td>
<td>69.6</td>
<td>198</td>
<td>72.5</td>
<td>306</td>
<td>71.5</td>
</tr>
<tr>
<td>&gt; 0.5 µg/g</td>
<td>14</td>
<td>9</td>
<td>18</td>
<td>6.6</td>
<td>32</td>
<td>7.5</td>
</tr>
<tr>
<td>≥ 1.0 µg/g</td>
<td>13</td>
<td>8.4</td>
<td>17</td>
<td>6.2</td>
<td>30</td>
<td>7.0</td>
</tr>
</tbody>
</table>

D: Detected, ND: Not detected, < 0.5 µg/g very low, > 0.5 µg/g low, ≥ 1.0 µg/g high
<table>
<thead>
<tr>
<th>Riyadh (n=268)</th>
<th>Detected</th>
<th>ND</th>
<th>Low</th>
<th>High</th>
<th>Have cat</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>244</td>
<td>24</td>
<td>232</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>%</td>
<td>91</td>
<td>9</td>
<td>86.6</td>
<td>4.4</td>
<td>4.5</td>
</tr>
<tr>
<td>Makkah (n=110)</td>
<td>Detected</td>
<td>ND</td>
<td>Low</td>
<td>High</td>
<td>Have cat</td>
</tr>
<tr>
<td>No.</td>
<td>106</td>
<td>4</td>
<td>92</td>
<td>14</td>
<td>10</td>
</tr>
<tr>
<td>%</td>
<td>96.4</td>
<td>3.6</td>
<td>83.6</td>
<td>12.7</td>
<td>9</td>
</tr>
<tr>
<td>Jeddah (n=50)</td>
<td>Detected</td>
<td>ND</td>
<td>Low</td>
<td>High</td>
<td>Have cat</td>
</tr>
<tr>
<td>No.</td>
<td>45</td>
<td>5</td>
<td>41</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>%</td>
<td>90</td>
<td>10</td>
<td>82</td>
<td>8</td>
<td>0</td>
</tr>
</tbody>
</table>

D: Detected, ND: Not detected, < 0.5 µg/g very low, > 0.5 µg/g low, ≥ 1.0 µg/g high

is rather difficult to understand as to why higher levels did not but medium or low levels were found to have association with allergic symptoms. If their findings are acceptable, then majority of our samples in Saudi Arabia had low to medium levels of Fel d 1 [28].

5. CONCLUSION

The study suggests that majority of homes (79%), without having cats, contained Fel d 1 allergen, and a small percentage of homes (7%) with higher levels, possibly entering through exchange of air or contaminated stuff such as school bags. Therefore, for in vivo and in vitro diagnostic profiles, it is advisable to include cat allergen even in patients not exposed to cat directly.

In addition, prevention strategies can be adapted with source removal, source control, (if any) and mitigation such as high-efficiency particulate air purifiers, allergen-proof mattress and pillow encasements. Education to patients and families is highly encouraged which can be delivered by primary care pediatricians, allergists, pediatric pulmonologists, other health care workers, or community health workers trained in asthma education and environmental control [31].

CONSENT

It is not applicable, as only house dust were provided by patients.

ETHICAL APPROVAL

It is not applicable.

ACKNOWLEDGEMENTS

This study was supported by a grant from National Scientific and Technology Innovation Planning (NSTIP) 13-BIO814-20 and approved by the American Association for the Advancement of Science (AAAS). The authors wish to acknowledge Ms. Cheryl Mijares-Oblea, Allergy and Medical Aerobiology Research at King Faisal Specialist Hospital and Research Centre, for her typographical assistance.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES


DOI: 10.1016/j.envint.2009.09.001


DOI: 10.1016/j.ijheh.2009.12.003


DOI: 10.2500/aap.2008.29.31372


DOI: 10.1007/s11882-016-0622-9


DOI: 10.1371/journal.pone.0124905


DOI: 10.1016/j.jaci.2014.08.026


DOI: 10.1016/j.ijheh.2009.12.003


DOI: 10.1016/j.jaci.2015.09.052


DOI: 10.1159/000250435


DOI: 10.1159/000322879


DOI: 10.1007/s10453-016-9433-7


DOI: 10.1016/j.jaci.2015.03.021.


DOI: 10.1111/pai.12198


DOI: 10.1186/s13223-016-0163-8


DOI: 10.1016/j.jaip.2013.08.008


DOI: 10.1074/jbc.M304740200


DOI: 10.1016/j.ymeth.2013.09.002


