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Quantification of House Dust Mites allergens in homes of Saudi Arabia

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Abstract

The prevalence of allergic diseases in Saudi Arabia is known to be increasing. Indoor allergens appear to play an important role in allergic sensitization and manifestations of symptoms. In order to identify and quantify common house dust mites, we investigated specific indoor determinants of some dust mite allergens and their concentrations in settled house dust samples in patients and control samples from coastal and non-coastal cities. A total of 560 house dust samples were collected from diagnosed respiratory allergic patients (n=164) and control homes (n=396) from eleven cities including coastal and non-coastal regions. The dust samples were collected in sterile plastic (ziploc) bags by 5 min vacuuming of the required areas. Der p1 from Dermatophagoides pteronyssinus, Der f1 from D. farinae, and Blo t5 from Blomia tropicalis allergens levels were quantified by dust extraction and ELISA using monoclonal antibodies from Indoor Biotechnologies, (Cardiff – UK). The analysis of data revealed comparatively higher concentration levels of all 3 allergens mentioned above in coastal cities compared to non-coastal ones. The patients' homes showed overall higher level of Der p1 (25%) while in control homes both, Der f1 (25%) and Blo t5 (17%) were higher. Coastal cities constituted higher level of all HDMs compared to non-coastal cities. Der p1 was found to be higher in patient homes compared to Der f1 and Blo t5 in control homes.

Keywords: *Asthma, House Dust Mite, Indoor Allergens, Allergic Disease, Coastal Cities*.

Introduction

Allergic diseases, particularly bronchial asthma and allergic rhinitis, have become very common in Saudi Arabia. (Nahhas et al., 2012); (Al Ghobain et al., 2012); (Al-Ghamdi et al., 2008); (Harfi et al., 2010); (Hussain et al., 2018); (Khawaji et al., 2017); (Alanazi et al., 2018); (Al Ghobain et al., 2018); (Horaib et al., 2018); (Alotaibi et al., 2018).

There are a number of sensitizing agents present in our outdoor and indoor environment as well as in settled dusts. These sensitizers (allergens) become airborne in higher concentration with human activities. A number of studies are available on outdoor allergens but very limited for indoor sensitizers in the kingdom. Therefore, in order to identify these allergic sensitizers, a study was conducted at different cities of Saudi Arabia including coastal and non-coastal regions.

House dust mites (HDM) have been shown to be important sources of indoor allergens associated with asthma and other allergic disorders such as rhinitis, rhino conjunctivitis and atopic dermatitis. (Henszel & Kuzna-Grygiel, 2006); (Fernández-Caldas, Puerta & Caraballo, 2014); (Hammad et al., 2009); (Gandhi et al., 2013); Wang, 2013); (Gregory & Lloyd, 2011).

The most common dust mite species around the world include *Dermatophagoides pteronyssinus (Dp), Dermatophagoides farinae (Df), Euroglyphus maynei (Em) and Blomia tropicalis (Bt)* (Fernandez-Caldas et al., 2004). *Dp* and *Df* are the predominant sources of inhalant allergens in most parts of the world. (Fereidouni et al., 2012); (Milián & Diaz, 2004) Prevalence data for HDM allergens sensitization vary from 65 to 130 million persons in the general population worldwide to as many as 50% among asthmatic patients. (Calderón et al., 2014)

Distribution of dust mites varies between geographic areas with different climate, and may be affected by housing characteristics. (Lim FL, et al 2015, Valdivieso R, et al 2006, Sharma D, et al 2011, Zheng YW, et al 2015, Wayne R Thomas 2010).

Many studies investigated the relationship between the level of environmental exposure to dust mite with sensitization and development of some allergic disease. (Prester L. 2012, Moghtaderi M, et al 2016, Manuyakorn W, et al 2015, Boquete M, et al 2006, Medeiros M Jr, et al 2002, Zheng YW, *et al* 2012, Lim FL, et al 2015, Zock JP, et al 2006, Mihrshahi S, et al 2002).

Mite proteins (mite allergens) that originate from mite faeces or decaying mite remains and are bound to dust particles are responsible for sensitization and the onset of symptoms. More than 20 allergens from *D. pteronyssinus* and *D. farinae* have been identified and, moreover, ac-accepted and listed by the World Health Organization and International Union of Immunological Societies (WHO/IUIS) Allergen Nomenclature Sub-Com-mittee. (Thomas WR, et al 2010).

The most important allergenic proteins in *Df* are Der f1 and Der f2, and for *Dp* are Der 1 and Der p2 as well. (Raulf et al., 2015); (Thomas, Smith & Hales, 2004); (Prester, 2012).

Blomia tropicalis, a Storage mite, was earlier found predominantly in agricultural environments but is now being recognized as an important contributor to the allergen content in house dust in indoor urban dwellings in subtropical and tropical regions (Montealegre, Goth & Hart, 2006); (Fernandez-Caldas & Lockey, 2004).

At least 23 IgE-binding components have been demonstrated for *B. tropicalis (*Johansson et al., 1997) among these, Blo t5, a 14 kDA Group 5 mite allergen, a homologue of Der p5 (Tsai et al., 2003); (Kuo et al., 2003). Blo t5 is a major allergen of *B. tropicalis*, with up to 92% of allergic

patients sensitized to it. (Yi et al., 2004). Evaluation of the prevalence of sensitization to B*t* indicated the importance of B*t* as an allergen source (Sade, Roitman & Kivity, 2010).

The prevalence of HDM in Saudi Arabia especially D*f*, D*p*, and B*t* is documented in some studies (Edrees, 2008); (Edrees, 2009); (Edrees, 2012); (Edrees, 2014). However, only one study was conducted about Der f1 and Der p1 levels (Al-Frayh et al., 1997).

The aim of our study was to determine levels of Der f1, Der p1and Blo t5 in house dust samples in patients and control homes from different regions of Saudi Arabia including coastal and non-coastal regions.

Materials and Method

Samples Collection

House dust samples (HDS) were randomly collected from allergic patients (individual suffering from bronchial asthma, allergic rhinitis, and/or rhinoconjunctivitis) attending allergy clinics. All samples were acquired through the regional participating clinics. Control samples were also provided by individuals through the clinics, these individuals were friends and relatives of allergy patients and no one was known to have any allergic symptoms in those homes.

Samples were collected from bedding, mattress, curtains and carpeted area in sterile plastic (ziploc) bags using vacuum cleaners. New vacuuming bags were used for each sample.

Out of 675 house dust samples collected 115 were not enough for extraction and analysis and thus discarded. A total of 560 samples from 164 patient homes and 396 control homes were accepted and analyzed by separating the bigger particles and sieving the samples.

Because of some logistic and cultural reasons, we were unable to receive an even number of samples from patients and control as well as coastal and non-coastal regions. Therefore, the paper highlights the concentration level of these allergens in both coastal and non-coastal regions without much emphasis on patients and control data.

Collection regions

Samples were collected from coastal and non-coastal cities in Saudi Arabia. This included: Riyadh, Qassim, Jouf, Arar, Abha, Makkah AlMukarama (non-coastal regions), Jeddah, Dammam, Jizan, Alwajh (coastal regions).

Antibodies selected

HDM antibodies were obtained commercially from Indoor Biotechnologies, (Cardiff – UK): Der p1 (***Der****matophagoides* ***p****teronyssinus*), Der f1 (***Der****matophagoides* ***f****arinae*), and *Blo* t5 (***Blo****mia* ***t****ropicalis*).

Extraction of dust samples

Extraction procedure was applied as per following steps:

- A 2 ml of phosphate-buffer saline with Tween (Phosphate buffer, 8.0 g NaCl, 0.2 g KCl, 1.15 g Na2HPO4, 0.20 KH2PO4, Thimerosal 0.10 g in 1 L distilled water, pH 7.4, contained 0.05 % Tween 20) were added to 100 mg of dust samples
- Constant shaking performed at room temperature for 2 hours.
- Dust extracts were centrifuged for 30 min at 4000 rpm.
- Supernatants were stored at -20°C until analyzed for allergen content.

Allergen levels (Der p1, Der f1, Blo t5) in the dust were measured using reagents for the ELISA assay purchased from Indoor Biotechnologies (Cardiff- UK).

Enzyme-Linked Immunosorbent Assay (ELISA)

ELISA plates were coated with anti-monoclonal antibody, covered and incubated at 4ºC overnight. Capture antibody was diluted immediately before use. After washing with PBS-T, the plates were blocked with BSA-PBS-T for 30 min and washed. After incubation the wells were washed with PBS-T and treated with

biotinylated antibody for 1 h and washed. All wells were then incubated with Streptavidin –HRP or Goat anti rabbit peroxidase for 30 min and washed. A substrate solution of ABTS/peroxide was added and color (green) was developed for 15 min.

The optical density was read after 10 min at 405 nm on BioTek ELISA micro plate reader (Gen5). Following the protocol of the kit controls were added to the respective wells. Measurements were done semi-automatically. (Hasnain, et al., 2017).

Results

The thresholds for sensitization (clinically significant levels) for HDMs (Chapman, 2010) are:

- Low < 0.3 µg/ gm dust
- Medium 2-10 µg/ gm dust
- High >10 µg/ gm dust

The analyzed samples in this study revealed that the detected levels of HDMs in all samples were low to moderate.

Patient and control samples

Figure 1 (A, B and C) display that all the 3 allergens (Der p1, Der f1, and Blo t5) were present in almost all samples with low concentration.

However, Der p1 emerged to be higher (25%) of the detected patients' home samples (Figure 1-B). In contrast, both Der f1 and Blo t5 were found to be higher with 20% and 12% respectively in control samples (Figure 1-A and 1-C).

Coastal and non-coastal samples

All coastal cities samples constituted higher level of Der f1, Der p1 and Blo t5 with 48%, 36% and 12% compared to non-coastal cities samples with 8%, 13% and 7% respectively Figure 2 (A, B and C).

Figure 3 - Exhibit results of all detected samples for the 3 HDMs mentioned above. The data for all 560 samples showed Der f1 with 25% and Blot t5 with 17%, ranked higher in control samples compared to Der p1 with 25% in patients' samples.

Figure 4 - Showing comparatively higher levels of detected samples for Der p1, Der f1, and Blo t5 in coastal cities than the non-coastal samples.

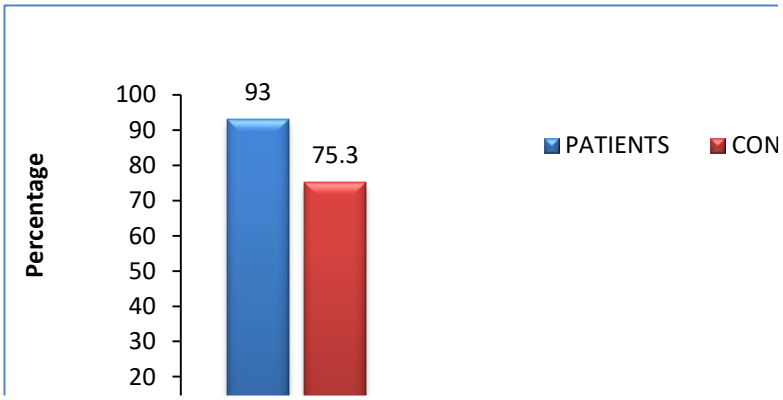

Figure 1-A: Der f1 levels in House dust samples in Patients and Control homes
(ND: Not Detected)

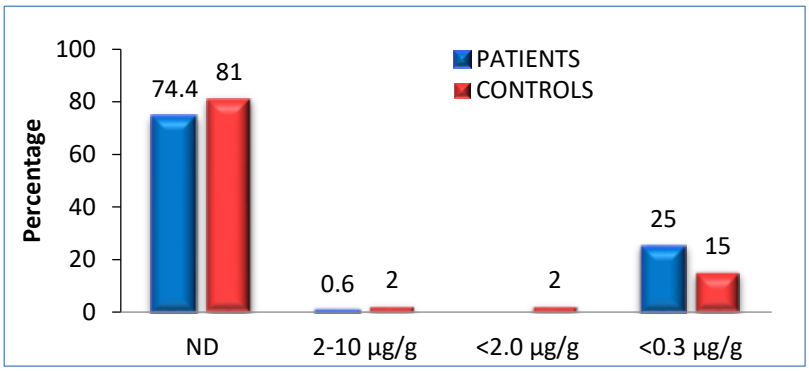

Figure 1-B: Der p1 levels in House dust samples in Patients and Control homes
(ND: Not Detected)

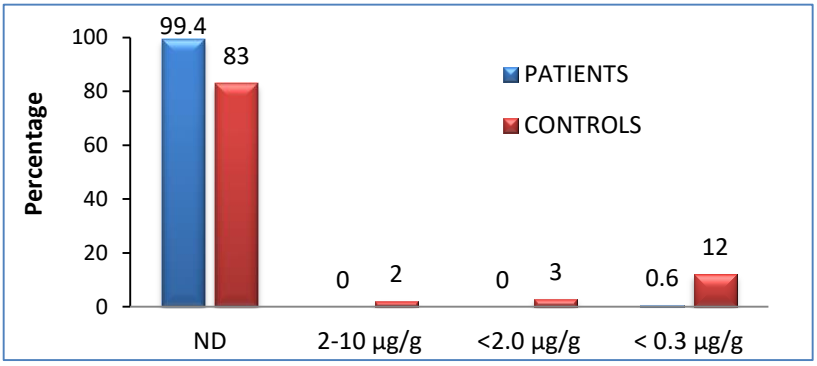

Figure 1-C: Blo t5 levels in House dust samples in Patients and Control homes
(ND: Not Detected)

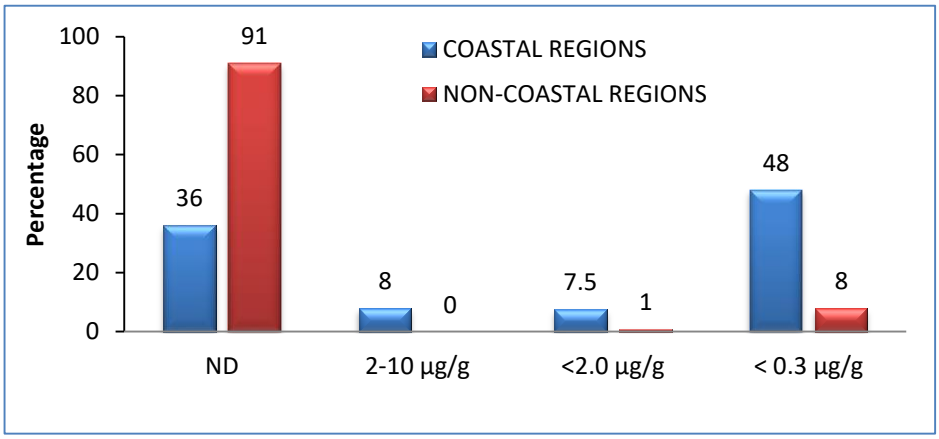

Figure 2-A: Der f1 levels in House Dust Samples in Coastal and Non-Coastal Cities

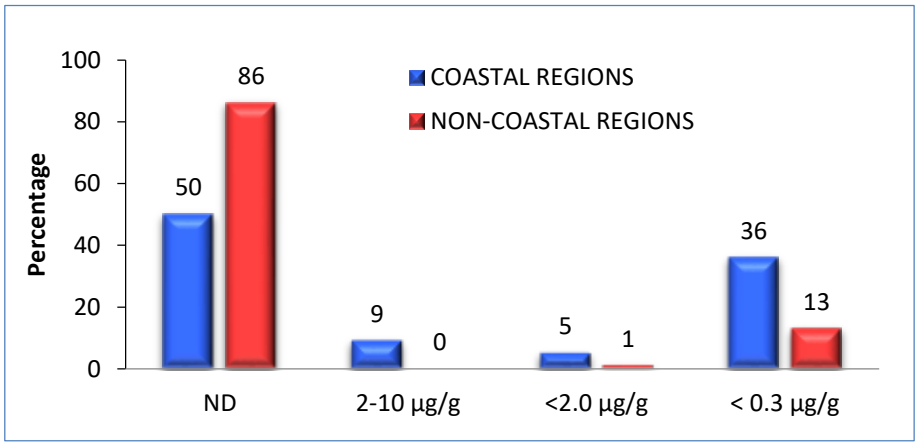

Figure 2-B: Der p1 Levels of House Dust Samples in Coastal and Non-Coastal Cities

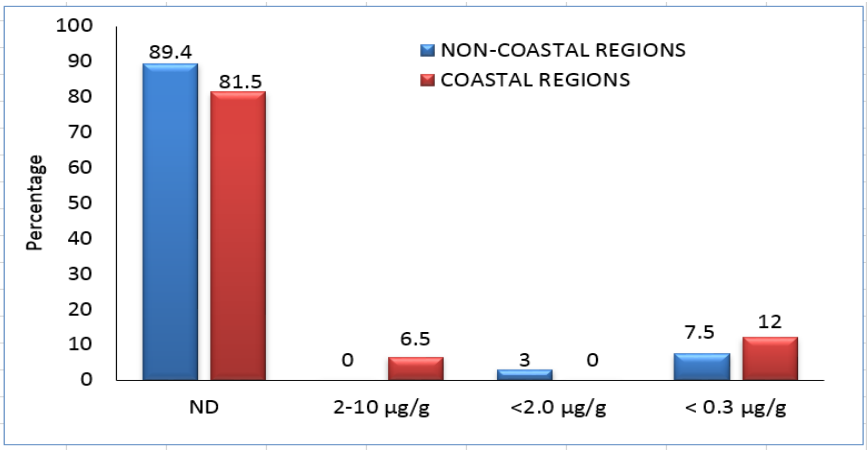

Figure 2-C: Blo t5 Levels in House Dust Samples in Coastal and Non-Coastal Cities

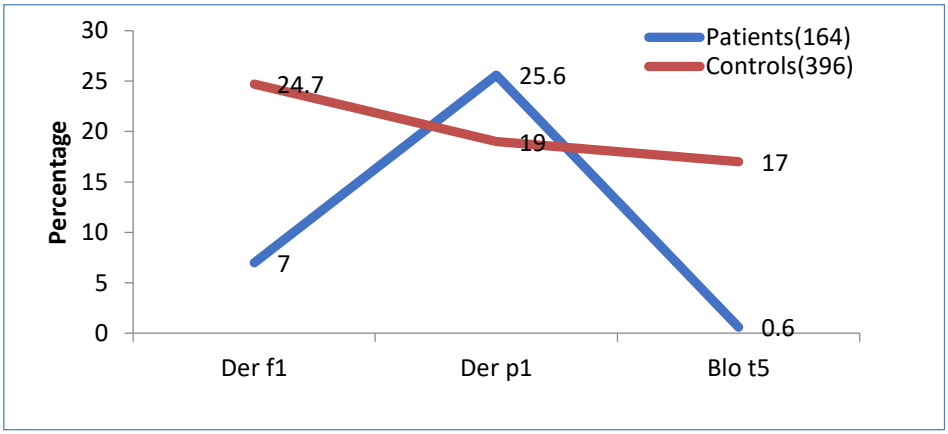

Figure 3: Overall Percentage of Detected HDMs Allergens in Patient & Control Homes (n=560)

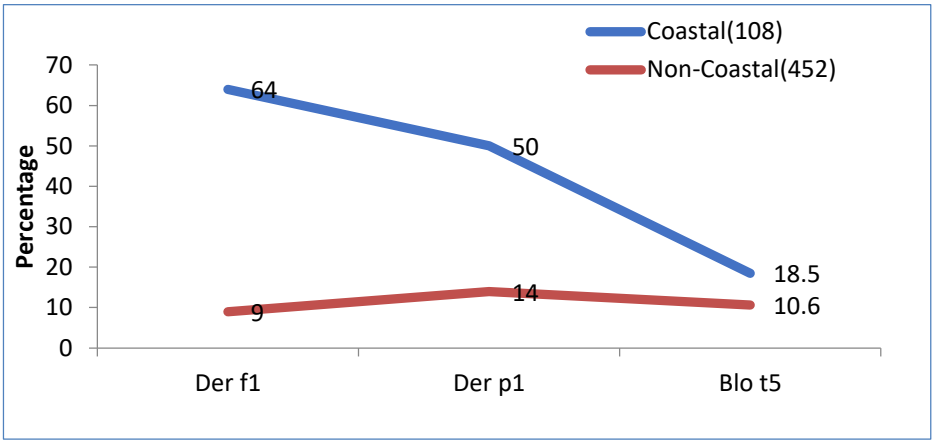

Figure 4: Overall Percentage of Detected HDMs Allergens in Coastal and Non-Coastal Cities (n=560)

Discussion

The concentration levels of HDMs allergens we detected in this study may be an underestimate of the actual levels because of the environmental parameters affecting the samples including handling temperature during transportations and storage before delivery to their respective clinics and to the main processing laboratory.

Most of the detected levels of HDMs allergens appear to be low to moderate. This is an interesting observation as Chapman's study (Chapman, 2010) hypothesizes that the "lower level" of any allergen at home does not reduce the risk of sensitization. He explains that high exposure level of allergens, for example Fel d1 with more than 20µg/g, give rise to a modified TH2 response. In other words, it induces tolerance in patient resulting in low prevalence of IgE antibody responses. He further explains that the low dose exposure to cat allergen (1-2 µg/g) is strongly associated with the development of IgE antibody.

Based on this finding it is not inconceivable that the low level of allergens detected in this study may be responsible for sensitization of many individuals rather than presence of high level. The finding that the coastal regions recorded higher HDMs allergens is an agreement with other international studies (Van et al., 2004). This is important to highlight that house dust mites thrives on high humidity, approximately >70%, and they are not able to survive <50% relative humidity (RH). Therefore, it is understandable that

coastal regions provide a conducive RH for the dust mites to survive and propagate. Studies have also shown that the increased humidity and temperature were associated with increased indoor allergens concentrations, and the influence of household characteristics of mites' allergens concentration, with a special emphasis on the measured relative humidity and temperature in the home (Matsui, Abramson & Sandel, 2016); (Van et al., 2004).

For non-allergist readers, it is important to mention that the HDMs themselves do not become airborne during human activities in homes and inhaled by the susceptible or sensitive individuals. But rather, the abdominal waste (fecal particles) of the HDMs, approximately (20-30 micron in diameter), that contain the allergenic protein become airborne which are responsible for human sensitization with subsequent development of IgE sensitization.

Another important point to note for allergy sufferers that the level of HDMs allergens may suddenly increase (10-100 times) with human activities such as vacuuming, bed making and children playing with the pillows and curtains etc. The sorting effects are high exposure probabilities under such environment.

The patient homes with higher Der p1compared to control homes and Der f1 and Blo t5 higher in control homes, can be interpreted in different ways. Firstly, there are some regional variations in the HDMs composition in coastal and mountains regions in Saudi Arabia (Al-Frayh et al., 1997). We are thus likely to have some variation in samples coming from these regions. Secondly, Der p1 and Der f1 (group 1 allergen) are known to have some cross-reactivity in skin prick testing. However, in Saudi Arabia, because of regional variation, and likely sensitization, a careful consideration in has to be given in diagnostic and therapeutic approaches.

Conclusions

We conclude that the 3 well known HDMs allergens are present in Saudi Arabia with a contrast quantitative variation between the coastal and non-coastal cities. In addition, one of the 3 allergens (Der p1) exhibited comparatively higher concentration level 25% in patient home compared to only 19% in control home. Likewise, one of the allergens (Der f1) displayed higher level 25% in control homes compared to only 7% in patient homes.

Since Saudi Arabian geography encompasses coastal, desert and mountainous regions, there are likely to be some qualitative variations as evident from a previous study in the region. Therefore, it is advised that selection of diagnostic and therapeutic extracts may be given extra consideration in coastal regions of the world.

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