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Cockroach exposure and its allergy sensitization in asthma patients

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Key words: Cockroach, asthma, skin prick test, specific IgE, total IgE

Abstract
Asthma is a heterogeneous disease with distinct phenotypes. Serum tIgE, SSIgE and SPT are the methods of evaluating allergen sensitization. The present study evaluates the exposure and sensitization to cockroach (Periplaneta americana) antigens in asthma patients in a metropolitan city of India. The study enrolled 200 consecutive bronchial asthma patients, diagnosed as per GINA guidelines. As per history of exposure to cockroaches, the patients are divided in two groups as exposed and non-exposed asthmatic. All the enrolled subjects underwent SPT against common aeroallergens including cockroach, spirometry and estimation of tIgE level and SSIgE against cockroach. Out of 200 asthma patients, a total of 114 (57%) asthmatic were found SPT positive against one of the common aeroallergens, of which 68 (34%) showed SPT sensitivity against cockroach. A total of 103 (51.5%) patients were found exposed to cockroaches. In the cockroach exposed group, the mean serum tIgE was found significantly higher than the non-exposed group (569.31±224.64 vs 479.29±237 IU/ml; p=0.007). The mean SSIgE against cockroach in exposed groups was found not significant than non-expose group (4.87±11.19 vs 4.11±8.39 KUA/L; p=0.589). The mean tIgE was also not significant in atopic compared to non-atopic asthmatic (553.25±218.12 IU/ml vs 489.1±251.16 IU/ml; p=0.056). The mean SSIgE against cockroach was 5.66±10.45 KUA/L for atopic and
2.96±8.98 KUA/L for non-atopic (p=0.054). The airway obstruction was almost the same in both groups. Asthmatic patients who were exposed to cockroach and atopic had high tIgE, SSIgE levels and SPT positivity against cockroach antigen compared to non-exposed patients.

Introduction
Over 4,000 species of cockroach exist worldwide and only a few of these species live in homes. [1] These cockroaches include *Periplaneta americana* (American Cockroach) and *Blattella germanica* (German cockroach). They are one of the most common sources of domestic indoor allergens. These two species of cockroach contained potent allergens that induce the formation of Immunoglobulin E (IgE) antibodies and trigger asthma in individuals. [1, 2] Cockroach sensitization plays an important role in the pathogenesis of bronchial allergy and it has been established as an important cause of asthma. [3] Cockroach sensitization and asthma has directly been associated with environmental exposure to cockroach. Various studies across the globe reported that cockroach sensitivity was 58% in the USA, 6.3-29% in European countries, 30% in India and Africa and 9.5-36% in Turkey. [4-6] We have reported earlier that the sensitivity to cockroach in bronchial asthma and allergic rhinitis was 24% among north Indian patients. [7] Various studies indicated that cockroach is crucial aeroallergen in pathogenesis of bronchial asthma and allergic rhinitis and a number of allergens from cockroach has already been identified and characterized. However, sensitization to cockroaches might differ widely within different regions of countries. [1, 7] The aim of this study is to determine the allergen sensitivity of American cockroach between exposed and non-exposed asthmatic and also to investigate and compare serum total IgE (tIgE), and Serum Specific IgE (SSIgE) level against cockroach in atopic and non-atopic asthma.

Materials and Methods
Subjects
The study was conducted at the Out-Patient Department of Viswanathan Chest Hospital, Vallabhbhai Patel Chest Institute, Delhi, India in years 2017-2018 after prior approval by the Institutional ethical committee. We enrolled 200 consecutive asthmatic patients. Diagnosis of asthma was done as per Global Initiative for Asthma (GINA) guidelines 2017 [8]. All enrolled patients were divided into two groups based on exposure to cockroach. The personal details of patients as well as information about asthma provided by them were recorded on a standard questionnaire as per Indian condition. Patients’ with pregnancy, lactating females, Chronic Obstructive Pulmonary Disease, tuberculosis, diabetes, hypertension, and heart diseases were excluded from this study. Details history of all enrolled patients was obtained for exposure to cockroach at their home or workplace. Patients given history of presence of cockroach at home or workplace for minimum 1-3 hours per day for minimum six months in last 2 years were considered as exposed group otherwise considered as non-exposed group. All enrolled patients undergone Skin Prick Test (SPT) for 57 common aeroallergens including cockroach, Pulmonary Function Test (PFT) and estimation of serum tIgE, and specific IgE against American cockroach after written consent.

Pulmonary Function Test
Spirometry was performed on a dry, rolling-seal spirometer of the benchmark model lung function machine by PK Morgan, Kent, UK. Maximal expiratory flow volume curves were obtained as per the ATS recommendations. FEV$_1$/FVC was taken for assessing the pulmonary obstruction and Forced Expiratory Volume 1 (FEV1) was taken for assessing severity of asthma was categorized as mild, moderate, moderate severe, severe and very severe. [9]

**Skin Prick Tests** SPT was performed with 57 common aeroallergens, which include cockroach allergen i.e. *Periplaneta americana*. The allergens extract (1:10 w/v, 50% glycerinated) were obtained from All Cure Pharma Pvt. Ltd., New Delhi, India. Glycerinated Buffered saline and histamine diphosphate in a glycerinated buffer were used as negative and positive controls, respectively. In brief, a volar aspect of the forearm cleaned with cotton balls soaked in 70% alcohol was allowed to dry. A small drop of antigen was placed on the forearm and each drop was numbered. Then skin was pricked using 26.5 gauge sterile needles. The skin test reactions were graded by calculating the mean diameter as \((D + d)/2\); \(D\) = largest diameter and \(d\) = orthogonal or perpendicular diameter at the largest width of \(D\) after 15–20 minutes in comparisons to the wheal size of positive control i.e. histamine diphosphate (10 mg/ml). To ensure uniformity, the same lots of cockroach extracts were used in all subjects, only one researcher performed all SPT. A positive result (2+ and above) to a specific allergen is indicated by a mean wheal diameter measuring 3 mm or more, greater than negative control (buffered saline) as per Standard Indian Guidelines. [10]

**Serum Total IgE Estimation** Serum total IgE estimation were carried out for each asthmatic patients by Enzyme Linked Immunosorbent Assay (ELISA) using Calbiotech ELISA kit (CalbiotechInc, CA) according to the manufacturer’s instructions with URIT-660 microplate reader (URIT Medical Electronic Co., Ltd., Guangxi, China. [Total IgE: >250 IU/ml (Male) &>175 IU/ml (Female) were considered positive].[11]

**Serum Specific IgE Estimation** Estimation of SSIgE, for *Periplaneta americana* were measured with ImmunoCAP system (Phadia, Sweden), according to the manufacturer’s instructions. Sensitization was considered to be present when SSIgE levels were >0.35 kUA/L [12]. The spirometry values, SPT, Serum tIgE and specific IgE against cockroach were compared between cockroaches exposed and non-exposed as well as atopic and non-atopic groups.

**Statistical Analysis**
We used descriptive statistics to examine the epidemiological and therapeutic features of our sample. Independent t-test was used to compare means and significance differences between all variables of groups. Statistical significance was set at 5% (p<0.05 value) considered as statically significant at 95% Confidence Interval (CI). Analysis of data obtained from all enrolled subjects was analyzed by using the Statistical Package for Social Sciences (SPSS statistics) version 16.0.

**Results**
The most of the enrolled subjects come from the metropolitan city i.e. Delhi-National Capital Region. The majority of enrolled patients 161(80.5%) were from urban areas while the remaining 39(19.5%) were from rural areas. The family history of asthma was positive in 121(60.5%) subjects. Breathlessness was the most common symptom (100%) followed by Wheezing (92.5%), cough (91.5%), nasal congestion (88%) and skin complaints (22%). The demographic of patients were shown in Table 1.

SPT, tIgE, SSiGE and spirometry pattern among cockroach exposed and non-exposed asthma patients

In cockroach exposed asthma patients, 37.86% (39/103) were found SPT positive against cockroach allergens while 29.89% (29/97) positive in non-exposed asthma patients, with no statistically significant (p=0.236). Overall cockroach SPT positive is 68(34%) (39 exposed + 29 non-exposed). In the cockroach exposed group, the mean serum total IgE was higher and statically significant as compared to the non-exposed group (569.31±224.64 vs 479.29±237 IU/ml; p=0.007). On the other side, the mean SSiGE against cockroach was not significantly higher in exposed group compared to the non-exposed group (4.87±11.19 vs 4.11±8.39 KUA/L; p=0.589). The airway obstruction was seen in 62 (60.19%) in the exposed group while 64(65.98%) in non-exposed asthma patients with not statically significant difference (p=0.4). The total IgE positivity among cockroach SPT positive patients was almost equally affected in cockroach exposed and non-exposed group. However, the mean total IgE was not significantly higher in exposed group compared to non-exposed group (564.67 vs 544.33; p=0.114).The level of specific SSiGE against cockroach antigen positivity was also similar between exposed and non-exposed asthmatic patients with no significant difference in mean specific SSiGE level among them (p=0.458).

The total IgE positivity in cockroach SPT negative patients was almost similar in the cockroach exposed (87.5%) and non-exposed (85.2%). The mean total IgE level was found significantly higher among exposed group compared to non-exposed group (615.25 vs 529.97; p=0.026). The level of specific SSiGE against cockroach in cockroach SPT negative patients was almost same between exposed and non-exposed patients. However, the mean specific SSiGE value was not significantly higher in exposed asthmatic patients (p=0.603) (Table 2).

Total IgE, SSiGE and spirometry age-wise distribution among cockroach allergic patients

Out of 68 cockroach SPT positive patients, higher percentage of positive patients (n=35; 51.47%) were found in age group 21-30 years, followed by 12-20 years (n=14; 20.59%), 31-40 years (n=13; 19.12%), 41-50 years (n=4; 5.88%) and >50 years (n=2; 2.94%). Out of all cockroach SPT positive patients, 64 (94.12%) patients were found total IgE positive in which highest (n=34; 53.17%) in 21-30 years age group, followed by 12-20 years (14; 21.88%), 31-40 years (11; 17.19%), 41-50 years (3; 4.69%) and >50 years (2; 3.13%). Among the cockroach SPT positive patients, 52 (76.47%) patients were found SSiGE positive against cockroach in which highest found in age group 21-30 years (n=28; 53.8%), followed by 12-20 years (11; 21.15%), 31-40 years (7; 13.46%), 41-50 years (4; 7.69%) and >50 years (2; 3.85%). Similarly, out of all cockroach SPT positive patients, airway obstruction at the time of enrollment was present in 36(52.9%) patients, in which highest obstruction was noticed in the age group 21-30 years (n=16; 44.4%). Overall the commonly affected age group with all parameter is 21-30
years with nearly 50% of patients fall in this age group. However, the p value for age group difference was not statistically significant (p=0.323) (Figure 1).

**Baseline PFT in cockroach exposed group and non-exposed group**
In cockroach exposed and non-exposed group, airway obstruction was found almost the same [62(60.19%) vs. 64(65.98%); p=0.4]. The airway obstruction was mild in 38(61.2%) among cockroach exposed group and 39(60.9%) in non-exposed group. Similarly, moderate obstruction was seen 10(16.2%) in cockroaches exposed and 11(17.2%) in non-exposed. While 14(22.6%) patients showed moderate severe to very severe obstruction in cockroach exposure and 14(21.8%) in non-exposed. Overall the severity of obstruction was similar in both groups (Figure 2).

**Atopic and non-atopic asthma patients with cockroach sensitization**
Out of 200 asthmatic patients, 114(57%) exhibited positive sensitization (atopy) against common aeroallergens (i.e. sensitization to at least one allergens on SPT) and rest were found as non-atopic 86(43%). Further, out of 114 atopic patients, including 68(34%) was found positive for cockroach antigen.

**Pattern of total IgE, SSiGE and spirometry in atopic and non-atopic patients**
The overall total IgE was raised in 178(89%) of asthma patients with significantly higher in atopic. The airway obstruction was almost similar between atopic and non-atopic patients. The various detail of total IgE, SSiGE and spirometry of atopic and non-atopic is shown in Table 3.

**Baseline PFT in atopic and non-atopic group**
The airway obstruction was seen in 68(59.7%) of atopic group and 58(67.4%) of non-atopic group. The airway obstruction was mild in 44(64.8%) among atopic and 33(56.9%) in non-atopic. Similarly, moderate obstruction was seen 12(17.6%) in atopic and 9(15.5%) in non-atopic. While 12(17.6%) patients showed moderate severe to very severe obstruction in atopic and 16(27.6%) in non-atopic (Figure 3).

**Discussion**
Cockroach has been identified as one of the most important allergen sources for the development of asthma and allergic rhinitis. It is one of the important causes of indoor allergens throughout the world [13, 14]. Cockroaches affect human health adversely through food contamination, transmission of bacterial and viral pathogens, psychologic stress and allergic sensitization. The most commonly encountered species are *Periplaneta americana*, *Blattella germanica* and *Blattella orientalis* [4,13]. Among these cockroach species, *Periplaneta americana* is known to have a high immunologic potency and is the most widely encountered one. [2,3] In India, *Periplaneta americana* is also one of the most frequently observed cockroach species and number of studies involving this sensitivity are growing up. Bernton and Brown in 1964 reported that the positive skin test to cockroach antigens was in 44% of 755 allergy clinic patients. [15] Mungan D et al. in a study of 206 asthmatic reported that 63% of their patients were atopic with cockroach antigen sensitivity was detected in 25.7% patients. [16] We used *Periplaneta americana* antigens for skin prick test, as it is one of the most
frequently found cockroach species in India. The cockroach sensitivity was detected in 34% of all asthmatic (atopic) patients with 37.86% in exposed and 29.89% in non-exposed asthmatic patients.

Atopy has historically been seen as a risk factor of having asthma. [17] The studies in more recent years have confirmed the association of cockroach exposure and increased asthma morbidity in inner city areas in the United States. [18, 19] The studies from Delhi have found that the SPT prevalence for cockroach varied between 19-25%, which in turn may increase the asthma rate [7,20]. Ronchetti et al. suggested that the incidence of atopy in asthma depends on certain environmental factors that may at the same time induce atopy in both asthmatic and non-asthmatic people [21]. Ige et al. reported that the positive SPT to some of the common environmental allergens and increased level of IgE suggests an important role of atopy in the expression of asthma in the growing country's city setting [22].

The cockroach sensitivity has been reported to affect young males and adult females more frequently; however, the reason for this observation is not clear. Variability in distribution of age and gender may be related to different allergen exposure depending on the lifestyle of the population groups. We found cockroach sensitivity in 43(21.5%) of males and 25(12.5%) of females in asthmatic (atopic) patients. The overall and cockroach sensitivity was highest among 21-30 age groups. Similarly the total IgE and specific IgE were also highest in the same age group with nearly 50% cases (Figure 1). Similar to our study, Ghaffari et al. reported that allergen SPT reactivity increases in the middle age group and then tends to decrease with increasing age [23]. Kumar et al. in a study revealed that younger adults were the most commonly affected age group [7]. Wang et al. also reported that the prevalence of aeroallergens sensitization decreased with increasing age [24]. Tayeb and Qutub in a study of 118 asthma patients concluded that the elevated total IgE have both high sensitivity and high negative predictive values for German cockroach sensitivity [25]. The higher prevalence of sensitivity among young age may be due to exposure duration, occupation, lifestyle and genetic susceptibility among this age group.

The development of a constant inflammatory process of the T\textsubscript{H2}-type, triggered by inhalation of a particular allergen, is the characteristic feature of allergic (or atopic) asthma leading to the synthesis of specific IgE in susceptible individuals [26], hence the detection of specific IgE in the serum is the key evidence of allergic asthma [27]. Finn et al. in 2000, has demonstrated a significant association between exposure to cockroach allergens in the first 3 months of life and the development of repeated wheeze in the first year among infants in metropolitan areas [28]. Our study also demonstrated that the total IgE and SSIGE against cockroach antigens were almost similar in atopic asthmatic patients, who were exposed to cockroaches at home/workplace than non-exposure asthmatic. This similar level of the specific IgE level may be due to the less number of enrolled patients and the different reactivity to allergens among atopic individuals. Overall the SPT against cockroach was positive in 68(34%) and the SSIGE was positive in 52(26%). This finding shows that the SPT has high sensitivity than SSIGE in cockroach reactivity amongst asthma patients.

It has been clearly accepted that exposure to cockroach allergen is one such risk factor for allergic diseases [4, 7, 29]. It is shown that the African-American, lower socioeconomic status, age more than 11years and exposure to cockroach are one of the independent risk factors predictive of cockroach allergic sensitization in children [4]. Sohn et al. reported that
measurement of Bla g 1 levels has been used to estimate exposure to cockroach allergens. Bla g 1 exposure above 2 U/g of dust is thought to be a strong risk factor for sensitization. Furthermore, asthma symptoms occur with increased frequency with exposure to 8 U/g of dust or more among sensitized individuals [30]. Kang et al. also reported association between cockroach allergy and asthma following the inhalation of cockroach allergens by sensitized asthma patients [31]. Later, several studies have demonstrated that patients with asthma are commonly sensitized to cockroaches in several urban or inner cities around the world. Cockroaches are among the most common pests prefer to live in damp but warm places and are generally found in homes, schools, offices and other buildings. At night they search for food in kitchens, food storage places, rubbish bins, drains and sewers. They are pests because of their filthy habits and bad smell. Their saliva, feces and shedding body parts act like dust mites, aggravating symptoms when they are kicked up in the air. Cockroach sensitivity can be observed even without the evidence of cockroach infestation[13]. The cockroach sensitivity is also related to outdoor contacts, therefore taking preventive measures such as sampling of indoor sources i.e. mat, mattress, curtains etc., could not be used as an evidence of cockroach sensitivity [14]. The sensitization is more common among exposed people as seen in our study. On the other hand genetic factors particularly genetic variants in TLRs, CLR, CD14, may also play an important role in conferring the susceptibility to cockroach sensitization. Several genes have been associated with cockroach sensitization and asthma-related phenotypes [32]. Avoidance is the most appropriate therapy for the cockroach allergen sensitivity. The common ways to treat prevent cockroach exposure is to eliminate these insects from home. The various methods of cockroach elimination are, keeping house clean including kitchen floor, store etc. and keep closed or sealed all food containers and garbage cans with regular interval cleaning. The pest management and removal of reservoirs of cockroach contaminants were the strongest recommendations to decrease cockroach exposure and reduce asthma morbidity. In addition to evidence of the presence of cockroaches by visual inspection or sticky trap placement, measurement of cockroach allergen levels could be useful to guide environmental interventions aimed at reducing cockroach exposure [33]. Furthermore, the immunotherapy with cockroach extracts can be considered the effective treatment if symptoms persist or avoidance is not possible.

There are few limitations of this study. The present study was a single centre study. The other limitation was we did not take the extract dust samples to be used to evaluate exposure to cockroach allergens. Hence, we advise for a larger multicenter study with dust extract sample for evaluation of cockroach exposure effect on asthma patients.

**Conclusion**

The sensitivity to cockroach allergen is almost similar in atopic and cockroach exposed asthma patients, however the mean total IgE was significantly higher in cockroach exposed group. The SPT, total IgE, specific IgE are important tools for diagnosis of cockroach allergy among asthmatic. Spirometry severity is not affect by cockroach sensitivity in asthmatic patients.

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### Table 1. Demographic data of asthmatic patients.

| Variables                      | Asthmatics (n=200) | Cockroach Exposure (n=103) | Cockroach Non-exposure(n=97) | Total | p-value |
|--------------------------------|--------------------|-----------------------------|-----------------------------|-------|---------|
| Number                         | 103 (51.5%)        | 97 (48.5%)                  |                             | 200   |         |
| Male/Female                    | 44/59              | 37/60                       |                             | 81/119| 0.606   |
| Age (mean in years)            | 29.60±9.52         | 31.79±10.18                 |                             | 30.69±9.85| 0.119   |
| BMI (mean in kg/m²)            | 24.9±5.01          | 26.9±5.94                   |                             | 25.75±5.48| 0.011   |
| Duration of Disease (mean in year) | 4.65               | 4.89                        |                             | 4.77  | 0.684   |
| Study Population               |                    |                             |                             |       |         |
| Urban area                     | 80 (77.66%)        |                             |                             | 161   | 0.388   |
| Rural area                     | 23 (22.33%)        |                             |                             | 39    | 0.388   |
| Food Habit                     |                    |                             |                             |       |         |
| Vegetarian                     | 77 (74.75%)        |                             |                             | 145   | 0.563   |
| Non-Vegetarian                 | 26 (25.24%)        |                             |                             | 55    | 0.563   |
| Symptoms (Year)                |                    |                             |                             |       |         |
| Breathlessness                 | 103 (100%)         | 97 (100%)                   |                             | 200   | -       |
| Wheezing                       | 97 (94.17%)        | 88 (90.72%)                 |                             | 185   | 0.511   |
| Cough                          | 94 (91.26%)        | 89 (91.75%)                 |                             | 183   | 0.890   |
| Nasal                          | 89 (86.40%)        | 87 (89.69%)                 |                             | 176   | 0.6197  |
| Skin Complaints               | 18 (24.41%)        | 26 (26.80%)                 |                             | 44    | 0.1554  |
Table 2. SPT Sensitivity against cockroach exposed and non-exposed asthma patients.

| Variables                      | Cockroach Exposed asthma patients (n=103) | Cockroach Non-exposed Asthma patients (n=97) | p-value |
|--------------------------------|-------------------------------------------|---------------------------------------------|---------|
| Total IgE (mean, IU/ml)        | 569.31±224.64                             | 479.29±237                                  | 0.007   |
| Specific IgE (Kua/l)           | 4.87±11.19                                 | 4.11±8.39                                   | 0.589   |
| PFT (Obstruction)              | 62 (60.19%)                                | 64 (65.98%)                                 | 0.4     |
| Cockroach SPT +ve              | 39 (37.86%)                                | 29 (29.89%)                                 | 0.236   |
| Total IgE (+ve)                | 37 (614.67)                                | 27 (544.33)                                 | 0.114   |
| Total IgE (-ve)                | 2 (197.5)                                  | 2 (121.8)                                   | 0.458   |
| Specific IgE (+ve)             | 30 (8.91)                                  | 22 (8.68)                                   | 0.950   |
| Specific IgE (-ve)             | 9 (0.064)                                  | 7 (0.134)                                   | 0.033   |
| Cockroach SPT –ve              | 64 (62.1%)                                 | 68 (70.1%)                                  | 0.236   |
| Total IgE (+ve)                | 56 (615.25)                                | 58 (529.97)                                 | 0.026   |
| Total IgE (-ve)                | 8 (130.83)                                 | 10 (81.28)                                  | 0.148   |
| Specific IgE (+ve)             | 37 (6.24)                                  | 41 (4.97)                                   | 0.603   |
| Specific IgE (-ve)             | 27 (0.09)                                  | 27 (0.091)                                  | 0.888   |

Table 3. SPT, total IgE, SS IgE and spirometry pattern in atopic and non-atopic patients.

| Variables                      | Asthmatic Patients (n=200) | p-value |
|--------------------------------|----------------------------|---------|
|                                | Atopic (n=114)             | Non-atopic (n=86) | |
| Airway Obstruction             | 68 (59.7%)                 | 58 (67.4%) | 0.258 |
| Cockroach SPT +ve              | 68 (34%)                   | 0        |       |
| Total IgE (mean, IU/ml)        | 553.25±218.12              | 489.1±251.16 | 0.056 |
| Total IgE +ve patients (n)     | 106 (92.9%)                | 72 (83.7%) | 0.049 |
| SS IgE (Kua/l)                 | 5.66±10.45                 | 2.96±8.98 | 0.054 |
| SS IgE +ve patients (n)        | 78 (68.4%)                 | 52 (60.5%) | 0.245 |
Figure 1. Age-wise distribution of cockroach allergic patients.

Figure 2. Baseline spirometry in cockroach exposed group and non-exposed group.
Figure 3. Baseline spirometry in atopic and non-atopic group.