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Comparison between Sensitivity of Autologous Skin Serum Test and Autologous Plasma Skin Test in Patients with Chronic Idiopathic Urticaria for Detection of Antibody against IgE or IgE Receptor (FcεRIα)

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ABSTRACT

Intradermal injection of autologous serum and plasma elicit a cutaneous reactivity in almost 45-60% of patients with Chronic Idiopathic Urticaria (CIU). This reactivity is associated with the presence of auto antibodies against IgE or IgE receptors. This study was carried out to compare the cutaneous reactivity of autologous serum and plasma skin tests in a series of patients with CIU for diagnosis of auto antibodies against IgE or IgE receptor.

Fifty eight patients with CIU were injected intradermally with autologous serum and plasma (anticoagulated by citrate). Histamine was used as positive control and normal saline as negative control. The study group was checked by routine laboratory tests (CBC, U/A etc), allergens with skin prick tests, and serum IgE level, and auto antibodies against thyroid as well. Duration of urticaria was another factor which was assessed.

There was no significant difference between positive ASST and positive APST patients for the above mentioned tests. 77.6% of the patients were Positive for APST and 65.5% were ASST positive. Duration of urticaria was longer in patients with positive ASST and APST than ASST and APST negative patients, although the difference was not statistically significant.

Autologous serum skin test (ASST) and autologous plasma skin test (APST) could be used for estimation of duration and severity of urticaria and planning for the treatment.

Keywords: Autologous Serum Skin Test; Autologous Plasma Skin Test; Chronic Idiopathic Urticaria; FcεRIα; IgE Receptor; Skin Prick Test

INTRODUCTION

Chronic urticaria is a condition which is defined as the daily or almost daily occurrence of wheals for at least 6 weeks. The term Chronic Idiopathic Urticaria (CIU) is used for definition of chronic urticaria cases in which neither signs of vasculitis nor causative drugs, foods, and/or physical factors could be specified. It has been identified from previous studies that chronic idiopathic urticaria (CIU) includes a heterogeneous

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subset (up to 46% of cases)who have a previously unidentified serum factor, which was discovered to be an antibody with an important role in the pathogenesis of CIU.[1] This antibody belongs to the IgG isotype that reacts against the α-chain of the high affinity IgE receptor (FcεRIα) of basophiles and mast cells, or alternatively against IgE itself.[2] Functional properties of these antibodies are evidenced in vivo by the intradermal injection of autologous serum and autologous plasma. This procedure induces a wheal-and-flare response in patients with CIU; further in vitro evidence is the release of histamine from basophils and mast cells elicited by addition serum of CIU patient on these cells.[3,4]

Since it is hypothesized that CIU has an autoimmune etiology, so CIU patients with positive ASST and in vitro assays have been known as Chronic Autoimmune Urticaria (CAIU). These patients frequently present an increased incidence of autoimmune disorders (e.g. thyroiditis) and more severe clinical features of urticaria than patients suffering from mild chronic urticaria.[5]

The literature includes several reports contributing to better understanding the pathogenesis and effector mechanisms of CIU, focusing on the different cellular populations that infiltrate urticarial lesions. A comprehensive review of the molecular immunopathology of CIU indicates early activation of mast cells, followed by a lymphocyte and granulocyte cell-mediated hypersensitivity reaction with a Th0 (or, alternatively, a combination of Th1/Th2- CD4) cytokine profile.[6-8]

Kaplan et al.[9] mentioned that warming sera to 56° c decreases sensitivity of ASST. It has been demonstrated that C5a amplifies IgG-dependent histamine release from basophils in chronic urticaria.[10] Asero et al mentioned that coagulation cascade might be involved in the pathogenesis of chronic urticaria.[11] Thrombin is the last enzyme of the coagulation cascade and is generated from prothrombin by activated factor X in the presence of activated factor V and calcium ions. Thrombin triggers mast cell degranulation and may activate protease activated receptor.[1] Some studies showed that heparin inhibits both the skin response to autologous serum and histamine release from human cultured basophils in vitro.[12,13] Importantly, heparin exerted its inhibitory effect in vivo also in ASST-positive patients whose sera did not induce any histamine release in vitro.[11] Metz and colleges [10] had confirmed their previous finding that plasmas from a proportion of patients with CU, particularly those with a more severe disease, showed signs of thrombin generation as evidenced by the elevated levels of fragment F_{1+2}. However, whatever the mechanism of coagulation activation (primary or secondary), the presence of thrombin, an enzyme that increases permeability of blood vessels, seems to play an important role in the pathogenesis of CU. In conclusion, the extrinsic pathway of the coagulation cascade is activated in chronic urticaria and this activation appears to lead to thrombin generation, and thus suggesting the efficacy of heparin in treatment of chronic urticaria. In fact, both heparin (which highly increases the antithrombin activity in plasma) and oral anticoagulant therapy (which reduces thrombin generation in vivo) have been shown to be effective in the treatment of chronic urticaria.[14] Thus it is predictable that APST generates more positive responses than ASST because plasma contains coagulation factors and complements. Therefore consumption of coagulation factors and formation of clot seems to be responsible for less positive results in ASST.

This study was aimed to compare the sensitivity of Autologous Serum Skin Test and Autologous Plasma Skin test in patients with chronic idiopathic urticaria for coordination clinical pattern of disease and probable presence of auto antibody against IgE or IgE Receptor (Fc**ε**RIα). The results could be used in predicting the course and severity of urticaria and selecting the best protocol for treatment of each patient.

MATERIALS AND METHODS

Fifty eight patients (17 male, 41 female; with mean age of 34 years) referred to asthma and allergy clinic located in children medical center during 2000-2007 were diagnosed with chronic idiopathic urticaria recruited for this test comparison study.

Sample size was calculated by $x = \frac{2(z\alpha + z\beta)^2\, p(1-p)}{(p_0 - p_1)^2}$

$=0.5 \rightarrow z\alpha = 1.96\alpha$

$=80\% \rightarrow z\beta = 1.28\beta$,

P1=0.86 $p_0 = 0.40$ $p = \frac{p_0 - p_1}{2}$

Fifty eight patients referred to asthma and allergy clinic recruited in this study. All the patients voluntarily accepted to participate in the study and signed the approval according to the Helsinki protocol. SPSS 17 was used for the statistical analysis.

All subjects had disease duration of more than 6 weeks. The severity of urticaria was scored on a scale from 0 to 4 as mentioned (Table 1).

The severity of itching was scored as mentioned (Table 2).[1]

Probable causes of urticaria were ruled out by means of history, prick test and lab data (CBC, LFT, UA, Stool Op&Ob, IgE, LFT, thyroid function tests, ANA, RF, CH50, C3, C4). Lab data, prick test and history were used also for detection of potential causes of allergies to drugs, foods, inhalations and infections and auto immune disease. All the patients underwent skin prick test for 6 allergens including: mite (Dermatophagoides pteronyssinus and Dermato-phagoides farina) and cockroach for indoor allergens and grass, trees, weeds for outdoor allergens.

In addition other prick tests were done individually for food, additives and inhalation allergens according to the history of the patients. Antihistamines and other medications were discontinued one week before skin testing in all cases. All the patients had an autologous serum skin test (ASST), and autologous plasma skin test (APST) as defined by Grattan, et al.[15]

The exclusion criteria were: chronic disease and malignant disease, pregnancy, hyper IgE syndrome (IgE <500 IU and absence of criteria for hyper IgE syndrome in history and physical exam and lab data), allergic urticaria (IgE-mediated). However accompanying diseases such as eczema and allergic rhinitis etc were not excluded. Five age- and sex-matched healthy cases were recruited as controls.

The autologous plasma skin test (APST) was compared with ASST to detect auto reactivity in patients with CU.[10,16,17] If the difference between positive and negative control was less than 3 mm or negative control was more than 3 mm the case was excluded and if the positive control was 3 mm more than negative control, the patients were included. Patients who had low titer ANA and RF without symptoms were included while high titer of ANA and RF or auto immune symptoms excluded the patients from this study.

Table 1. Scale of severity of urticaria

Scale of Severity of urticaria	Clinical feature
Score 0	No wheals
Score 1	1 to 10 small (<3 cm in diameter) wheals
Score 2	10 to 50 small wheals or 1 to 10 large wheals
Score 3	More than 50 small wheals or 10 to 50 large wheals
Score 4	Virtually covered with wheals

Table 2. Scale for severity of itching

Scale of severity of itching	Severity of itching
Score 0	No itching
Score 1	Mild itching
Score 2	Moderate itching
Score 3	Severe itching
Score 4	Very severe itching

Two blood samples (2 ml each) were obtained in sterile conditions. For the ASST, after separation of serum, 0.05 ml of serum was injected intradermaly on the volar surface of forearm. For the APST citrated blood was centrifuged 1250 r/min for 3 minutes in room temperature to separate the plasma. Immediately after separation 0.05 ml of plasma was injected intradermaly on the volar surface of the forearm. All patients received intradermal 0.05 ml normal saline as negative control and 10 mg/ml histamine skin prick test as positive control simultaneously.[10] Test was considered positive if the wheal of ASST was at least 3 mm more than those of negative control with saline and also if histamine wheal was at least 3 mm more than the negative control.[16]

RESULTS

The results were categorized as follows: Autologous serum skin test was positive in 38out of 58 patients (65.5%), while the autologous plasma skin test was positive in 45 out of the 58 patients (77.6%). 37 patients were positive for both ASST and APST and 12 out of 58 patients were negative for both skin tests. One of these 58 patients was negative for APST and positive for APST, while 18 patients were APST positive and ASST negative (Table 3, 4).

The results of positive skin prick test for allergens are mentioned (Table 3).

Twenty five out of 45 patients with positive APST were positive for an allergen prick test, while 8 out of 13 patients with negative APST were positive to an allergen prick test. The results did not have significant difference between APST negative and positive patients (p=0.7). Twenty two out of 38 patients with positive ASST were positive for an allergen prick test, while 11 out of 20 patients with negative ASST were positive for an allergen prick test. The results did not have significant difference between ASST negative and positive patients (p=0.832).

There was no significant specific result regarding to the variation of allergens used for skin prick test, therefore it was not mentioned.

Mean IgE value for the patients with positive ASST was 115 IU and 350 IU for negative ASST group. The mean value for positive APST patient was 122 IU and 434 IU for negative APST patients.

According to scale of urticaria[1] 5% of the patients showed score 2 urticaria, 84.4% showed score 3 and 10.3% showed score 4 urticaria at the beginning of the study. One month after the beginning of study and doing the ASST and APST the patients reevaluated for urticaria scale, the results were as follows:23 patients (39.6%) evaluated as score 0-1 ,9 patients (15.5%) as score 2 ,20 patients (34.4%) as score 3 ,6 patients (10.3%) as score 4.

In the course of study and 1 month follow up thereafter, the patients with positive APST demonstrated a history of mean urticaria duration of 32 months. This duration was 26 months in negative APST patients (p=0.646). Mean duration of urticaria in positive and negative ASST were 38 and 20 months respectively (p=0.353) in this study. Urticaria duration did not differ between these four groups (Table 5).

Ten out of the total 58 patients showed an abnormal thyroid function test or revealed anti thyroid antibody (p=0.743),which include 7of ASST positive patients (18%) and 3 of ASST negative patients. 10 of 45 APST positive patients (22%) showed abnormal thyroid function tests ,while none of the APST patients had abnormal thyroid function tests test. There was not significant difference between ASST positive patients and ASST negative patients (Fisher's exact test p=0.097).

Nine out of 38 patients with positive ASST and 4 of the negative ASST had a history of dermographism (p=0.749) without interfering with skin prick test and their negative control test revealed no wheal. This ratio was 10 of the 35 positive APST patients and 3 of the 13 negative APST patients (p=0.948).

Three out of 58 patients were ANA or RF positive. These patients were not excluded from the study because of low titer ANA or RF. They did not have any other criterion of collagen vascular disease but one patient was excluded because of high titer ANA and RF and symptoms of collagen vascular disease.

Table 3. Results of positive skin prick test for allergenes

Allergenes	Soya	Milk	Walnut	Hazelnut	Peanut	Alternaria	Feather	Cat fur	Wheat	Fish
Number of patients	2	3	2	2	2	3	2	1	2	1

Table 4. Total number patients with autologous plasma skin test and autologous serum skin test

Topic		Serum group		
		Wheal negative number	Wheal positive number	Total
Plasma Group	Wheal positive	12 (20.6%)	1 (1.7 %)	13 (22.4%)
	Wheal negative	8 (13.7%)	37 (63.7%)	45 (77.5%)
Total		20 (34.3%)	38 (65.4%)	58 (100%)

Table 5. Results of allergen sensitivity, duration of urticaria and mean IgE levels in patients

Skin prick test	Positive allergen prick test (%)	Urticaria duration(month)	Mean IgE value(IU)
Positive APST	55.5%	32	122
Negative APST	61%	26	434
Positive ASST	57.8%	38	115
Negative ASST	55%	20	350

DISCUSSION

As Asero et al.[11] and Greaves[18] had reported in their previous studies, APST is a reliable in vivo skin test, but dos Santos et al , Asero et al, Fagolio et al and Sichere et al reported the accuracy of the test to be 30-53%.[13,19-21] Caproni et al mentioned that ASST alone is not sufficient to define CIU as an autoimmune disease. However, ASST has often been chosen as an experimental model[1,22] because it has an effect very similar to that of the physiological stimulus that induces whealing in urticaria.[8]

The results of the current study seem to be similar to the previous studies, which indicate that autologous plasma skin test is a sensitive method for detection of functional auto antibodies in patients with urticaria.[16] There is an agreement between ASST and APST in positive or negative results. Since 84.4% of the cases were positive or negative for both tests, 37of the 58 cases were positive and 12 were negative for both tests. 8 cases were positive for APST and negative for ASST. Only one case proved to be positive for ASST and negative for APST. Although the literature includes various results, these variations depend on multiple factors including: methods of skin test, interpretation criteria of skin test results, type of anticoagulant, centrifuging blood specimen and methods of serum separation. For example Asero et al in 2007[16] used the EDTA as anticoagulant, so the positive results reported to be 97% of the cases. He and his colleges repeated another study in 2009,[20] when they considered the wheal as positive response while Pachlopnic and colleagues[23] consider a wheal volumes >9 mm as a positive response. Consideration of flare or redness as positive response was responsible for 52% prevalence of positive response in urticaria patients and 55% in healthy controls. The test result was considered positive when the mean diameter of the wheal with autologous serum was at least 2.5 mm larger than the mean diameter of the wheal elicited by the negative control.[24]

Impaired thyroid function and anti thyroid antibody were detected in 10 cases. The correlation of the results with positive APST seems to be considerable because these cases were all APST positive, although the relation was not significant. Reported results of ASST in these cases were 7 patients ASST positive and 3 patients ASST negative. Realizing the fact that thyroid auto antibodies are present at a relatively high frequency in patients with positive APST Chronic Idiopathic Urticaria (CIU).[21,25,26]

A relation between IgE ratio and duration of urticaria could not be proved in this study. IgE ratio and allergen sensitivity do not play a significant role in the duration of urticaria.[2,9,18,27] It seems that in contrary to some reports, in this study existence of allergen sensitivity showed no relation to the false positive or negative result. Surprisingly the patients who suffered a long term urticaria had a lower IgE ratio than the patients with short duration of the disease. The possible explanation indicates that allergen sensitivity in chronic urticaria attains less important role and the cause of urticaria is autoimmune antibodies against IgE and IgE receptor.

There was no significant difference in the case of relation between the duration of urticaria and positive result of autologous plasma and serum skin tests.

Duration of CIU seemed to be longer in patients with positive ASST and APST tests, but the difference was not significant.

Another finding of this study was that after ASST and APST, the patients experienced significant improvement in their signs and symptoms .Either they had been cleared and had not relapsed for 2-4 weeks or experienced mild disease. Evaluation scoring of the patients after 4 weeks follow up showed : 23 patients (39.6%) evaluated as score 0-1 ,9 patients (15.5%) as score 2 ,20 patients (34.4%) as score 3 ,6 patients (10.3%) as score 4 , indicating improvement in the score of the patients' urticaria. In these circumstances the need for drugs is minimized, indicating that ASST and APST could be an effective therapy,[22,28,29] the effect was more significant in patient with larger wheals and flare size. Interestingly, ASST results were found to have more coordination with clinical symptoms of the patients, whereas positive APST does not show coordination with clinical symptoms in spite of more positive results comparing with ASST.

Although H1-antihistamines are the first line of treatment in all urticaria cases, the first generation antihistamine drugs were poorly tolerated in this study. We could not prescribe diphenhydramine more than 25 mg/day due to low compliance of our patients while in some studies the prescribed dose reported 100-200 mg/day.[29]

Suggestions

Considering the agreement of ASST and APST in 84% of the cases for negative or positive results, it is suggested to check both tests simultaneously to confirm the result of either test. Combination of ASST and APST with an in vitro test such as expression of CD63 or CD203 could be a reliable diagnostic approach and a strong predicting tool for determining the severity and duration of CIU, and a valuable aid in planning the treatment protocol as well.

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