ORIGINAL ARTICLE

Comparison of Serum Specific IgE with Skin Prick Test in the Diagnosis of Allergy in Malaysia

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SUMMARY

We compared a newer serum specific IgE (SSIgE) test with skin prick testing (SPT) in the diagnosis of allergy in Malaysia. Ninety newly diagnosed allergic patients were enrolled for both tests. Using SPT as a clinical gold standard, the sensitivity, specificity, positive, and negative predictive values (PPV, NPV) were calculated for SSIgE for each of the common allergens tested. The highest positive results for both SPT and SSIgE were for house dust mite and cat. Compared to SPT, SSIgE showed better sensitivity but poorer specificity, low PPV and good NPV in all the allergens tested. Significant positive correlation was seen between the diameter of wheal and flare of SPT and the SSIgE results.

KEY WORDS:
Comparison, Skin prick test, Serum specific IgE, Allergy, Malaysia

INTRODUCTION

Skin prick test (SPT) is the most widely used diagnostic test in allergy. The test is simple, quick and is regarded as the gold standard method for allergy testing. However, anaphylaxis is a potential complication and the emergency resuscitation equipments should always be available at the test vicinity. Serum specific IgE (SSIgE) has now emerged as an alternative test and is gaining popularity in the field of allergy diagnosis as it offers fewer complications and more objective results.

Prior studies comparing these diagnostic modalities indicated that SPT is more sensitive than SSIgE [1-3]. These studies used different in vitro technologies with varying results and were done in Western population. However, the clinical characteristics of allergic diseases differ by genetic and geographical milieu, and the study from Asian population is scarce. We found no previous study in Malaysia specifically comparing the SPT and SSIgE in allergy testing. However, prior study on SPT has shown that the overall clinical profiles of our allergy patients are comparable to the ones from other high temperature/humidity countries in terms of the allergen types, sensitivity and specificity [4].

The knowledge of the correlation between these two diagnostic tests would be important in the scenario where the patient's history is unclear and SPT is equivocal or contraindicated. SSIgE should be considered as an alternative test, particularly before making immunotherapy recommendations. In this scenario, the data on the extent of agreement or disagreement between the two tests would be vital before starting treatment.

In this study, we compared the sensitivity and specificity of SSIgE versus the gold standard SPT in the diagnosis of allergic diseases in our Asian community. Additionally, we would also determine the correlation between the diameter of the wheal and flare of SPT and the patient's total serum IgE level.

MATERIALS AND METHODS

The study was conducted over one year duration. The source population was new patients referred to an allergy clinic in a tertiary referral hospital in North-East Malaysia. All patients aged more than 18 years old with informed consent were included in the study. The patient's detailed history and examination were carried out, and another appointment was given for the SPT and SSIgE tests two weeks later. Those patients who are on antihistamines were asked to stop treatment at least one week prior to the SPT and SSIgE tests. Patients with negative SPT to all the allergens tested were excluded from the study.

Both tests were done by the same investigator. Eight common allergens in our community were used for the skin prick test namely Dermatophagoides pteronyssinus (house dust mite), Felis domesticus (domestic cat), Mucor mucedo(fungi), wheat flour, peanut, egg yolk, egg white and chicken meat. Numbered strip (1.5 cm apart) were applied onto the forearm area. Drops of selected allergen were then placed on the forearm skin next to the numbered strip. A sterile lancet (ALK–Abello skin prick test kit, Bege Alle, 2970 Horsholm, Denmark) was used to prick the skin gently through the drops of allergen. Excess allergen was then wiped off. Histamine (as positive control) and diluents (as negative control) were included for control testing. After 20 minutes, the forearm skin was examined. The presence of a wheal and flare of at least 3 mm and 10 mm larger than the negative control were regarded as a positive reaction.

The same patient was earlier underwent SSIgE test. The test was done using the CLA Allergen Specific IgE Assay (Hitachi Chemical Diagnostics Inc, Japan). The machine permits

This article was accepted: 10 July 2011
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simultaneous determination of the patient's IgE level to a multiple numbers of specific allergens. Within the machine, a small plastic device known as a Test Chamber (TC) exposes patient's serum simultaneously to a number of allergens or allergen mixed. The TC contains discrete segments of cellulose thread, each with an allergen mixed covalently to it. Each TC also contains one negative blanking control and one positive procedural control.

Again, the test was done by the same investigator and followed strict test protocols. A venous blood sample (7 ml) was collected into a 10 ml red top plain tube. The blood was allowed to clot in the tube for 1 hour at room temperature. The clotted blood is centrifuged for 10 minutes at 2500 rpm. The machine is run by filling the TC with patient serum. Serum filled TC was then placed upright in a workstation rack and incubated at room temperature for 16 to 24 hours. IgE in the serum binds to the allergen-coated cellulose threads during the incubation period. The TC is then washed with buffer solutions to remove unbound serum components. Enzyme labeled anti IgE was then added to the chamber and coupled with the serum IgE bound to the threads. After a second wash, the TC is filled with a photo reagent mixture that reacts with the labeled antibody to produce chemiluminescence. The amount of light emitted by each thread is directly proportional to the amount of allergen specific IgE in the patient serum. The Luminometer in this machine measures the amount of light emitted in the TC in luminescence unit (LUs). To calculate the patient's IgE response, the instrument automatically subtracts the emission level of each specific IgE thread. CLA Class values are assigned from 0 to 4 based on the amount of light emitted by the individual thread in the TC. These values made up the CLA Class Allergy Scoring System of the CLA Allergen Specific Assay (Table I). In this study CLA class 1 and above is considered a positive allergy reaction.

Sensitivity is defined as the proportion of diseased patients who were reported as positive by the test. Specificity is defined as the proportion of non-diseased patients who were reported as negative by the test. The data obtained in this study were analysed using SPSS 13 software (Chicago, Illinois). The chi-square test or Pearson correlation was used whenever appropriate to evaluate statistical significance, where p value of less than 0.05 was considered significant.

RESULTS

A hundred and fourty-nine patients were enrolled in this study, with ninety patients completed them successfully. Fifty-five patients are females (61.1%) and 35 (38.9%) are males. The age group ranges from 18 to 66 year-old with the mean age of 32.58 years. Majority of the study population is Malay, which account for 76.7%, followed by Chinese at 15.6% and Indian at 1.1%. More than half of the cases (54.4%) had positive family history of atopy. In terms of occupation, majority of the study subjects were students (27.8%) followed by housewives (24.4%), professional workers (13.4%), teachers (10%), retiree (5.6%) and the rest were either odd job workers or unemployed. Majority (67%) of the patients were living in a city area with population of over 300,000. Clinically, majority of patients came with nasal allergy alone (54%), followed by eye allergy (12%), skin hypersensitivity (10%) and the rest had mixed complaints.

The prevalence of positive SPT and SSIgE is shown in Table II. The overall trend was quite similar in both as the highest prevalence of the positive results was for the house dust mite, followed by cat. The overall percentage of positive responses was higher in SSIgE in all the allergens tested. Table III showed the sensitivity, specificity and positive and negative predictive values (PPV and NPV) of SSIgE when tested with the same allergens as determined by SPT set as the gold standard. The sensitivities of the test for all the aero-allergens (cat, house dust mites and fungi) were excellent (85-100%). In contrast, the sensitivities for the food allergens were poorer (33-85%). Despite high sensitivities for the aeroallergens, the PPV for all of them were poorer (20-70%). Although the overall specificities were at an average between 45-70% for all of the allergens tested, the average NPV was high at 89% and above.

The mean diameter of the wheal and flare in the SPT and the mean result of the SSIgE together with their correlation are shown in Table IV. There were significant correlations between the diameter of wheal and flare of SPT and SSIgE result in all the tested allergens. Good correlation (r = 0.51 to 0.75) were shown for house dust mite, mucor mucedo, egg yolk and egg white while a fair correlation (r = 0.26 to 0.50) were shown for cat, wheat flour, peanut and chicken respectively.

DISCUSSION

The presence of positive clinical history of allergy and positive allergometric tests (*in vivo* and/or *in vitro*) forms the basis of the diagnosis of allergy [5]. While the clinical history may be sufficient to judge the severity and identify the causes of some allergic diseases, sometimes the history is complex and many allergens may be involved. Clinical tests such as direct challenges or avoidance can be applied to identify the etiologic allergen. However, these tests are complex and usually only performed for food allergies.

This study compared SPT and SSIgE results for 8 common allergens in 90 allergy patients in the tropical climate population of Malaysia. Only ninety patients out of a hundred and fourty nine enrolled for the study completed the tests successfully. The main reason for exclusion was a failure to attend the complete tests. Among all patients, the average number of positive results was higher in SSIgE for all the tested allergens. Possible explanation is that the detection of SSIgE antibodies simply establishes that sensitization has occurred, but does not always indicate the presence of allergic diseases [6]. The very high prevalence of positive SSIgE particularly to food allergens in our data is also puzzling, and could point to a fault in the SSIgE assay with non-specific binding and high false positive readings. Other studies in different ethnicities and climates have shown that the average number of positive results was almost similar between the two testing modalities [2,7,8]. Regardless of this, our findings showed that the overall frequency of positive results followed similar trends between both tests as the highest positive results fell for the aero-allergens.

Table I: Interpretation of serum specific IgE result using CLA – 1 Luminometer. Positive test is taken at CLA class 1 and above

CLA class	LU	Allergen specific IgE concentration
4	> 242	Very high
3	143 – 242	high
2	66 – 142	Moderate
1	27 – 65	Low
1/0	12 – 26	Very low
0	0 – 11	Non-detectable

Table II: Prevalence of positive result of SPT and SSIgE among study subject (n=90)

Type of allergen	SPT n (%)	SSIgE n (%)
Cat	21 (23.3)	57 (63.3)
Wheat flour	8 (8.9)	39 (43.3)
House dust mite	57 (63.3)	67 (74.4)
Mucor mucedo	8 (8.9)	41 (45.6)
Peanut	11 (12.2)	42 (46.7)
Egg yolk	7 (7.8)	39 (43.3)
Egg white	7 (7.8)	32 (35.6)
Chicken meat	3 (3.3)	38 (42.2)

SPT: Skin prick test, SSIgE: Serum specific IgE

Table III: Sensitivity and Specificity of SSIgE versus SPT

Type of allergen	Sensitivity (95%CI)	Specificity (95%CI)	PPV(%)	NPV(%)
Cat	90.5	44.9	33.3	93.9
Wheat flour	50.5	57.3	10.3	92.2
House dust mite	86.0	45.5	73.1	65.2
Mucor mucedo	100.0	59.8	19.5	100.0
Peanut	54.5	54.4	14.3	89.6
Egg yolk	85.7	60.2	15.4	98.0
Egg white	85.7	68.7	18.8	98.3
Chicken meat	33.3	57.5	0.26	96.2

PPV: positive predictive values, NPV: negative predictive values, CI: confidence interval

Table IV: Mean diameter (mm) of wheal and flare of SPT and mean result of serum IgE (LU) and their correlation among study subject (n=90)

Type of allergen	SPT Mean(sd)	SSIgE Mean (sd)	r*	p* value
Cat	2.68 (1.71)	68.17 (75.24)	0.396	<0.01
Wheat flour	2.09 (1.32)	40.93 (53.46)	0.325	0.002
House dust mite	5.81 (3.81)	135.27 (113.31)	0.536	<0.01
Mucor mucedo	2.27 (1.49)	46.47 (60.41)	0.528	<0.01
Peanut	1.83 (1.59)	40.24 (48.20)	0.397	<0.01
Egg yolk	1.85 (1.41)	46.37 (57.78)	0.571	<0.01
Egg white	1.70 (1.41)	37.03 (50.26)	0.628	<0.01
Chicken meat	1.74 (1.17)	37.56 (49.26)	0.365	<0.01

* pearson correlation; r = 0 to 0.25 indicates poor or no correlation; r = 0.26 to 0.50 indicates fair correlation; r = 0.51 to 0.75 indicates good correlation and r = 0.71 to 1.0 indicates excellent or perfect correlation

In our study, house dust mites had the highest prevalence of positive results among all studied allergens. The warm and humid tropical climate provides a favourable condition for them to live, thus explains the high prevalence of sensitization towards them. We found the SSIgE for house dust mite had a higher sensitivity and PPV than the specificity and NPV compared with SPT as the clinical gold standard (Table III). Ricci *et al.* also showed similar finding in that the SSIgE test for house dust mite when using UniCAP showed higher sensitivity but lower specificity in comparison to the SPT [9]. Similarly, a study in Thailand comparing SPT and SSIgE against the standard intradermal test in allergic rhinitis patients have also shown that SPT was more specific but SSIgE was more sensitive when tested against house dust mites [10].

Among pets, cat allergen is the most thoroughly described allergen. In this study, the SSIgE test for cat had a sensitivity of 90.5%, with specificity of 44.9%, while PPV is 33.3% and NPV is 93.9% (Table III). Another study in the US showed the similar trend in their result that SSIgE (UniCAP) for cat has a sensitivity of 100%, specificity of 71%, PPV of 25% and NPV of 100% [9]. From here, we can see that the pattern of sensitivity and specificity of SSIgE in subjects with clinical allergies is almost similar regardless of difference ethnicity and climates. Mucor mucedo, another aero-allergen used in this study also yielded similar results of SSIgE, with high sensitivity and low specificity in comparison to SPT (Table III). For cat and fungi, although SSIgE showed high sensitivity to detect the allergens, the PPV results were low which indicate that there is a high possibility of getting false positive results here.

Of food allergens, the SSIgE for egg white and egg yolk showed a similar trend with high sensitivity but low PPV, and lower specificity but high NPV (Table III). For comparison, Ricci et al. study showed that the SSIgE for hen egg (without specifying egg white or egg yolk) when using UniCAP has a sensitivity of 94.0%, specificity of 64.0%, PPV of 55.0% and NPV of 96.0% [9]. Considering the low PPV of most in vitro tests (a positive result would usually require an oral challenge to confirm the diagnosis of food sensitivity), it is quite helpful if we can provide the diagnostic decision point value for food allergens (not done in this study). Sampson *et al.* suggested these point values which he described can be used to eliminate the need for oral challenges by providing cut-off reference values [11]. If the specific IgE level exceeds the diagnostic decision point value, it indicates that the patient is more than 95% likely to experience an adverse reaction to the food. The challenge is to provide universal standard values that can fit all individuals regardless of genetics and environmental differences.

Our results showed a positive correlation between the total levels of serum IgE and the diameter of wheal and flare of the SPT in all the test allergens (Table IV). The bigger the diameter of the wheal and flare of SPT, the higher the levels of serum IgE observed. Chinn *et al.* studied on the relationship between the wheal diameter of SPT and serum IgE levels on 100 field-workers showed that a wheal diameter of 3 mm or more is associated with greater SSIgE levels than a wheal diameter of 2 mm [12]. Another study in Sweden showed the correlation between SPT and IgE levels (CLA antigen) were varies between aero-allergens and food allergens [13]. Different population genetics, environmental factors and test methodology may contribute to the difference in their findings and ours. We found in this study that patients with higher total IgE levels have more positive SSIgE results that outnumbered the positive SPT results. This finding was also observed in another study by Calabria *et al* [2]. Thus, we may suggest that although total IgE levels are not routinely obtained on patients in the in vitro testing, it should necessarily be done in order to interpret the SSIgE results better in cases of positive SSIgE but negative SPT test.

Skin testing has been used in the management of allergy since the 19th century. SPT has proven advantages over SSIgE as it is more convenient, least expensive, and more specific screening method for detecting the presence of IgE antibodies in patients who had appropriate exposure history [14]. However, the drawbacks are that the results depend on the skill of the tester, the amount of allergens injected during the procedure, the reliability of the device, the potency and stability of the test extract, the depth of the puncture and the duration and angle of application devices. Thus, there are instances in which SSIgE is preferred to SPT. These include when testing patients with extensive skin lesions, patients on prolonged antihistamines, patients with severe sensitivity to specific food, especially by skin contact or inhalation and when we need to measure the levels of IgE as SSIgE is quantitative, while SPT is not. The disadvantages of SSIgE in comparison to SPT include delayed results, higher potential false negative and false positive results (negative result does not rule out an allergy as it may be non-IgE-mediated; a positive result does not always indicate a clinical reaction) and the test's reliability can vary from one manufacturer to another.

CONCLUSION

From our results and comparing with findings from the literatures, we conclude that SPT being more specific and good sensitivity is still a better test to diagnose allergy compared to SSIgE. Although SSIgE is more sensitive, the potential high false positive results made it a less accurate method in comparison to the SPT. Despite all the drawbacks of SPT and the continuing improvement of the in vitro testing, we still believe that SSIgE will not totally replace the conventional method of skin testing in the near future.

ACKNOWLEDGEMENT

We would like to thank the staffs in the Otolaryngology clinics of HUSM and HTAA for providing the assistance throughout the data collection period. This study did not receive any sponsorship from any pharmaceutical company or grants from any institution.

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