



Full Length Research Paper

A study on the prevalence of allergy markers in Nigerian asthmatic patients

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Studies have shown that allergens are important sensitizing agents in asthma, but the study on prevalence of allergy markers in Nigerian asthmatic patients is scarce. Also, no study on serum concentration of allergen specific immunoglobulin E (IgE) in Nigerian was encountered. This study was therefore designed to investigate this dearth of knowledge. Serum samples of eighty adult asthmatic patients recruited for this study was analyzed for specific IgE using enzyme linked immunoassay (ELISA) method. Also, skin prick test (SPT) to 8 common allergens was performed on these subjects. Sixty five percent of the asthmatic patients had positive SPT reaction to at least one allergen. *Dermatophagoides pteronyssinus* (house dust mites (HDM)) sensitization was the most common, occurring in 45% of asthmatic patients. There was a positive correlation between the wheal diameter of SPT and concentration of specific IgE in one of the 3 allergens tested ($r = 0.545$, $p = 0.001$ for HDM). The observations of this study showed that a large proportion of asthmatic patients in Nigeria are sensitive to *D. pteronyssinus* and measurement of allergen-specific IgE is an effective method to determine sensitivity to HDM.

Key words: Asthma, immunoglobulin E (IgE), skin prick test (SPT), environmental allergens.

INTRODUCTION

Asthma is a global disease and one of the most common chronic diseases in the world. It is estimated that around 300 million people in the world currently have asthma (Masoli et al., 2004). Asthma has become more common in both children and adults around the world in recent decades. The prevalence of asthma varies with geographical location. Asthma is a common respiratory disease in Nigeria with a prevalence of 5.4% (Masoli et al., 2004). The International Study of Asthma and Allergies in Childhood (ISAAC) in Nigeria revealed that cumulative prevalence rate for wheezing within the immediate 12 months has 10.7% (Falade et al., 1998).

The diagnosis of allergy is usually established either by allergen skin test or by *in vitro* allergen-specific immunoglobulin E (IgE) measurements (Nepper-Christensen, 2003). However, some patients cannot have skin tests, because of dermatographism, severe eczema, and medication (Li, 2002).

Eosinophilia and elevated serum IgE was found to correlate with allergy skin test reactivity and have been used by some investigators as alternative methods to characterize atopic patients (Tullerud et al., 1991). However, in tropical countries such as Nigeria where the prevalence of intestinal helminth is high, serum IgE levels appear to be much higher than in subtropical countries and this might affect the interpretation of total IgE results (Arinola, 2008). In addition, total IgE value is influenced by many factors such as gender, age, race, and smoking habits (Arinola, 2008).

Therefore, measurement of allergen-specific IgE is indicated in our environment for evaluating allergy. The use of specific IgE antibodies as a diagnostic tool in allergic disease has not been investigated in our environment, though many of these studies have been done in other parts of the world

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Table 1. Percentage positivity and wheal diameter of SPT in asthmatic patients.

Allergen	No. of patients with positive SPT	%	Wheal diameter (mm) $\bar{X} \pm SD$
<i>D. pteronyssinus</i>	36	45	4.05 \pm 1.17
Cockroach	23	28.8	3.56 \pm 0.78
Dog dander	9	11.3	3.0 \pm 0
Mixed feathers	6	7.6	3.83 \pm 0.96
Birch	6	6.3	3.8 \pm 0.83
Wheat grain	2	2.5	4.0 \pm 0
Cow milk	3	3.8	3.0 \pm 0
Cotton flock	2	2.6	3.5 \pm 0.71

(Santoso, 1998; Khadadah et al. 2000). It is imperative to assess the role of specific IgE antibodies as an alternate diagnostic tool for evaluating allergy among asthmatic patients in our environment.

METHODOLOGY

Patients

This study was conducted at the Chest Clinic of University College Hospital, Ibadan, Nigeria from January to June, 2006. Patients were adults of age between 15 and 50 years (33.01 ± 10.50) who were diagnosed with bronchial asthma. Patients were recruited to the study only if they fulfilled the criteria for the diagnosis of asthma by the National, Heart, Lung and Blood Institute Expert Panel Report II.

Patients who were on prednisolone in the last 3 months, or who had been taking antihistamine in the last 30 days were excluded from the study. Patients with history and findings suggestive of cardiovascular, renal, or hepatic disease were excluded from the study. All patients recruited were non-smokers.

A comprehensive history of the patients using a structured questionnaire was taken. This included history of cough with or without sputum, shortness of breath, chest tightness, wheezing, duration of symptoms, frequency of acute attacks, history of allergy, and previous history of asthma/other chest diseases, family/occupational history of asthma, history of previous and current drug therapy. All participants gave informed consent. The study was approved by the Joint University of Ibadan/University College Hospital Institutional Review Committee.

Blood sample

Blood sample (5 ml) was collected from all subjects by venopuncture before the SPT was performed. The blood sample was put into a container to clot at room temperature. The specimen was then centrifuged for 5 min at 3000 g. Serum was collected and stored at -20°C for the assay of specific IgE.

Skin testing

Allergy SPTs were carried out using eight allergens prevalent in this geographical region as described by the manufacturer (Allergy Therapeutics Ltd UK). Glycerol was used as the negative control and histamine as the positive control. The allergens applied were *Dermatophagoides pteronyssinus* (house dust mites (HDM)), birch pollen, dog hair, cockroach, mixed feathers, cotton flock, milk, and

wheat grain. The diameter of wheal reaction was read after 20 min. A wheal diameter of at least 3 mm, after subtraction of the diameter size of the negative control was taken as a positive reaction.

IgE assay

The concentrations of allergen-specific IgE antibodies to HDM, cockroach, and dog dander (allergens with most prevalent positive skin test reaction) were determined using the enzyme linked immunoassay (ELISA) method with RIDASCREEN spezifisches IgE kit (R-Biopharm AG Germany) based on the instruction of the manufacturer. Briefly, 50 μl of standards, control and sera samples were pipette into the appropriate wells of IgE plates. These plates were incubated at 37°C for 60 min and was washed 6 times with 700 μl of buffer per well. After which 50 μl of conjugate was added into the wells and was incubated at 37°C for another 60 min, and was washed 6 times again with 700 μl of washing buffer per well. Substrate (50 μl) was added and incubation was done for 15 min at 37°C . Thereafter, 50 μl of stop solution was pipette into the wells and optical density was read at 405 nm against a reference wavelength of 602 nm.

The intensity of the yellow color is proportional to the quantity of allergen-specific antibodies in the serum. The optical density values of the test sample were used to extrapolate the concentration of specific IgE in the test sample from the standard curve.

Statistical analysis

The statistical package (SPSS version 12.0) was used for data entries. All entries were double checked and analyzed using SPSS and EPI-Info version 6. Frequency distributions were generated for nominal and ordinal variables. Chi-square test was used to test for significance between proportions. Statistical significance was said to have been achieved when the p-value was equal to or less than 0.05. The Spearman rank correlation was used to relate skin test wheal diameter and the serum concentration of specific IgE.

RESULTS

Adult patients (80), 28 males and 52 females were recruited for the study. Their ages ranged from 15 to 50 years with a mean of 33.01 ± 10.50 years. SPT was positive to at least one allergen in 52 (65%) cases.

Table 1 shows the SPT results in asthmatic patients. The prevalence positivity of the skin reaction was highest

Table 2. Prevalence of allergen specific IgE in asthmatic patients that were SPT positive for particular allergen.

Allergen	No. of patients with positive SPT	No. of patients with positive specific IgE	Specific IgE concentration (IU/ml) \pm SD
<i>D. pteronysinnus</i> (HDM)	36	31	32.41 \pm 21.45
Cockroach	23	5	2.38 \pm 1.78
Dog dander	9	5	12.45 \pm 20.09

Table 3. Correlation between SPT wheal diameter and specific IgE concentration (IU/ml).

Variable	Spearman's correlation	p value
<i>D. pteronysinnus</i>	0.545	0.001
Cockroach	-0.084	0.460
Dog dander	-0.063	0.582

with HDM allergen (45%), followed by cockroach allergen (28.8%) and dog dander allergen (11.3%). Table 2 shows the prevalence of allergen specific IgE in asthmatic patients that were SPT positive for 3 allergens. SPT was positive in 36 cases of HDM. It was observed that the concentration of specific IgE was positive in 31 cases. For the cockroach allergen, 23 cases had positive SPT reaction, while only 9 cases had positive specific IgE concentration. A positive SPT was observed in 9 cases of dog dander, while 5 showed positive specific IgE concentration.

Table 3 shows the correlation between SPT wheal diameter and the specific IgE concentration of the 3 allergens (HDM, cockroach and dog) with the highest SPT positivity in this study. Only *D. pteronysinnus* showed significant positive correlation. ($r = 0.545$; p value 0.001).

DISCUSSION

Allergen-specific IgE determination is widely used in the diagnosis of IgE-mediated allergic diseases, but the relative merits of *in vitro* measurement of IgE antibody in comparison to *in vivo* skin tests are still debated (Plebani et al., 1995). In this study, aeroallergen sensitivity was found to be 65% of cases in this environment. This was similar to the findings reported by Aderole and Oduwale (1981) among asthmatic patients. HDM was the most prevalent positive skin test reaction (45%). This finding is similar to the observations reported by Onadeko and Sofowora (1979) where 43% of asthmatic patients had positive reaction to HDM. Awotedu et al. (1992) also reported that 58% of the asthmatic patients had positive reactions to HDM. The larger percentage reported by Awotedu et al. (1992) may be due to the large number of patients (746) studied. Cockroach allergen was the second most commonly recognized allergen in this study, because 28.8% of the asthmatic patients had positive

reaction. Adanijo and Bandele (2000) reported a prevalence of 44% in Lagos. The higher prevalence of cockroach allergen sensitivity obtained in Lagos may be due to dense population and cockroach infestation. Studies on cockroach allergen reported varying prevalence and this may be a reflection of the level of cockroach infestation in their environment (Fraser, 1999; Khadadah et al., 2000).

Dog dander was the third commonest allergen in this study. A prevalence of 11.3% was observed in this study. This was similar to the finding reported by Onadeko and Sofowora (1979) (14%), Awotedu et al. (1992) (12.9%), and Aderole et al. (1981) (7%).

It was observed from this study that only a proportion of patients with positive skin sensitivity test also had positive RIDA Screen-specific IgE to the same allergen. A significant proportion (86%) of the asthmatic patients who had positive SPT to HDM also showed positive specific IgE to the same allergen. The corresponding figure for dog dander and cockroach were 55.5 and 21.7%, respectively. This demonstrated that serum allergen specific IgE test was less sensitive than SPT. Positive skin test reactions which are not accompanied by a positive specific IgE concentration are probably caused by IgE antibodies that are not detectable in the serum by the method of assay. Although, characterization and standardization are advanced for most of the commercial allergen and specific IgE assay kit; the lower sensitivity of specific IgE as compared to SPT may be due to modifications of the antigen or reagents by manufacturers. Ezeamuzie et al. (2000) and Khadadah et al. (2000) in their respective studies reported that specific IgE positivity was lower than SPT positivity.

Correlation between the size of wheal of SPT and concentration of specific IgE to the 3 common allergens was carried out. There was significant correlation with only HDM, but not for cockroach and dog dander. In a study by Santoso (1998) in Indonesia, there was a positive correlation between wheal size and specific IgE

concentration to HDM and similar to the finding in this study, he noted that HDM was the prevalent allergen in their environment. The observation of lack of correlation between the size of wheal of SPT and specific IgE concentration to cockroach in this study is in contrast to the finding in Khadadah et al. (2000) study. In their study, they observed a correlation between the size of wheal of SPT and specific IgE concentration to cockroach, though cockroach was the most prevalent allergen in their environment. The reason for the lack of correlation in this study is not clear, because cockroach infestation is quite rampant in this environment, especially among the low socioeconomic class. Perhaps, genetic and environment factors may be responsible for this observation. The relatively low positive SPT to dog dander might have accounted for the absence of correlation between the size of wheal of SPT and specific IgE concentration.

Conclusively, this study has revealed that SPT is a better tool for detecting allergen sensitivity, though specific IgE to aeroallergens may also be useful in assessing allergy in patients with asthma. In addition, most asthmatic patients considered for this study were sensitive to HDM.

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