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Full Length Research Paper

Indoor sensitizers of allergy and asthma in coastal and non-coastal regions of Saudi Arabia

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ABSTRACT

Allergic diseases such as bronchial asthma and allergic rhinitis have increased in the pediatric and adults populations in Saudi Arabia. Apart from traditional lifestyle, the hot temperatures force families to spend more time indoors, resulting in high probability of individuals' exposure with the indoor allergy sensitizers. In order to evaluate their impact in the allergic population, a nationwide study of various allergy and asthma sensitizers was conducted simultaneously in several cities of Saudi Arabia including coastal and non-coastal regions, during 2015-2016. A total of 560 house dust samples (HDS) from 164 allergic patients and 396 control homes were collected in sterile ziploc bags, by vacuuming from seven regions. Samples were sieved, extracted in PBS-Ph8 and analyzed by ELISA using seven different antibodies from Indoor Biotechnologies (Cardiff-UK). The targeted allergens included *Dermatophagoides pteronyssinus (Der p1), Dermatophagoides farinae (Der f1), Blattella germanica (Bla g1, Bla g2), Felis domesticus (Fel d1), Rattus norvegicus (Rat n1)* and *Blomia tropicalis (Blo t5).* Chi-square test and odd ratio to test the association between patients and controls as well as detection rate in coastal and non-coastal cities were conducted. The analyses of data between patients and controls as well as coastal verses non-coastal regions revealed quantitative variations in their threshold values. *Der p1, Der f1* and *Blo t5*, the three house dust mites (HDM) antigens were higher in the coastal regions compared to non-coastal. While the other allergens viz. *Bla g1, Bla g2, Fel d1* and *Rat n1*, exhibit an opposite trend. Significant levels for *Bla g1* in Makkah ($p < 0.0001$) and Riyadh ($p < 0.0006$), *Rat n1* ($p < 0.0001$) and *Blo t5* ($p < 0.0038$) for Riyadh were obtained. The results are expected to help physicians, allergists and hospitals in selection of appropriate diagnostic test panels and may further help in therapeutic and preventive approaches on a regional basis.

Keywords: Asthma, indoor allergens, allergic disease, sensitizers, house dust.

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INTRODUCTION

A number of factors play a role in the development of allergy and asthma, including genetic, environmental, dietary, and occupation. Indoor factors may include house dust mites, cats, dogs, cockroaches and mice (Moorman et al., 2007; Custovic and Simpson, 2012; Mukherjee and Zhang, 2011; Custovic et al., 2010).

Bronchial asthma is the leading cause of morbidity and mortality among allergic individuals, and indoor allergen exposure is an important risk factor for asthma in children. Strong evidence has revealed associations between indoor allergens and initiation, promotion and exacerbation of allergic respiratory disease (Sheehan et al., 2017; Asher and Pearce, 2014). The prevalence of asthma is increasing despite advances in its treatment and understanding of its pathogenesis (Yan et al., 2016; Chen et al., 2016; Alavinezhad and Boskabady, 2018).

Studies have shown links between the concentration of allergens in homes and asthma. Levels of exposure, determined by house dust analysis, are important determinants of sensitization (Raja et al., 2010).

Some of the most important indoor allergens are house dust mites, *Der p1* (*Dermatophagoides pteronyssinus*), *Der f1* (*Dermatophagoides farina*), *Blo t5 (Blomia tropicalis*). *Fel d1 (Felis domesticus*) from cats, *Bla g1, Bla g2* (*Blattella germanica*) from cockroaches and *Rat n1* (*Rattus norvegicus*) from mice.

Sensitization to house dust mites (HDM) appears to play an important role in the progression from allergic rhinitis to asthma in children, and is associated with asthma in all age groups (Biagtan et al., 2014; Loo et al., 2016). Cat allergy is also of great importance, and its prevalence is increasing worldwide. Cat sensitization and allergy are known risk factors for rhinitis, bronchial hyper-reactivity and asthma (Kelly et al., 2012; Wang et al., 2016; Patel et al., 2013; Eder et al., 2016).

Cockroach allergens were found to be a common source of allergens. Exposure to high levels of cockroach allergens is a major risk factor for sensitized individuals, leading to worst asthma control, and increased airway inflammation (Bassirpour and Zoratti, 2014; Uzela et al., 2005; Arruda et al., 2001).

Rats and mouse allergens have long been recognized as important cause of allergy and have been implicated in asthma/allergic diseases in community settings (Matsui, 2009).

In Saudi Arabia, limited studies have been conducted as regard to indoor allergens (Almogren, 2009; Al-Qurashi, 2006; Hasnain et al., 2001; Hasnain et al., 2004; Koshak, 2006; Hasnain and Al-Frayh, 2015; Hasnain et al., 2012).

One study has indicated that 75% of allergic patients reacted to one or more allergen extracts. The most frequent reacting indoor allergen was house dust mite (77.8%), followed by cat (33.6%) and cockroach (19.2%) (Almogren, 2009).

MATERIALS AND METHODS

Collection of samples from patients (P) and control (C) homes

House dust samples were randomly collected from allergic patients attending regional allergy clinics and reporting symptoms of bronchial asthma, allergic rhinitis, and/or rhinoconjunctivitis. The physicians in the clinics were responsible for their diagnosis and treatment.

The samples were collected using a non-hepa filter vacuum cleaner (5970121 Shop.Vac ® Model: K12-SQ14, 1400 Watts). However, because of the cultural reasons, entry to every family home was a difficult task. Thus, we had to request a number of patients (identified by clinics) and control occupants in Riyadh to collect the samples using their own vacuum cleaners. Same procedure was adopted for patients by other regional allergy clinics. The protocol for dust sample collection was based on using a new vacuuming bag for each sample (home) and transferring the dust in a sterile plastic (ziploc) bag. The selected locations within any premises were vacuumed for a total of 5-10 min mainly bedding, mattress, curtains and carpeted areas.

Control homes samples from 11 different cities 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.

As the aim of this study was to detect indoor/sensitizers by analyzing the house dust samples only, this did not include any diagnostic procedure on any patient at any region. However, the results obtained in our study are being provided to all participating clinics for future diagnosis and follow up. It must, therefore, be noted, that no patient was recruited for diagnostic purposes and as such no patient inclusion and exclusion was adopted. The only inclusion and exclusion applied in this study was, that there must be one or more people suffering from respiratory allergy symptoms (pre-determined by the clinics) living in the home. While for the control homes no individuals were known to have any allergic symptoms.

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.

All collected HDS were cleaned in the laminar flow cabinet, separating the bigger particles and sieving the samples. For each sample all information, where possible, such as collection date, name, address, location and contact person, were recorded in the database.

Sampling regions

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

Antibodies selected

These antibodies were purchased from Indoor Biotechnologies, (Cardiff – UK): Der p1 (*Dermatophagoides pteronyssinus*), Der f1 (*Dermatophagoides farinae*), Blo t5 (*Blomia tropicalis*), Fel d1 (*Felis domesticus*), Bla g1, Bla g2 (*Blattella germanica*) and Rat n1 (*Rattus norvegicus*).

Table 1 is adapted from (Chapman, 2010) as a guideline only for the risk of sensitization for various groups of allergens.

The thresholds for sensitization levels (clinically significant levels) are different for each indoor allergen (Chapman, 2010).

Dust extraction

A 100 ± 5 mg dust samples (sieved) were extracted with 2 ml of phosphate-buffer saline with Tween 20 (PBS-T). 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 (3, 22). Extraction was performed at room temperature for 2 h, with constant shaking. Dust extract was 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, Fel d1, Bla g1, Bla g2, and Rat n1) in the dust were measured using ELISA assay.

Enzyme-Linked ImmunoSorbent Assay (ELISA)

Microtiter plates (NUNC Maxisorp. Cert- Thermo scientific, USA) were coated with anti-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.

Table 1. Allergen Exposure threshold for sensitization (Chapman, 2010).

Risk for sensitization	Mite group 1 (µg/g)	Fel d 1 (µg/g)	Can f 1 (µg/g)	Bla g 1 (U/g)	Bla g 2 (µg/g)
High	> 10	1-8	1-8	> 8	> 1
Medium	2-10	8-20	8-20	1-8	0.08-0.4
Low	< 0.3	<0.5	<0.5	<0.6	<< 0.08

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 or Goat anti rabbit peroxidase for 30 min and washed. A substrate solution of ABTS/peroxide was added and colour (green) was developed for 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 allergens from the calibrating curve prepared by dilution of standard stock solution. Results were calculated as microgram of allergen per gram of dust (µg /g).

As per the antibodies manufacturer, the lower limit of detection was 1.01 µg /g dust for Der p1, Der f1, Blo t5, Bla g2. And were 0.004 µg /g for Rat n1, Fel d1 and Bla g1.

RESULTS

As the samples were collected from both patients and control homes in coastal and non-coastal regions, the results obtained are summarized in Figures 1 to 3 and Table 2. Table 2 shows coastal and non-coastal cities and the number of patients and control homes samples were collected through their regional clinics.

Figure 1 displays that house dust mite allergens (Der p1, Der f1, and Blo t5) were higher in coastal regions, whereas other allergens (Fel d1, Blag 1, Bla g2 and Rat n1) were higher in non-coastal regions.

Figure 2 exhibits results of all samples in low, medium and high concentration levels. This data for 560 samples was summarized in three categories in order to correlate the quantitative values presented in Table 1. The mean value of all allergens detected was 85% for low level, 11% for high level and only 4% for medium level.

Because of the variations in threshold level of different allergens and the known sensitizing effect of low to medium level, and the known effect of high concentration level in desensitization (Chapman, 2010), a clear comparison between the patients and control for the collected samples is only possible by individual allergens and not with all allergens. Therefore, these comparative data have been provided in the statistical part of the publication (Forest Plot, Figure 3), showing 3 allergens viz. Blo t5, Der p1 and Der f1 with medium level having significant level for detection and exposure.

In addition, the detection rate of the three individual allergens mentioned in figure 3 (forest plot) emphasizes that allergic patients are likely to have more exposure probability and possibilities with the 3 allergens mentioned above compared to others. Since these were not found in significant detection rate in non-coastal regions, therefore their exposure possibility in patients or susceptible patients is likely to be limited.

Table 2 exhibits a comparative data of detection rate (DR) between the patient and the control samples. The significant detection rate was obtained for Der p1 (< 0.0001), Rat n1 (< 0.0001) and *Blo t5* ($p < 0.0038$) in Riyadh region. The significant detection rate for Bla g1 was obtained in both Makkah ($p < 0.0001$) and Riyadh ($p < 0.0006$).

Statistical analysis

Chi-square test (SAS) was used to test the association between cases and controls for all levels of each allergen. The comparative data for patients and control were available only for 4 cities. This included Riyadh, Makkah, Dammam and Jouf while rest of the regions provided either patient or control samples, making a comparative analysis irrelevant. The results are summarized in Table 3. We also used the Odds ratio to test the association between coastal and detection rates for each level of each allergen. The results are summarized in a forest plot (Figure 3). We detected significant odds ratio (association between detection rates and region (coastal versus non-coastal) for only for Blot5_L, Derf1_M, and Derp1_M.

DISCUSSION

This study is first of its kind for the analyses of various indoor allergens conducted simultaneously for the comparison of data between coastal and non-coastal homes in Saudi Arabia.

The results revealed that seven different allergens were present in Saudi Arabia but with quantitative variation and regional diversity. The data further revealed that there was a high prevalence of house dust mites (HDM) in the coastal regions compared to the non-coastal regions. This trend was quite opposite at non-coastal cities where the other allergens appeared to be more common and frequent than the coastal cities.

Most of the detected level of allergens was low. This is an interesting observation as Chapman's study (Chapman, 2010) hypothesizes that the "lower level" of any allergens at home does not reduce the risk for

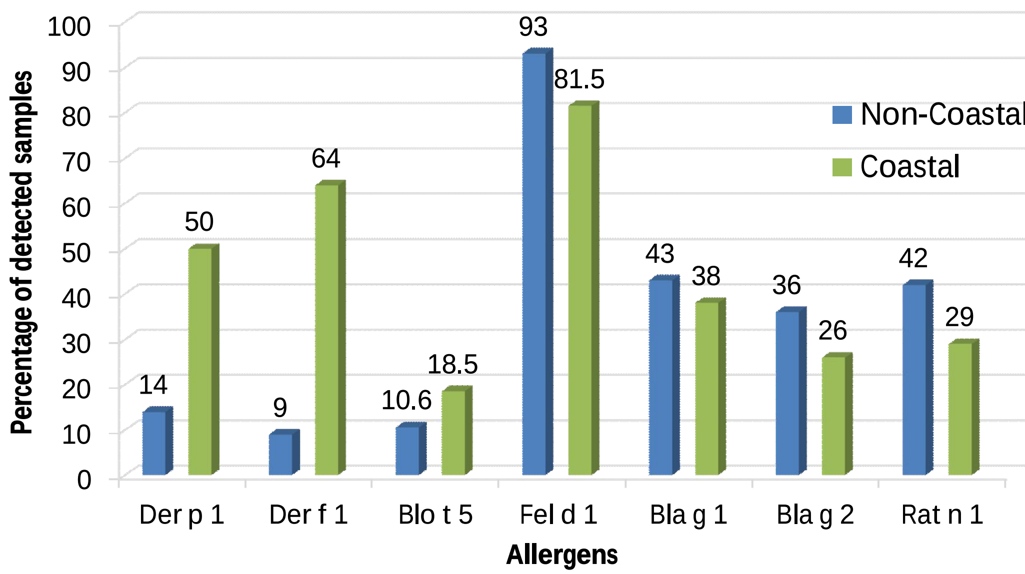

Figure 1. Percentages of multiple sensitizers in coastal and non-coastal samples (n = 560).

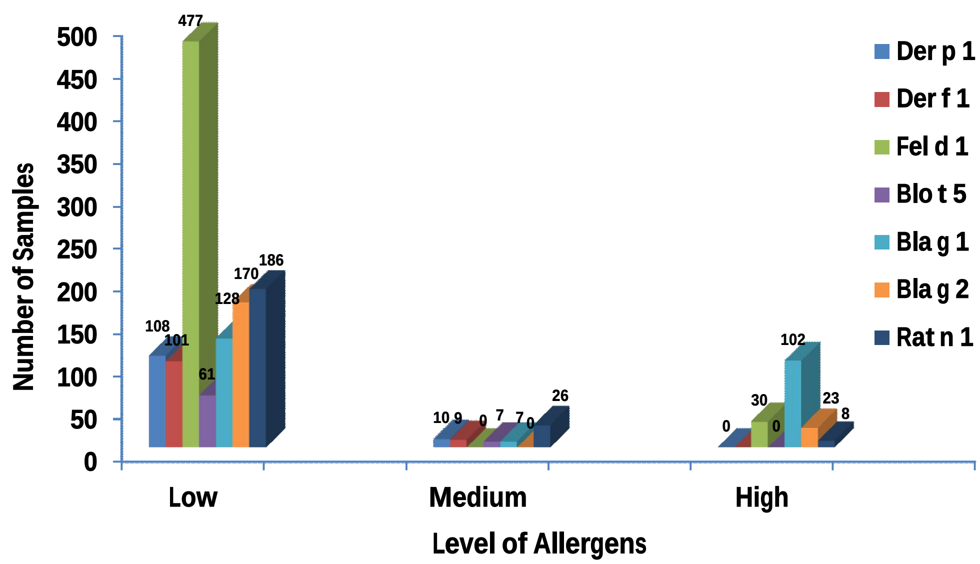

Figure 2. Low, medium and high levels of indoor allergens in patient and controls samples (n = 560).

sensitization. He explains that high exposure level of allergens, for example *Fel d1* with more than 20 µg/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 to 2 µg/g) is strongly associated with the development of IgE antibody.

HDMs allergens level in coastal regions is consistent to international finding that relative humidity >70% help thrive HDMs which is generally expected in all coastal regions world over (Biagtan et al., 2014).

Studies have shown that mouse allergen is detectable in most US homes, with strikingly high levels in some inner cities (Matsui, 2009). However, sensitization seems to occur at low levels of exposure (Pongracic et al., 2008) which supports our findings.

Allergen exposure is not limited to private homes. Mite, cat, and dog allergens were measured in day care centers (Matsui et al., 2016; Sander et al., 2016), and schools where domestic animals are kept as pets and for education. Sensitive children in schools and individuals working in the animal industries, animal farming etc. may be exposed to higher level of allergens. Schools play an important role in harboring various indoor allergens

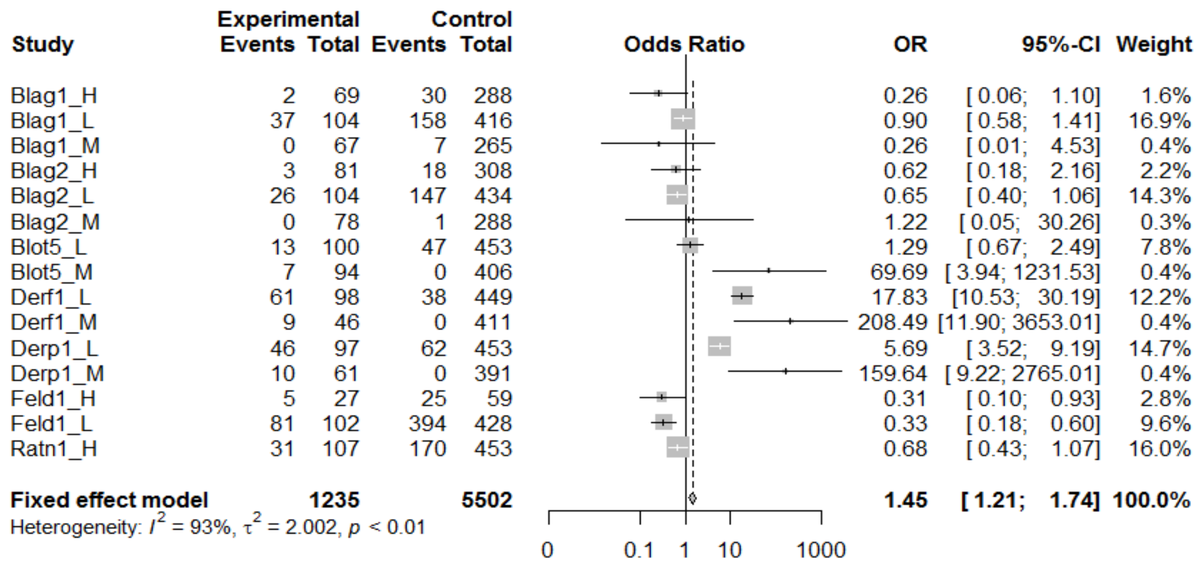

Figure 3. Forest plot for the odds ratio showing significant difference in detection rates between coastal and noncoastal for Blot5_LDerf1_M, and Derp1_M.

Table 2. Number of samples for patients and control from each city

City		No. of controls samples	No. of patients samples	Total	
Non-coastal cities	Riyadh	238	44	282	453
	Makkah	48	62	110	
	Qassim	-	25	25	
	Abha	-	12	12	
	Al-Jouf	6	14	20	
	Arar	4	-	4	
Coastal cities	Dammam	9	2	11	107
	Jeddah	50	-	50	
	Wajh	41	-	41	
	Jizan	-	5	5	
Total		396	164	560	

carried in school bags and stuffs from different socio-economic group (Cyprowski et al., 2013; Zhao et al., 2006; Salo et al., 2009; Lim et al., 2015).

Avoidance of exposure to these allergens continues to be important especially given that the vast majority of children with asthma are sensitized to at least one indoor allergen (Sheehan and Phipatanakul, 2016).

A significant association was found between the visual observation of dust inside homes and the sensitivity of children to dust mites (Alvarez-Chavez et al., 2016).

The advent of new advances in technology, molecular biology and proteomics has led to the identification, cloning, and expression of new indoor allergens, which has facilitated research to elucidate their role in allergic diseases (Pomes et al., 2016). For example, understanding cat allergens from scientific name Fel d1 to Fel d 8 is the gift of molecular biology and proteomics. The Fel d1, proteins come from saliva while Fel d2 proteins come from the cat urine. Hens, it will be helpful to allergist and physicians to know that Fel d1 is more relevant as diagnostic allergen than Fel d2 because the salivary protein becomes airborne and are inhaled.

Research progress in indoor allergens is likely to result in the development of new diagnostic tools and the design of coherent strategies of immunotherapy, as well as aid the design of future public health interventions.

CONCLUSION

We conclude that there are different types of indoor allergenic sensitizers present in Saudi homes with

Table 3. Detection rates (DR) in patient and control with their P value.

Allergen		Riyadh	Makkah	Dammam	Jouf
	DR patient	54.55	1.61	100	100
Der p 1	DR control	10.92	0	0	100
	P value	< 0.0001	0.3767	0.0009	0
	DR patient	9.09	4.84	50	10
Der f 1	DR control	10.92	4.17	44.44	0
	P value	0.7171	0.8667	0.8865	0.4237
	DR patient	0	100	100	100
Blo t 5	DR control	13.83	100	100	100
	P value	0.0038	0	0	0
	DR patient	88.64	95.16	100	70
Fel d 1	DR control	92.44	91.67	88.89	100
	P value	0.3974	0.4565	0.621	0.1366
	DR patient	65.91	20.97	100	80
Bla g 1	DR control	38.24	62.5	11.11	100
	P value	0.0006	< 0.0001	0.0107	0.2416
	DR patient	52.27	19.35	100	50
Bla g 2	DR control	33.61	47.92	77.78	0
	P value	0.0182	0.0014	0.4611	0.0367
	DR patient	65.91	35.48	0	0
Rat n 1	DR control	34.45	58.33	44.44	16.67
	P value	< 0.0001	0.017	0.2373	0.1824

Note: Since we have 7 different antibodies and the unit of each AB is different, we included Chapman's table as a reference for both unit and levels of each allergen.

quantitative variations. HDMs allergens are dominant in the coastal regions, while other allergens are more prevalent in non-coastal regions.

As mentioned earlier, the low level of allergens contributes more towards sensitization than the higher level which may induce desensitization, (medically known as reversal of TH2 to TH1 responses). Thus, our findings emphasize the contribution of low level sensitizers towards allergic sensitization and disease manifestation.

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