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IMMUNOBLOTTING TECHNIQUE VERSUS SKIN PRICK TEST FOR DETECTION OF ALLERGEN SPECIFIC IMMUNOGLOBULIN E IN ALLERGIC CONJUNCTIVITIS

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ABSTRACT

Introduction: Skin prick test (SPT) is the most sensitive and reliable method of detecting the causative allergens and considered the gold standard method for allergy testing, it is also simple, quick and cheap. However it has an invasive nature requires multiple skin pricks, painful for children and difficult if skin diseases coexist. SPT can be affected by antihistaminics and corticosteroids. Hence, an immunoblotting technique as an alternative test for IgE determination has been developed, which is lesser invasive. **Aim:** To evaluate the immunoblot test by comparing it to SPT in diagnosis of allergic conjunctivitis. **Patients and Methods:** Immunoblot test was done for 32 patients clinically diagnosed as allergic conjunctivitis, either alone or associated with allergic rhinitis and / or bronchial asthma and who gave positive SPT with one or more of 11 natural allergenic extracts. **Results:** Overall diagnostic performance of immunoblot test in comparison to SPT for detection of all studied aeroallergens showed 27.5% sensitivity, 98.0% specificity, 97.0% positive predictive value, 33.2% negative predictive value and 46.2% diagnostic accuracy. **Conclusion:** Since immunoblotting technique has low sensitivity and high specificity, hence it can be used as confirmatory test secondary to SPT for diagnosis of causative allergens.

Keywords: Allergic diseases, IgE, Skin prick test, Immunoblott.

INTRODUCTION

Allergic conjunctivitis is often underdiagnosed and consequently undertreated except when it is severe and the chief complaint of a consultation in a specialty clinic. It is common for ocular symptoms to be associated with allergic nasal symptoms, rhinoconjunctivitis. Previous population studies estimated prevalence of 15-20% of allergic conjunctivitis, but more recent studies implicate rates as high as 40%. Ocular allergies rank secondary and at times may overcome the primary complaints of nasal congestion in rhinoconjunctivitis patients which contribute to the burden of allergic rhinitis and lower quality of life. However, ocular symptoms are less well studied as an independent entity and much of the clinical information is commonly buried within the rhinoconjunctivitis literature[1,2]. However the prevalence of asthma and allergic rhinoconjunctivitis symptoms in Cairo, Egypt in 2006 was 15.3%.[3] Skin prick testing (SPT) is recommended as the primary method for the diagnosis of IgE mediated allergies in most allergic diseases and considered the gold standard method for allergy testing. It is the most widely used diagnostic test in allergy as it has advantages of high sensitivity and specificity, rapid results, flexibility, low cost, good tolerability, and clear demonstration to patients of their allergies. However it is subject to some operator, observer and interpretation variability. SPT must be performed by trained and experienced medical and paramedical staff, in centers with appropriate facilities to treat systemic allergic reactions and anaphylaxis[4,5]. To overcome limitations of SPT, in vitro tools enzyme immunoassay have been developed to identify serum IgE and allow the identification of a wide spectrum of sensitizing allergens. It is straightforward, fast and has been widely used in outpatient clinics in Korea[6]. Enzyme immunoassay can help in proper diagnosis and determination of the specific causative allergen through an easy way[7]. The aim of this study is to evaluate immunoblot test using skin prick test as the standard in detection of causative aeroallergens among allergic conjunctivitis patients.

PATIENTS AND METHODS

The study recruited 32 patients who was referred to the Allergy Lab in the Research Institute of Ophthalmology. All patients were clinically diagnosed as allergic conjunctivitis, either alone or associated with allergic rhinitis and / or bronchial asthma and who gave positive SPT for one or more allergen(s). Blood samples were collected from them, left to clot for 30-40 minutes, centrifuged at 2,000g for 10 minutes and stored at -20°C for immunoblotting assay. All patients gave their written consent after being fully informed about the purpose and nature of the study. The study was approved by the Ethics Committee of the Institute. Eleven natural allergenic extracts were used for SPT. Except for mite, mould and cockroach which were purchased from Ain Shams University, 8 allergens had been prepared in our Allergy Lab of RIO according to Haggag[8] as follows: 1. Preparation of the extracting fluid which was coca solution as published by Ortolani and co-authors[9] by dissolving 20 gm sodium chloride and 11 gm sodium bicarbonate in one liter distilled water and sterilized. 2. Standardization of the allergenic extract by weight to volume ratio (w/v) method according to Fischer et al[10]. The extract was 1:20 w/v for the prepared allergens; wool, hairs of cat, dog, cow and goat, feathers mixture (duck, goose & chicken), pollens of Bermuda grass (El-Negeel) and pollens of olive tree. Afterwards, they were thoroughly mixed by

shaking in electric shaker for more than two hours for two successive days. 3. Primary filtration by the usual filter paper and secondary filtration by Millipore Syringe filter (Ministart) 0.2 µm into sterilized sealed glass bottles (vaccine bottle) were performed. Checking the sterility of the allergenic extract was done by culturing on blood agar aerobically and anaerobically, by then it was ready to be used for SPT, **(Figure 1 and Figure 2).**

Figure 1. Preparation of the natural allergenic extracts from cru materials in the Allergy Lab, Microbiology & Immunology Unite, RIO

Figure 2. Secondary filtration of the allergenic extract using Millipore Syringe Filter.

SPT was performed for each patient using the 11 allergenic extracts with histamine as positive control and coca solution as negative one. Prior to SPT, patients were asked to avoid first-generation short-acting antihistamines for at least 2-3 days and second-generation antihistamines for 5-7 days, while for corticosteroids 2 weeks at least. The skin of the volar side of both forearms was cleaned with alcohol and allowed to dry. Test sites were marked as circles and labeled at least 2 cm apart to avoid the overlapping of positive skin reactions. A drop of each allergenic extract was aseptically placed onto its specific labeled circle and a sterile blood lancet needle was inserted through the drop into the superficial skin and withdrawn with slight lifting of the skin. The allergen was wiped off after one minute. Test result was read after 15-30 minutes as the largest diameter measured in millimeter with transparent ruler. Reactions with wheal diameters of 3mm or more are considered to be positive[11]. In clinical practice, it may be preferable to express SPT results as the diameters of the wheals without adjusting them by histamine reaction,[12] **(Figure 3).** Measurement of specific IgE by immunoblot technique was performed for patients with positive skin test using EUROLINE inhalation "Turkey 1" IgE test kit, which contains one positive control and the following allergens:

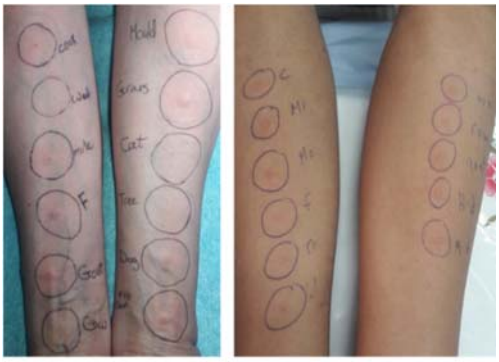


Figure 3. Skin-prick test

House dust mite, (Dermatophagoides pteronyssinus and Dermatophagoides farina), mold (Penicillum notatum, cladosporium herparum, alternaria alternate and aspergillus

fumigates), grass mix, grain pollen mix, cultivated rye, tree mix, mulberry tree, flower mix and weed mix, olive tree, cockroach (German), animal hair (dog, cat, goat, horse, hamster, cow and sheep wool), feather mix (chicken, duck, and goose), cage bird mix and exotic mould. For facilitating interpretation of results some allergens in the panel of the same nature would be collected together, e.g. grass mix early and grass mix late. These were represented as one group of grass mix, the same was done for tree mix (tree mix early and tree mix late), weed mix (weed mix 1 and weed mix 3), mould mix (Penicillum notatum, Cladosporium herparum, Aspergillus fumigates and Alternaria alternate) and mite mix (Dermatophagoides farina and Dermatophagoides pteronyssinus). Test strips are first moistened and then incubated in the first reaction step with patient serum. If samples are positive, specific antibodies of class IgE will bind to the allergens. To detect the bound antibodies, a second incubation is carried out using an enzyme-labelled monoclonal anti-human IgE (enzyme conjugate) catalyzing a color reaction. The color intensity on the test field is directly proportional to the amount of specific IgE in the serum of the patient for the allergens. For quantification, the instructions in the EURO Line Scan user manual were followed. When using the digital evaluation system 'EURO Line Scan', the intensity of the bands was calculated in EAST (Enzyme-Allergo-Sorbent Test) classes of 0-6 with respect to the concentration grades identical to the well-known RAST system (Radio-Allergo-Sorbent Test) used in allergy diagnostics, **(Figure 4 and Figure 5)**. Descriptive and analytical statistics were performed on IBM-compatible computer using SPSS 21 software package for windows 7 operating system. Continuous data were presented in the form of mean ± SD. Categorical data were presented in the form of number and percentage. Associations between studied parameters were performed using Chi square. Power of significance (probability): $P > 0.05$: non-significant, $P < 0.05$: significant, $P < 0.01$: highly significant. Diagnostic performance of immunoblot test [Calculation of sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and diagnostic accuracy (DA)] was compared to skin test as 'gold standard test'. Sensitivity = TP / (TP + FN). Specificity = TN / (TN + FP). Positive predictive value (PPV) = TP / (TP + FP). Negative predictive value (NPV) = TN / (TN + FN). Diagnostic accuracy (DA) = (TP + TN)/Total. TP: True positive = positive results in both tests. TN: True negative = negative results in both tests. FN: False negative = negative results in immunoblot test but positive in SPT. FP: False positive = positive results in immunoblot test but negative in SPT. PPV: Positive predictive value. NPV: Negative predictive value. P value is significant at 0.05. Kappa value reflects measure of agreement between results of SPT and immunoblotting test. The value of kappa index was interpreted according to the following scale: <0: poor agreement, 0 – 0.2: slight agreement, 0.21–0.40: fair agreement, 0.41–0.60: moderate agreement, 0.61–0.80: substantial agreement, and 0.81–1.00: perfect agreement.

Figure 4. Test strips coated with allergens in the incubation tray.

Figure 5. Test strips placed on the evaluation protocol ready for digital evaluation.

RESULTS

The study included 32 patients, 17 males and 15 females, their age ranged between 8 and 65 years. Eight patients (25%) presented with allergic conjunctivitis alone, while 10 (31.3%) had associated allergic rhinitis (rhinoconjunctivitis) and 7 (21.9%) presented with allergy triad meaning rhinoconjunctivitis associated with asthma, **(Table 1).** All patients in our study showed polysensitization pattern to at least 6 allergens. The allergens that were most frequently positive in SPT were house dust mite, mould and cockroach (96.9%, n = 31), followed by pollens of grass (87.5%, n = 28). On the other hand, the most prevalent allergens using immunoblot test were mould (37.5%, n = 12) followed by pollens of grass (31.3%, n=10), **(Table 2)** and **(Figure 6)**. According to the data shown in **(Table 3)** and **(Table 4),** the overall diagnostic performance of immunoblot test in comparison to SPT in detection of all studied inhalant allergens showed 27.5% sensitivity, 98% specificity, 97% PPV, 33.2% NPV and 46.2% DA. They were calculated according to equations (kappa statistics) mentioned earlier.

DISCUSSION

In the present study, 20 patients (62.5%) had positive family history. Our results more or less agree with two studies, one was done on allergic conjunctivitis patients and revealed that 66% had positive family history of atopy[13] and another one on allergic rhinitis in which 64.4% of patients had positive family history of allergic disease[14]. Our study revealed slight predominance of male patients (53.1%) which is lower than that reported by Nagrale and coworkers[15] (82.5%). While female preponderance was reported by Kosrirukvongs et al [13] and also by Malu[16] as 51.2% females among patients with allergic conjunctivitis. Regarding SPT pattern of sensitization, in the present study all allergic patients showed polysensitization pattern for at least 6 allergens. Multiple sensitizations to aeroallergens was a common finding in three previous studies[8,17,18]. Bogomolov[19] reported that 86.7% of allergic rhinitis patients had multiple sensitizations to aeroallergens, while Nyembue and co-authors[11] reported lower percentage of polysensitization (40.9%). Regarding number of allergic patients detected by immunoblot test in the present study 29 patients (90.6%) were found to have one or more positive responses to aeroallergens in the panel, while the remaining 3(9.4%) showed no response to any allergens. Rasheed and coworkers[20] reported that out of 128 patients with respiratory allergies, 80 (62%) had positive serum specific Ig (ssIgE) for one or more than one of the tested inhalant allergens; 36 (45%) were sensitive to one allergen and the rest 44 (55%) were sensitive to two or more inhalant allergens using EUROLINE Mediterranean inhalation specific IgE kits which examined 20 inhalant allergens simultaneously by immunoblotting method. While Kim et al[21] reported lower percentage as 54.4% of allergic patients

tested positive for one or more allergens in MAST-immunoblot assay. In our study, the most frequently positive aeroallergens according to SPT results were mites, molds and cockroach as 96.9% followed by grass 87.5%, then animal hairs. In Congo, a study made by Nyembue and his coworkers[11] using SPT showed that mites 68.5% and cockroach 36.2% were the most common allergens among sensitized patients. In Egypt 2016, another study was made by El Shabrawy [22] reported that mould allergy was 43% among allergic patients. While in Morocco 2017, a study made by Serhane and coworkers[23], found that skin sensitization was most commonly associated with cockroach which corresponded to 44.8% of allergic cases, grass sensitization was 27.6% and mite was 24.1%. Their findings were almost the same like ours but with much lower percentages. Caraballo and co-authors[24] explained that allergies are frequent in the Tropics with particularities which are related to climate conditions that favor permanent exposure to mite allergens, helminthic infections and stinging insects. In the present study immunoblot test results showed that the most frequently positive aeroallergens were mould mix (37.5%), followed by pollens of Bermuda grass (31.3%), then animal hairs and cockroaches. Rasheed et al[20] found that Bermuda grass pollens represented the commonest offender allergen followed by animal dander and mite, while mould came last, using the immunoblotting method (EUROLINE). This study revealed that overall diagnostic performance as regard sensitivity, specificity, PPV, NPV and DA of immunoblot test when comparing it with SPT were 27.5%, 98.0%, 97.0%, 33.2% and 46.2% respectively. These results are more or less in concordance with Park et al[25] who reported that overall sensitivity and specificity of immunoblot in comparison to SPT for detection of specific IgE against inhalant allergens were 38.4% and 93.9% respectively. While Hamid and co-authors[7] reported that overall diagnostic performance of immunoblot test in comparison to SPT in detection of aeroallergens showed 63.6% sensitivity, 100% specificity, 100% PPV, 91.4% NPV and 92.5% DA. Also Kim et al[21] reported that sensitivity of MAST-immunoblot test over SPT was 63.16%, specificity was 65.57% and efficacy was 63.92%. O'Brien and Head[26] explained that the higher sensitivity of SPT compared to immunoblot test may be due to the presence of the majority of specific IgE in the body bound to mast cells, or other cells bearing high-affinity IgE receptors, which are abundant under the skin with little amount present in the circulation. Additionally, the test allergens fixed onto the immunoblotting strips are mostly proteins which render them more liable for external physical and chemical effects.

CONCLUSION

SPT remains the gold standard and the primary test for diagnosis of the offending causative allergens because of its high sensitivity and good reproducibility. Immunoblot test cannot replace SPT as it lacks sensitivity but since it has very high specificity it can be used as confirmatory secondary test to SPT in determining the specific IgE responsible for allergic disease.

Table 1. Demographic and clinical characteristics of the 32 allergic patients.

Variables		No.	%
Age Range: Mean ±SD	(8-65) years (34.5±16)		
Sex	Male	17	53.1%
	Female	15	46.9%
No. of patients with allergic conjunctivitis		8	25.0%
No. of patients with allergic conjunctivitis +Bronchitis		7	21.9%
No. of patients with allergic rhinoconjunctivitis		10	31.3%
No. of patients with allergic rhinoconjunctivitis +Bronchitis		7	21.9%
Duration of disease (years)	10 (1-40)		
Family history	Positive	20	62.5%
	Negative	12	37.5%

Table (2): Comparison between SPT and immunoblot test as regard number of positive cases in each of them.

Allergens	Skin prick test (No. of positive patients)		Immunoblot test (No. of positive patients)	
	No.	%	No.	%
Cat	20	62.5%	3	9.4%
Dog	24	75.0%	4	12.5%
Sheep	22	68.8%	6	18.8%
Goat	24	75.0%	6	18.8%
Cow	15	46.9%	9	28.1%
Olive	5	16.1%	3	9.4%
Feather	23	71.9%	1	3.1%
Grass	28	87.5%	10	31.3%
Cockroach	31	96.9%	7	21.9%
Mould	31	96.9%	12	37.5%
Mite	31	96.9%	8	25%

Table 3. Statistical analysis showing sensitivity & specificity of immunoblot test using results of SPT as the standard.

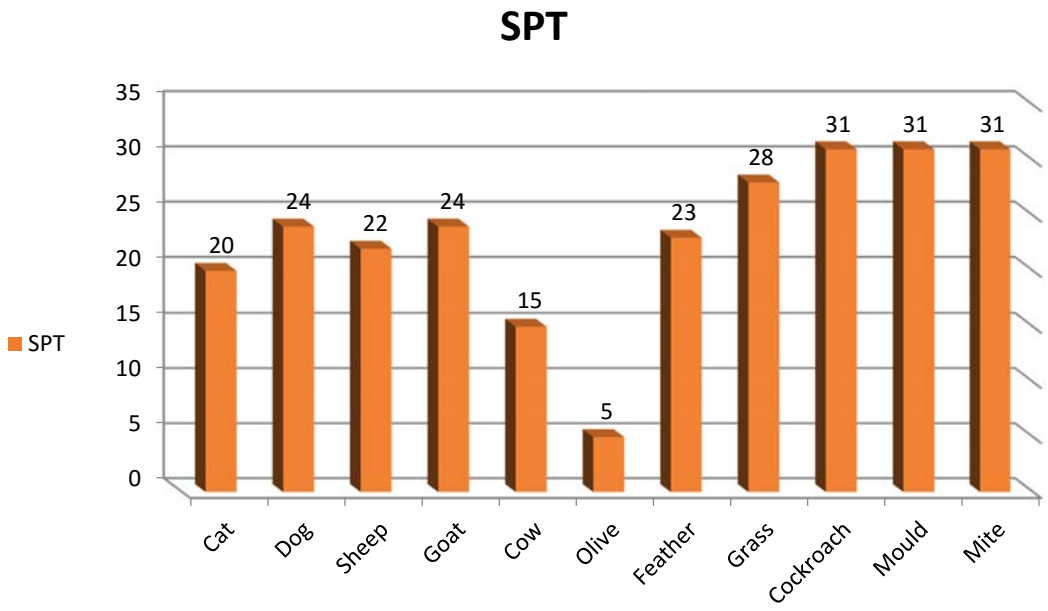
Immunoblot Test	TP	TN	FN	FP	Sensitivity	Specificity	PPV	NPV	P value	Kappa
Cat	3	12	17	0	15.0%	100%	100%	41.4%	0.27	0.12
Dog	4	8	20	0	16.7%	100%	100%	28.6%	0.5	0.09
Sheep	5	9	17	1	22.7%	90.0%	84.6%	34.6%	0.39	0.09
Goat	8	6	18	0	25.0%	100%	100%	30.8%	0.12	0.14
Cow	8	16	7	1	53.3%	94.1%	88.9%	69.6%	0.003	0.49
Olive	3	26	2	0	60.0%	100%	100%	92.9%	0.002	0.72
Feather	1	9	22	0	4.3%	100%	100%	29.0%	0.71	0.025
Grass	10	4	18	0	35.7%	100%	100%	4.0%	0.28	0.12
Cockroach	7	1	24	0	22.5%	100%	100%	7.5%	0.47	0.018
Mould	12	1	19	0	38.7%	100%	100%	5.0%	0.32	0.04
Mite	8	1	23	0	25.8%	100%	100%	4.2%	0.45	0.021

TP: True positive=positive results in both tests. TN: True negative= negative results in both tests. FN: False negative= negative results in immunoblot test but positive in SPT. FP: False positive= positive results in immunoblot test but negative in SPT. PPV: Positive predictive value. NPV: Negative predictive value. P value is significant at 0.05. Kappa value reflects measure of agreement between results of SPT and immunoblotting test. The value of kappa index was interpreted according to the following scale: <0: poor agreement. 0–0.2: slight agreement. 0.21–0.40: fair agreement. 0.41–0.60: moderate agreement. 0.61–0.80: substantial agreement. 0.81–1.00: perfect agreement.

Table 4. Overall diagnostic performance of immunoblot test in comparison to skin prick test.

		Skin Prick Test		
		Positive	Negative	Total
Immunoblot Test	Positive	69	2	71
	Negative	187	93	280
	Total	256	95	351

Figure 6. Comparison between SPT and immunoblot test as regard number of positive cases in each of them.



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