Original Article

A Comparative Study of Skin Prick Test *versus* Serum-Specific IgE Measurement in Indian Patients with Bronchial Asthma and Allergic Rhinitis

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Abstract

Background. Skin prick testing (SPT) is the 'gold standard' in the assessment of allergic sensitivity to inhalant allergens. Serum-specific immunoglobulin E (SSIgE) measurement is a complementary test. SPT is performed with antigen extracts from India while SSIgE utilises extracts derived from European antigens.

Objective. To evaluate the performance of allergic assessment by SSIgE against cockroach, housefly and mosquito aeroallergens which are frequently implicated in driving respiratory allergies in India considering SPT as the 'gold standard'.

Methods. Twenty patients (mean age 28.5 years; range 15-50 years) diagnosed to have bronchial asthma and/or rhinitis underwent SPT. The SSIgE levels were obtained at the same visit. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of SSIgE testing were calculated using SPT as the 'gold standard'. The correlation between SPT grading and SSIgE levels was also evaluated.

Results. The sensitivity of SSIgE testing to each of the 3 aero-allergens was >85%. The PPV of cockroach and mosquito SSIgE was >85%; housefly SSIgE had PPV of 68.7%. The two tests were in agreement in 85% (cockroach), 90% (mosquito) and 55% (housefly). There was a significant correlation between the grades of SPT reactions and SSIgE levels.

Conclusions. The SSIgE has higher sensitivity and PPV, but lacks specificity. Higher sensitivity with low specificity leads to increased false positive diagnosis of allergic disease. Unlike allergenic pollens, however, insect antigen extracts from different regions seem to give comparable results, and can thus, reliably be used in the evaluation of allergy.

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Key words: Allergy, Asthma, Rhinitis, Serum specific IgE, Skin prick test.

Introduction

Immunoglobulin E (IgE)-mediated allergic diseases are common in India. The majority of the patients suffer from bronchial asthma, allergic rhinitis or both. The diagnosis of these conditions is based on clinical history and examination, however establishment of allergy requires either skin prick testing (SPT) or measurement of serum-specific IgE (SSIgE) levels to relevant allergens. The diagnostic modalities measuring *in vitro* SSIgE levels include radioallergosorbent test (RAST) and ImmunoCAP methods.[1-4] Results from western literature have shown SPT to be more sensitive than SSIgE.[2-4] Hence, SPT is more commonly used in allergy testing, while *in vitro* tests are considered to be complementary.[3]

Skin prick testing has been considered to be the 'gold standard' in the assessment of inhalant allergen sensitivity. Results are interpreted in the context of clinical history and epidemiological profile. Allergy is diagnosed when the history correlates well with the testing results. Patients in whom this correlation is not found or SPT cannot be properly interpreted (due to equivocal response, dermatographia, or inability to discontinue medications which interfere with SPT), *in vitro* testing is a useful alternative. The SSIgE testing is helpful in these cases such that recommendations regarding avoidance measures and immunotherapy can still be made. It is clearly important that SSIgE provides data that correlates with gold standard SPT results when considering introduction of allergen immunotherapy.[5] Thus, we have to consider how the antigen extract preparation of SPT and SSIgE from different flora and fauna affect the test results in the population under consideration.

To the best of our knowledge there have been no studies correlating SSIgE and SPT done in India. Hence, the present study was undertaken in Indian population to compare the sensitivity and specificity of SSIgE *versus* gold standard SPT against 3 common aeroallergens, cockroach, housefly and mosquito.

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Material and Methods

This study was conducted in the outpatient department of the Vallabhbhai Patel Chest Institute, Delhi. The study group consisted of 20 patients with bronchial asthma and/or allergic rhinitis with positive SPT result to at least one of the three aero-allergens (vide infra). Patients were diagnosed to have bronchial asthma as per Global Initiative for Asthma (GINA) 2010 guidelines[6] and rhinitis was diagnosed in accordance with the Allergic Rhinitis and its Impact on Asthma (ARIA) guidelines.[7]

Both SPT and SSIgE tests against cockroach, housefly and mosquito were performed on the same visit by a single investigator. The antigens were obtained from All Cure Pharma Pvt Ltd, New Delhi and derived from Indian species of these insects [Cockroach (*Periplaneta americana* spp), mosquito (*Anopheles* spp), housefly (*Musca* spp). To perform the skin test, a small drop of test reagent (allergen extract) was placed on the surface of the forearm. Then a disposable hypodermic needle (26G) was passed through the drop with its bevel facing up and inserted into the skin about 1mm at a low angle. The needle tip was gently lifted upwards a bit without inducing bleeding and then withdrawn slowly. After about 2 minutes, the drop was gently wiped off with dry cotton. The test reading was done after 15-20 minutes. Atopy was defined as a positive SPT in which the wheal diameter is >3 mm as compared to the negative control (buffer saline) for at least one aeroallergen. Grading of the positive reaction was done as per the Indian guidelines.[8] An allergen-induced skin wheal response equivalent to the histamine response was graded as 2+. Skin responses of 2 mm less or 2 mm more than the histamine reaction were graded as 1+ and 3+, respectively. A wheal response of greater than 3+ was graded as 4+.

The SSIgE measurement was done by ImmunoCAP system (Phadia, Sweden). ImmunoCAP specific IgE test is designed as a sandwich immunoassay. The antigens used in this method were extracted from the European antigens. In this technique the allergen, covalently coupled to the solid phase, reacts with the specific IgE in the patient sample. After washing away non-specific IgE, enzyme-labelled antibodies against IgE are added to form a complex. After an incubation period, unbound enzyme-labelled anti-IgE is washed away and the bound complex is then incubated with a developing agent. After stopping the reaction, the fluorescence of the eluate is measured. The higher the fluorescence, the more specific IgE is present in the sample. The SSIgE $\geq$0.35 kU_A/L was considered to be positive.

Statistical Analysis

The end-point of the study was to compare the performance of SSIgE by ImmunoCAP SPT as the gold standard. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated for comparison with the SPT. The Z test for proportions was used to compare the yield of the two tests. One-way analysis of variance (ANOVA) was used to calculate the correlation of SPT grade with SSIgE levels. The data analysis was done by Statistical Package for the Social Sciences (SPSS; 14 software) (Chicago, Illinois, USA).

Results

Twenty-two patients were screened for inclusion into the study and 20 met the inclusion criteria. Two were excluded since they had negative SPT against all 3 tested aero-allergens. Their mean age was 28.5 years (range 15-50 years); there were 16 males. Six had allergic rhinitis, 3 had bronchial asthma, and 13 had both allergic rhinitis and bronchial asthma. The mean duration of the disease was 14 years (range 1-30 years).

Table 1 shows the proportion of positive SPT and SSIgE results in the study patients. The overall SSIgE response was positive in a higher number of patients when compared to SPT. The differences between SSIgE and SPT testing result rates were not statistically significant (Table 1).

Table 1. Proportion of positive SPT and SSIgE results

Allergen	SPT No. (%)	SSIgE No. (%)	p-value
Cockroach (n=20)	14 (70)	15 (75)	1
Mosquito (n=20)	18 (90)	18 (90)	1
Housefly (n=20)	12 (60)	16 (80)	0.30

Definitions of abbreviations: SPT=Skin prick testing; SSIgE=Serum-specific immunoglobulin E

The sensitivity of SSIgE for all 3 aero-allergens (cockroach, housefly and mosquito) was more than 85% (Table 2). Although the PPV of cockroach and mosquito was more than 85%, housefly had a lower PPV of 68.7%. The specificity of SSIgE for all three aero-allergens was at an average between 37% to 67%, whereas NPV ranged between 50% to 80% (Table 2).

Table 2. Performance characteristics of SSIgE for 3 aero-allergens considering SPT as 'gold standard'

Allergen	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Cockroach (n=20)	92.8	66.6	86	80
Mosquito (n=20)	85	50	94	50
Housefly (n=20)	91.6	37	68.7	75

Definitions of abbreviations: SSIgE=Serum-specific immunoglobulin E; SPT=Skin prick testing; PPV=Positive predictive value; NPV=Negative predictive value

Concordant and discordant test results are shown in table 3. For cockroach allergen, positive SPT and SSIgE results were concordant in 92.8% of patients and negative results were concordant in 66.6% of patients. Concordance of positive and negative mosquito testing

was found in 94.4% and 50% of patients, respectively. Testing for housefly sensitivity with SPT and SSIgE provided concordant positive results in 91.7% of patients but concordant negative results in only 37.5% of patients.

Table 3. Concordant and discordant SPT and SSIgE results

SPT and ImmunoCAP Comparison	Cockroach (n=20)	Mosquito (n=20)	Housefly (n=20)
Concordant Results (Both + or both –)	17 / 20 (85%)	18 / 20 (90%)	11 / 20 (55%)
SPT+ among those with +SSIgE	13 / 15 (86.6%)	17 / 18 (94.4%)	11 / 16 (68.7%)
SPT– among those with +SSIgE	2 / 15 (13%)	1 / 18 (5.6%)	5 / 16 (31.2%)
SPT+ among those with –SSIgE	1 / 5 (20%)	1 / 2 (50%)	1 / 4 (25%)
SPT– among those with –SSIgE	4 / 5 (80%)	1 / 2 (50%)	3 / 4 (75%)
+SSIgE among those with +SPT	13 / 14 (92.8%)	17 / 18 (94.4%)	11 / 12 (91.7%)
–SSIgE among those with +SPT	1 / 14 (7.1%)	1 / 18 (5.6%)	1 / 12 (8.3%)
+SSIgE among those with –SPT	2 / 6 (33.3%)	1 / 2 (50%)	5 / 8 (62.5%)
–SSIgE among those with –SPT	4 / 6 (66.6%)	1 / 2 (50%)	3 / 8 (37.5%)

Definitions of abbreviations: SPT=Skin prick testing; SSIgE=Serum-specific immunoglobulin E; +=Positive; –=Negative

We also evaluated the correlation, between grade of SPT positivity and levels of serum specific IgE. In patients of proven cockroach allergy both by SPT and SSIgE, one-way ANOVA showed statistically significant positive correlation between the levels of IgE and SPT grading (p=0.003). A similar significant relationship was found for mosquito allergen (p=0.045) and housefly allergen (p=0.040) (Figure).

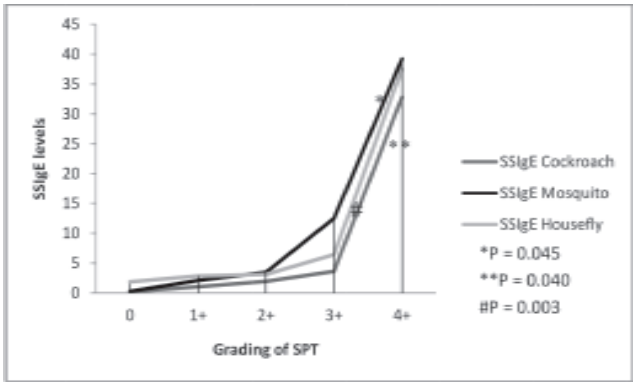

Figure. Correlation of skin prick testing (SPT) grades and serum-specific immunoglobulin E (SSIgE) values.

Discussion

The diagnosis of allergy is made on the basis of positive correlation between clinical history of symptoms on exposure to allergens and positive allergy tests to clinically relevant allergens (*in vivo and/or in vitro*).[9] In the present study of patients with allergy driven asthma and rhinitis, comparison of SPT and SSIgE results for 3 common aero-allergens in 20 patients was evaluated.

Cockroach allergens are frequently found in the kitchen and also in dust collecting on the surface of mattresses, bed linen and carpet.[10,11] The perennial nature of exposure to this allergen makes it more difficult to establish a temporal relationship of exposure with symptom exacerbation, thus, increasing the reliance on ancillary testing to make a diagnosis of cockroach allergy.[12] In our study, cockroach-SSIgE elevation was found at a slightly higher rate as compared to rate of positive SPT against cockroach (75% *vs* 70%, respectively). Similar discordance in rates of cockroach sensitivity detected by SPT and SSIgE were found in another study[13] whereby SPT against allergens from two cockroach species (*B. germanica and P. americana*) were positive in 27.6% asthmatic children whereas cockroach-specific IgE at concentration >0.35 kU_A/L were found in 42.1% of children with bronchial asthma. In a similar study,[14] SPT was positive in 30% while SSIgE was positive in 76% of patients with bronchial asthma exposed to cockroach allergens. The present study also showed higher sensitivity and specificity rate for SSIgE in detecting sensitisation to cockroach allergen in bronchial asthma patients as has been reported in the published literature.

In addition to cockroach allergen, we focussed our study on detection of mosquito sensitivity since emanations and detritus of mosquitoes released in the environment are a source of potent inhalant allergens causing IgE-mediated allergic respiratory disorders.[15,16] In a prior study of 200 patients[17] diagnosed with bronchial asthma, allergic rhinitis or both, 35% (n=70) had elicited a positive SPT reaction against mosquito extract and 18.5% (n = 37) demonstrated a markedly positive response (i.e., SPT grade of ≥2+). ImmunoCAP testing was performed to estimate allergen-speciûc IgE in patients' sera. Elevation of SSIgE against mosquito was detected in 52.9% of patients with positive SPT (1+ to 4+) and elevated in 78.4% of patients with markedly positive SPT (2+ to 4+). Only 1 patient with negative SPT response had a positive ImmunoCAP result. In the present study, among the 3 aero-allergens studied, mosquito tested positive most frequently. The sensitivity and PPV for the SSIgE was ≥85%, when

compared with SPT as the gold standard and is similar to as previously reported study.[17]

Other than cockroaches and dust mites, inhalant allergy to insects was described initially during the 1950s. In later years, the trend of increasing sensitisation rates for many allergens has been observed. In a study,[18] 60 out of 200 patients tested positive to at least one of five insects (caddis fly, mayfly, moth, carpenter ant, and housefly). Of the 60 patients with insect sensitivity, 36 subjects had positive SPT reactions to housefly. The sensitivity of SPT was 18% and the IgE-mediated sensitisation rate was about 30%. In our study, SPT had sensitivity of 60%, while SSIgE had higher sensitivity rate 80% in detecting allergen sensitisation to housefly antigen. The current study concluded that SSIgE has higher detection rate for sensitisation to housefly antigen.

We found that more patients had positive allergen sensitivity identified by SSIgE testing as compared to the SPT results for cockroach, mosquito, and housefly. A plausible explanation of more frequent allergen sensitivity identified by *in vitro* studies has been postulated.[19] It has been hypothesisssed that the discrepancies between negative SPT and positive SSIgE detection can be associated with the presence of cross-reactive carbohydrate determinant (CCD) SSIgE. This CCD-SSIgE seems to be incapable of triggering an allergic reaction *in vivo*, thus, causing a negative skin test response. Therefore, detection of SSIgE antibodies in these cases represents a cross reaction to an allergen and not necessarily clinically significant specific allergic sensitivity.

There are several other plausible factors contributing to the noted differences in SPT and SSIgE results for same allergen. First, the antigenic material used for SPT and SSIgE testing were from different sources. Non-standardised extracts may have variable potency due to contamination with other proteins, allergens and enzymes, thus leading to more variable results.[20] Other factors which influence the results of SPT includes the skills of the investigator, technique of puncture, amount of allergen injected, accuracy of interpretation and the possibility of cross-reactions amongst the various allergens. On the other hand, errors in determination of SSIgE may result from type and amount of allergen, destruction of epitope during binding on solid phase media, poor IgE binding, and increased levels of total IgE leading to false positive results due to non-specific binding.[21]

The advantages of skin testing over SSIgE are its specificity in screening patients for the presence of IgE antibodies and its cost-effectiveness. But sometimes SSIgE is preferred over skin testing especially in patients with extensive skin lesions and for those who are unable to discontinue use of antihistamine therapy. The SSIgE is a quantitative test while SPT is not. The SSIgE has its disadvantages over SPT, due to higher potential for both false-positive and false-negative results. We found that the precise sensitivity of serum speciûc IgE immunoassays compared with skin prick/puncture tests is approximately 70% to 75%.[19] In fact, our study showed that SSIgE has higher sensitivity when compared with SPT for all the three aero-allergens. The tests were done using antigen extract from two different regions which differ vastly in flora and fauna; SPT used the extracts prepared from antigen in India, whereas SSIgE had extracts from European antigens. The analysis showed sensitivity and PPV rates $\geq$85% when compared with SPT in detection of allergen sensitisation against cockroach and mosquito allergens. Hence, we hypothesise that SSIgE may be used as a marker for atopy in Indian patients, wherever indicated, despite the SSIgE antigen extract not being prepared in India.

Conclusions

We found that SSIgE has a higher sensitivity and PPV when compared to SPT which is in direct contrast to prior studies from medical literature. Unlike pollen extracts, insect antigen extracts from different geographic locations seem to give comparable results, and hence, SSIgE can be effectively used in the evaluation of these types of allergic sensitivities. Admittedly, the test lacks specificity and its higher sensitivity leads to increased identification of false-positive cases. Thus, SPT being more specific continues to be the 'gold standard' in allergy testing. However, the present study was conducted in a small number of patients and further adequately powered large scale studies are required to draw a definite conclusion.

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