

Immunoblotting technique: a new accurate in vitro test for detection of allergen-specific IgE in allergic rhinitis

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Abstract Allergic rhinitis is the most common manifestation of an atopic reaction to inhaled allergen. It accounts for 80% of rhinitis in children and 30% in adults. The objective of the study is to evaluate the presence of serum-specific IgE to a variety of common allergens using a new immunoblotting technique in order to detect the specific allergen causing allergic rhinitis. The study included 32 patients with allergic rhinitis (20 females and 12 males) with age ranging from 15 to 60 years. Measurement of specific IgE by immunoblotting technique was performed to patients with positive skin test. Overall diagnostic performance of immunoblot test in comparison to skin test in detection of all studied allergens showed 56.1% sensitivity, 100.0% specificity, 100.0% positive predictive value, 93.9% negative predictive value and 94.4% diagnostic accuracy. It may help in proper diagnosis and determination of the specific causative allergen through an easy and inexpensive way.

Keywords Allergy · Immunoblotting · IgE · Allergen

Introduction

Allergic rhinitis is the most common manifestation of an atopic reaction to inhaled allergen. It accounts for 80% of rhinitis in children and 30% in adults [12].

Allergic rhinitis is characterized by an inflammatory reaction secondary to an initial immunoglobulin E (IgE)-dependent mast cell sensitization [3]. Cross linking of IgE molecules on mast cell and basophil surface membrane by allergens triggers the release of multiple mediators leading to the development of IgE-mediated immediate and late hypersensitivity reactions [1].

The presence of atopy could be demonstrated through increased levels of total and specific serum IgE, skin test positivity and increased number of peripheral blood eosinophils [7].

As allergy test has a potential for false-positive and false-negative results, it must be interpreted in the context of the patient's clinical history. A thorough history will often reveal whether a patient is atopic or not [2].

The principle advantage of in vivo skin tests is that, they measure the whole biologic response to the allergen. Also it is more sensitive than in vitro tests. However, compliance, practicability, interpretation, hypersensitivity, and the need to stop medication prior to testing are its major drawbacks.

Serum IgE level assessment, using RAST or ELISA, is of limited value due to wide age-dependent normal range, absent clear cut-off levels, expensive, time consuming and have variable sensitivity and specificity [2]. Therefore, a new alternative test for IgE determination had been developed applying immunoblotting technique [7].

This study aims to evaluate the value of immunoblot test in comparison to skin tests.

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Subjects and methods

A total of 32 patients with allergic rhinitis were enrolled in this study. This group included 20 females and 12 males with age ranging between 15 and 60 years.

All patients were diagnosed as allergic rhinitis by history, clinical examination, and skin testing.

Blood samples were collected from patients with positive skin test. It was left to clot (30–40 min), and then centrifuged at (2,000g for 10 min). Serum was kept in the refrigerator at -20°C .

Measurement of specific IgE by immunoblotting technique was performed to patients with positive skin test using the RIDA allergy screen panel 1 TW (Art No. A 2142 TW/A2141TW), which contains one positive control and the following 20 allergens:

- House dust mite I (*Dermatophagoides pteronyssinus*).
- House dust mite II (*Dermatophagoides farinae*).
- House dust.
- Crab, shrimp, shellfish, codfish, egg white, egg yolk, milk, wheat flour, soya bean, peanut (food).
- Cat, dog (epithelial hair).
- *Alternaria alternata*, *Candida albicans* (moulds, fungi).
- Rye pollens, grass pollens (pollens) and cockroach (insect).

Principle of the used test is based on an immunoblot assay. Special allergens are bound to the surface of nitrocellulose membrane lying in a reaction trough. The patients' serum is pipetted into the reaction trough and incubated at room temperature. During this time, the allergen-specific IgE antibodies react with the allergens and bind to the nitrocellulose membrane via the allergen. Non-bound material was removed by washing. After this, an anti-human IgE antibody coupled with biotin was added and incubated at room temperature. This binds to the respective specific IgE in the test fields from the first incubation. Non-bound detector antibodies are removed by washing. Next, streptavidin is added which is conjugated with alkaline phosphatase and incubated at room temperature. This binds to the biotin from the second incubation in the test fields and to the positive control. Non-bound streptavidin conjugate was removed by washing. After adding the substrate and incubating at room temperature, a specific enzymatic color reaction of the alkaline phosphatase takes place, which results in the formation of precipitates on the test strips. 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. Quantification was done by using the RIDA[®]X screen appertaining PC software.

Descriptive and analytical statistics were performed on IBM-compatible computer using SPSS 11.5 software package for windows XP operating system.

Continuous data were presented in the form of mean $\pm$ SD (median, range). Categorical data were presented in the form of number and percentage.

Associations between studied parameters were performed using Fisher exact test (χ^2 value).

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)] in comparison to skin test as a "golden standard test" was done.

- Sensitivity (SEN) = $\text{TP}/(\text{TP} + \text{FN})$.
- Specificity (SPC) = $\text{TN}/(\text{TN} + \text{FP})$.
- Positive predictive value (PPV) = $\text{TP}/(\text{TP} + \text{FP})$.
- Negative predictive value (NPV) = $\text{TN}/(\text{TN} + \text{FN})$.
- Diagnostic accuracy (DA) = $(\text{TP} + \text{TN})/\text{Total}$.

True positive (TP): number of patients that are both skin test positive and immunoblot positive.

True negative (TN): number of patients that are both immunoblot negative and skin test negative.

False positive (FP): number of patients that are immunoblot positive and skin test negative.

False negative (FN): number of patients that are immunoblot negative and skin test positive.

Agreement between results of skin test and immunoblotting was assessed by kappa statistics. 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.

Results

The present study was conducted on 32 patients with allergic rhinitis. The determining symptom in all studied patients was rhinitis (100%) with variable expression of other different manifestations. Rhinorrhea was the second most evidenced manifestation (30 patients), followed by nasal obstruction (28 patients), sneezing (16 patients), nasal itching (12 patients), and nasal congestion (4 patients). Eight patients had bronchial asthma and six patients had both epiphora and urticaria.

All patients showed negative results (by both tests) for the following allergens: *Alternaria alternata*, peanut, soya beans, wheat flour, cat, dog, shrimp, shellfish, and cod fish allergens with 100% specificity.

The overall diagnostic performance of immunoblot test in detection of food allergy showed 25% sensitivity, 100% specificity, 100% positive predictive value, 96.2% negative predictive value, and 96.3% diagnostic accuracy with fair agreement between both methods.

The overall diagnostic performance of immunoblot test in comparison to skin test in the detection of aeroallergens showed 63.6% sensitivity, 100% specificity, 100% positive predictive value, 91.4% negative predictive value, and 92.5% diagnostic accuracy and substantial agreement between both methods. Table 1 shows the results of allergens studied.

The overall diagnostic performance of immunoblot test in comparison to skin test in detection of all studied allergens showed 56.1% sensitivity, 100% specificity, 100% positive predictive value, 93.9% negative predictive value, and 94.4% diagnostic accuracy with substantial agreement between both tests (Table 2).

Discussion

Skin testing had been used in the diagnosis of allergy since the nineteenth century. Skin testing was the only allergy-testing method available until approximately 30 years ago, when technology and innovation created an in vitro test for allergy [15]. Results of skin testing are influenced not only by the level of IgE antibodies and their allergen-binding characteristics, but also by the number of mast cells, mast cells activity in addition to high variability and subjectivity [15].

The interpretation of skin test results depends on the skill of the tester, the amount of allergen injected by the

prick test, the reliability of the device, the potency and stability of the test extract, the depth of the puncture, and the duration and angle of the application device. Despite these inherent difficulties with skin testing, the prick/puncture method is generally considered to be the most convenient, least expensive and most specific screening method for detecting the presence of IgE antibodies in patients who have appropriate exposure histories [13, 14].

Unfortunately, the clinical history has been used almost exclusively as the gold standard key for diagnosis. This practice has led to several aberrations in this field. Allergists often employ intra-dermal skin tests using potent allergen extracts to verify suspicions raised by history. But, it was found that this adds little to the accuracy of the diagnosis [10].

In vitro allergy testing offers another alternative tool for diagnosis [9]. Today's technology has improved in vitro testing so that it became an efficient and accurate tool in allergen identification [11].

Serologic tests for specific IgE (e.g., RAST), formerly criticized for their lack of sensitivity, have been constructed to agree better with the history by lowering the cut-off value for positivity of the assay. This also has the drawback of increasing the incidence of false-positive results [5]. Although these practices might result in better agreement with the clinical history used as the gold standard, this apparent agreement has been misleading [15]. Furthermore, for licensing purposes of the new in vitro products for allergy screen, the FDA demands performance characteristics of tests to be judged using the clinical history as the standard [15].

Crobach et al. [4] concluded that asking patients the questions "Do you think you have allergy?" and "What do you think you are allergic to?" can provide useful information, especially if the response is negative or "Don't know". They recommended that the diagnosis of allergic rhinitis

Table 1 Results of allergens studied

	TP	TN	FN	FP	Sensitivity (%)	Specificity (%)	Kappa	Significance
DF	8	18	6	–	57.1	100	Moderate	–
DP	10	18	4	–	71.4	100	Substantial	HS
HD	8	20	4	–	66.7	100	Substantial	S
Co	4	28	–	–	100	100	Perfect	HS
EW	4	28	–	–	100	100	Perfect	HS
EY	–	28	4	–	–	100	–	–
M	–	30	2	–	–	100	–	–
CA	4	22	6	–	40	100	Moderate	–
GP	4	26	2	–	66	100	Substantial	–
RP	4	26	2	–	66.7	100	Substantial	–
Cr	–	26	6	–	–	100	–	–

DF *Dermatophagoides farinae*, DP *Dermatophagoides pteronyssinus*, HD house dust, Co cockroach, EW egg white, EY egg yolk, M milk, CA *Candida albicans*, GP grass pollen, RP rye pollen, Cr crab, S significant, HS highly significant

Table 2 Overall diagnostic performance of immunoblot test in comparison to skin test

Overall allergen	Skin test	
	Positive	Negative
Immunoblot		
Positive	46	0
Negative	36	558
χ^2 value		168.633
<i>P</i> value		<0.01
Significance		HS
TP		9.1
TN		279
FP		0
FN		18
Sensitivity		56.1%
Specificity		100.0%
PPV		100.0%
NPV		93.9%
DA		94.4%
Kappa Index		0.690

and allergic asthma should be made using both medical history and allergy-testing.

The current study revealed that immunoblotting had good agreement with clinical picture and skin test without compromising the result as could be done with RAST where decreasing the cut-off point is needed to increase the sensitivity, yet also increasing the false positive results [8].

On the other hand, immunoblotting showed 63.6% sensitivity, 100% specificity, 100% positive predictive value, 91.4% negative predictive value, and 92.5% diagnostic accuracy in detection of aeroallergens and substantial agreement between both methods.

Kersten et al. [7] found that sensitivity, specificity, and diagnostic accuracy of immunoblot for aeroallergens (rye pollen and grass pollen) were 98.3, 87.1, and 92.7%, respectively.

Erin [6] had used immunoblotting in studying allergy to natural rubber latex. Those studies had shown that IgE from sera of latex-allergic patients binds heterogeneously to many different proteins ranging from 4 to 200 kDa.

It is noteworthy to mention that very few articles were available on the subject of immunoblotting in nasal allergy using the Medline computer search till August 2008. Therefore, there were very few available data to compare with our results.

As there were no false positive results, specificity was always 100% (i.e. if the test is positive it is positive). So immunoblot test may be considered as a good diagnostic and confirmatory test.

A highly sensitive test is needed when there is a reason to suspect a diagnosis of a treatable condition (such as TB and early carcinoma), when there is a possibility for missing a disease, also could be used for screening and early diagnostic work up to differentiate between possibilities. A sensitive test is helpful, when the probability of the disease is low and the purpose of the test is to discover the disease. In allergy, clinical history is the gold standard tool for achieving a diagnosis. So, there is no need for a highly sensitive test.

Specific tests, on the other hand, are useful to confirm (or ruling out) a diagnosis that has been suggested by clinical data; because a highly specific test is rarely positive in the absence of a disease (few false-positive results). Highly specific tests are needed when false-positive results can harm the patient physically, emotionally or financially.

Therefore immunoblotting, being a specific test, is very suitable for work-up of allergy used for confirming (or ruling out) the diagnosis. RAST or ELISA are more like a general allergen test and need a large number of patients to run the test, otherwise it would be very expensive to use for a single patient.

Immunoblotting is a simple, fast, and inexpensive test to run (i.e., it could be done for a single patient). The immunoblot strip contains many allergens that makes it more comprehensive and specific.

Conclusion

The immunoblot test may be a promising tool that may help in proper diagnosis and determination of the specific causative allergen. Further studies are recommended on a larger number of patients with different forms of allergy to establish its efficacy. This may help in performing new strategies for immunotherapy of allergic patients based on the results of the immunoblotting technique.

Conflict of interest statement Informed consent was obtained from every patient and the Institutional Review Board of Ain-Shams University Hospital approved the study protocol. The researchers themselves supported this work fund. Noteworthy to declare that there is no claims or conflicts of interest.

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