

ALLERGIC IMPLICATIONS OF BLA G 1 AND BLA G 2 CONCENTRATIONS IN HOUSES OF SAUDI ARABIA

SYED M. HASNAIN^{1*}, ALANOUD AL-QASSIM¹,
ABDULRAHMAN AL-FRAYH², HUSSEIN AL-MEHDAR³, SULTAN AL-SEDAIRY¹
AND FUTWAN AL-MOHANNA¹

¹Allergy and Medical Aerobiology Section, Department of Cell Biology, King Faisal Specialist Hospital and Research Centre, P.O. Box 3354, Riyadh 11211, Saudi Arabia.

²Department of Pediatric, College of Medicine, King Saud University, P.O. Box 7805, Riyadh 11472, Saudi Arabia.

³Department of Biology, Faculty of Science, King Abdulaziz University, P.O. Box 80203, Jeddah 21589, Saudi Arabia.

AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between all authors. Author SMH as the first author (corresponding author) contributed in writing the manuscript, analysis of data and data presentation. Author AAQ contributed in

ELISA procedure, calculation, data analysis, writing and references. Author AAF contributed in manuscript review, advice and guidance of the project. Author HAM contributed collection of samples from controls houses in Jeddah. Author SAS contributed the idea, managed the project, review and discussion. Author FAM contributed idea, obtained fund approval, participate review and discussion. All authors read and approved the final manuscript.

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ABSTRACT

Aims: As the higher cockroaches (CKRs) concentrations in houses are associated with allergic diseases, our objective was to determine the concentration levels of CKRs allergen.

Study Design: Quantitative and qualitative determinations of CKRs' antigenic component in indoor environment of diagnosed allergic patients and non-allergic individuals.

Methodology: A total of 560 House Dust Samples (HDS) were randomly collected from patient and control houses and grouped under coastal and non-coastal regions. HDS were extracted in Phosphate Buffered Saline (PBS) and analyzed by ELISA using antibodies obtained from Indoor Biotechnologies, USA.

Results: Bla g 1 and Bla g 2, being two main allergenic components in *Blattella germanica*, constituted 42.3% and 35.5% respectively. Bla g 1 with 28.7% and 23.6% of Bla g 2 were present in control houses compared to 13.6% of Bla g 1 and 11.4% of Bla g 2 in patient houses. A comparison of data resulted in 7.3% of Bla g 1 and 5.3% of Bla g 2 in coastal compared to 35.3% and 29.4% in non-coastal regions. As the clinical threshold levels of both allergens are different, Bla g 2 marginally higher (2.3 µg/g) in patient than control samples (1.8 µg/g). By contrast, Bla g 1 was less prevalent in patient (1.5 U/g) compared to control houses (16.5 U/g).

Conclusion: There appear to be no major variation in the concentration levels of Bla g 1 and Bla g 2 between patients and control houses. Nevertheless, Bla g 2 was found to be marginally higher in patients' samples.

Keywords: Cockroaches; allergic diseases; asthma.

1. INTRODUCTION

Respiratory allergies, particularly asthma and allergic rhinitis, are among the most common allergies all over the world and its incidence continue to increase. The traditional lifestyle and living environment have affected the prevalence of asthma [1,2]. Studies have shown prevalence rate of respiratory allergic diseases between 10-30% in different parts of the world, especially in the developed countries. The data for respiratory allergies from the Kingdom of Saudi Arabia [3] revealed prevalence of allergic rhinitis and bronchial asthma to be 25% and 23% respectively. In Australia, 27% of the children had wheeze and among the Greek population around 9% asthmatics have been reported. In India, 20–30% individuals suffer from allergic rhinitis [4].

Aeroallergens play a major role in the sensitization of respiratory allergic diseases, particularly asthma and allergic rhinitis. The role of the different allergens which trigger asthma and allergic rhinitis varies with environmental conditions, such as climatic factors, pollution and degree of exposure to aeroallergens [4, 5]. Exposure to aeroallergens is an important environmental factor in allergic sensitization and the development of allergic diseases which has a profound impact on quality of life [6]. Indoor allergens are of particular importance as triggering factors and well-studied among the main cause of respiratory allergy [7,8]. Indoor allergens are considered as important risk factors in asthma having a significant influence on human health [9]. Increased rate of asthma has been associated with exposure to high concentrations of indoor allergens. The development of sensitivity to indoor allergens and severity of asthma are directly related to exposure to increased level of allergens [10,11].

Cockroaches (CKRs) are commonly found in urban places with over 3,500 species worldwide. German species, *Blattella germanica* is one of the most common CKR found in many developing countries and have also been reported as a common indoor pest in low-income housing [12,13].

They produce several allergens that induce sensitization. Exposure to high levels of cockroach allergens in houses is a major risk factor for exacerbation in sensitized individuals [14,15] leading to worse asthma control, reduce lung function and airway inflammation [16]. German cockroach is amongst the indoor allergens frequently found in countries with traditional floor dining such as Saudi Arabia and other Gulf Countries [17,18].

In the Middle East, one or two available data signifies the importance of CKRs' allergens in the indoor

environment [19]. Some investigations in Saudi Arabia have reported IgE mediated sensitizations to CKRs with 33% [20] and 19.2% [21].

2. MATERIALS AND METHODS

2.1 Collection of House Dust Samples

HDS were collected, where possible, using a non-hepa filter vacuum cleaners. (5970121 Shop.Vac ® Model: K12-SQ14, 1400 Watts) for the samples collection from Riyadh region. Because of the cultural reasons, entry to family houses was a difficult task. Thus, we had to request the occupants to collect the samples using their own vacuum cleaners. Same procedure was adapted for patients by the regional allergy clinics. The protocol for dust sample collection was based on using a new vacuuming bag/filters (a separate clean bag/filter for different areas were used for each sample if any) and transfer the dust in a sterile plastic (ziploc) bag. The selected areas were vacuumed for a total of 10 min for bedding, including the mattress, curtains and carpeted area.

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.

2.2 Sampling Locations

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

2.3 Extraction of Dust Samples

A (100±5) mg sieved dust samples were extracted with 2 mL of phosphate-buffer saline with Tween (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 (3, 22). Thimerosal was added as preservative in the PBS-T. Extractions were performed at room temperature for 2 h, with constant shaking. Dust extracts were centrifuged for 10 min at 3000 rpm. Supernatants were stored at -20°C until analyzed for allergen content.

2.4 Analysis of Samples

For detection of Bla g 1 and Bla g 2, an Enzyme-Linked Immunosorbent Assay (ELISA) was employed as per following steps:

- 96 wells plates (NUNC Maxisorp. Cert-Thermo scientific) were coated with monoclonal antibody (10 µL per 10 mL of 50 mmol L-1 sodium carbonate buffer, pH 9.6), covered and incubated overnight at 4°C.
- Plates were washed 3X with PBS-T [PBS-0.05% Tween 20, pH 7.4], blocked by adding 1% BSA-PBS-T (100 µL) for 30 min and washed.
- Diluted dust samples and allergen standards were prepared in 1% BSA PBS-T to make 10 serial doubling dilutions with 100µl/well and incubated again for 1 hour.
- The plates were washed again 3X with PBS-T then biotinylated antibody (10 µL per 10 mL of BSA-PBS-T) were added and incubated for 1 hour then again washed.
- Plates were incubated with Peroxidase conjugated Goat anti-Rabbit IgG for 1 hour then washed.
- A substrate solution of ABTS/peroxide was added and when the color changed, the plates were read at 405 nm on a BioTek ELISA microplate reader (Gen5).
- Computer-based curve-fitting statistical software was employed to calculate the concentration of the sample.

3. RESULTS AND DISCUSSION

The Table 1 and Table 2 provide overall concentration levels of both allergens.

Though there is a limited data on IgE Mediated reactivity, appear to be no published data on the prevalence and frequency of CKRs allergens in the HDS in Saudi Arabia. As such, this publication is the first presenting detail qualitative and quantitative analysis on CKRs from 560 indoor samples from different parts of the country. The collection of samples included both from patients and control houses in the coastal and non-coastal regions. A few references available for the Kingdom provide skin prick test (SPT) data on allergic patients [19,20,23] without specifying CKRs species in some cases, and

their quantitative levels in the indoor allergens. In normal setting, 3 CKR species, viz. American species (*Periplaneta americana*) or German (*Blattella germanica*) and Oriental species (*Blattella orientalis*) are included in the test. A large number of patients show multiple sensitizations (poly-sensitization) with different allergens, which may be a cross-reactivity [24,25].

The data generated in this project, which was approved by the American Association for the Advancement of Science (AAAS), provide quantitative information on the two potent indoor allergens Bla g 1 and Bla g 2, originating from the source of *Blattella germanica*. *Periplaneta americana* (Per a1) and *Blattella orientalis* (Bla o) were not included because unavailability of commercial antibodies.

An attempt was made to group all data from coastal regions (n=108) and non-coastal regions (n=452). Because of the limitations imposed by logistics, the number of samples from coastal regions did not match the samples from non-coastal regions. Therefore a realistic comparison between the 2 regions will remain inconclusive till further data are collected. However, within the same number of samples between the 2 regions, the data revealed that 12% samples in coastal regions and 45.3% in non-coastal regions did not contain Bla g 1. However, 7.3% samples in coastal regions and 35.3% in non-coastal regions contain Bla g 1. Likewise, 14% samples in coastal regions and 51.2% samples in non-coastal regions did not contained Bla g 2. Only 5.3% samples in the coastal regions and 29.4% in the non-coastal regions had Bla g 2.

Careful evaluation of the two allergens indicates that, though Bla g 2 was marginally higher in patients' houses, no allergic impact of either allergen can be clearly drawn on patients at this stage. Further investigation may be required with a higher number of patients' houses sample. Therefore, it is advisable that, Per a 1 and Bla o should also be included for any diagnostic approach on allergic patient.

Table 1. Overall concentration levels of Bla g 1 in houses for all samples

Conc. U/g	Samples (n)	%	Control houses (n)	%	Patient houses (n)	%
ND	323	57.7	235	42.0	88	15.7
Low 0.6	128	23.0	66	11.8	62	11.2
1-8	7	1.3	2	0.4	5	0.9
High > 8	102	18.0	93	16.5*	9	1.5**
Total	560	100	396	70.7	164	29.3

Clinically significant levels of Bla g 1 (> 8 U/g) were found in 18% of houses [22].

* High level in control houses ** Low level in patient houses

Table 2. Overall concentration levels of Bla g 2 in houses for all samples

Conc. µg/g	Samples (n)	%	Control houses (n)	%	Patient houses (n)	%
ND	367	65	267	47.7	100	17.8
Low < 0.08	170	30.4	119	21.3	51	9.1
> 0.08 – 0.4	0	0	0	0	0	0
High > 1	23	4.1	10	1.8**	13	2.3*
Total	560	100	396	70.8	164	29.2

Clinically significant levels of Bla g 2 (> 1 µg /g) were found in 2.3% of patient house samples [22].

**High level in patient houses ** Low level in control houses*

The result discussed above is a guideline for future diagnostic, therapeutic and preventive approaches in the field of allergic diseases. For example, when it comes to determination of cross-reactivity with other allergens, the nature of proteins found in different allergens determines the cross-reactivity in polysensitized patients. In a number of cases, the nature of such allergenic proteins, have been characterized and listed. However, the Bla g 1 protein is still not fully characterized and listed by the International Union of Immunological Society (IUIS). Nevertheless, they have listed the identified Bla g 2 protein as Aspartic protease with molecular function of endopeptidase activity [26]. Therefore, it appears that the two allergens may cause individual reactivity and not cross-reactivity unless otherwise characterized to be the same protein. Future emerging characterization of allergenic proteins may have a great impact in the cross-reactive allergenic components. It is therefore concluded that, while considering diagnostic, therapeutic and preventive approaches to indoor allergens in allergy and asthma patients in the country, a careful evaluation and addition of relevant extracts must be considered.

The results of 560 house dust samples analyzed are presented in Figs. 1 and 2.

The composition of Bla g 1 and Bla g 2 were found to be 28.7% and 23.6% in control houses. The patient houses contained 13.6% of Bla g 1 and 11.4% of Bla g 2.

Fig. 1 provide data that 42% control houses and 15.7% patient houses did not contain Bla g 1 and likewise 47% control houses and 18% patient houses did not contain Bla g 2.

The composition of Bla g 1 and Bla g 2 were respectively 7.3% and 5.3% in houses in coastal regions. The composition of Bla g 1 and Bla g 2 in the non-coastal houses were 35.3% and 29.4% respectively.

Quantitative Levels of Bla g 1 and Bla g 2 allergen: Please note that there is a difference in threshold or the clinical significant levels of Bla g 1 and Bla g 2.

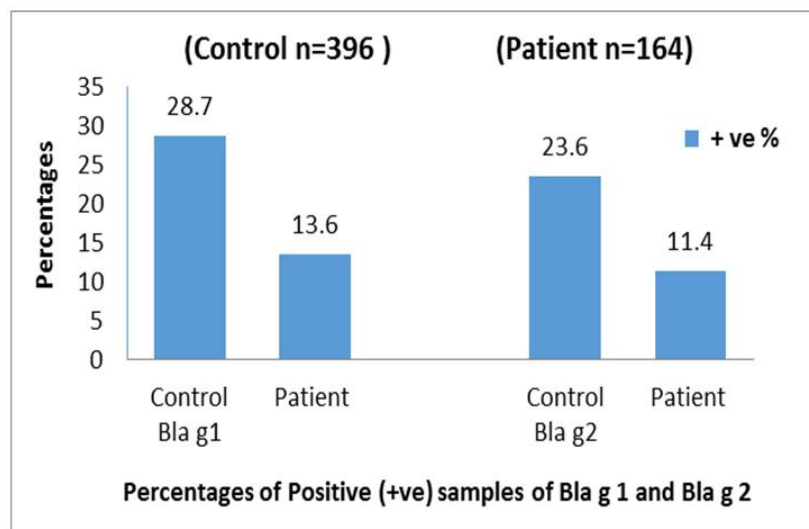


Fig. 1. Overall percentages of Bla g 1 and Bla g 2 in control and patient houses in KSA

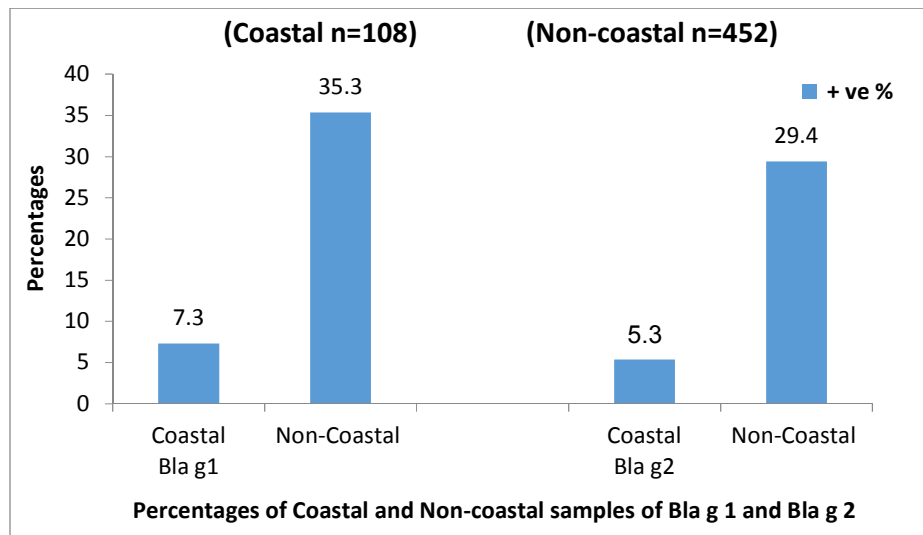


Fig. 2. Overall percentages of samples showing Bla g 1 and Bla g 2 concentration levels in houses in coastal and non-coastal regions

The results were divided as follows:

The quantitative or concentration level of both Bla g 1 and Bla g 2 were divided as follows:

ND: below the limit of detection (not detected)

Bla g 1 (Table 1)

- Low (0.6 U/g)
- Moderate (1-8 U/g)
- High (> 8 U/g)

Bla g 2 (Table 2)

- Low (0.08 µg/g)
- Moderate (0.08-0.4 µg/g)
- High (> 1 µg/g)

These values are according to the allergen exposure sensitization thresholds as mentioned in indoor allergens [22] (Chapman MD) the manufacturer of the antibodies.

4. CONCLUSION

Though the Bla g 2 allergen was marginally higher in patients' houses, however, in general, there appear to be almost an equal concentration level of *Blattella germanica* allergens in both patients and control houses. It is therefore recommended that Per a 1 and Bla o should also be included for any diagnostic approach on allergic patient. The authors were unable to determine their concentration levels in this study,

since antibodies to Per a 1 and Bla o were not commercially available.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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