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Original Article

Assessment of cellular antigen stimulation test in diagnosis of drug allergy

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ABSTRACT

Identification of the etiology of drug allergy is a crucial step in its management, and it depends mainly on the study of the patient's history; this is because skin testing is not always reliable, and drug challenge tests are hazardous. Cellular antigen stimulation test (CAST) is a diagnostic tool that is used for the detection of sulfidoleukotrienes released by basophils stimulated by allergens *in vitro*. A case-control study was conducted to evaluate the efficacy of cellular antigen stimulation test in the diagnosis of patients with a history of allergy to drugs. The study included 90 patients, divided equally into 2 groups (45 in the drug allergic group, and 45 in the control group). Full allergy history was taken, skin prick test (SPT), and patch test for penicillin and non-steroidal anti-inflammatory drugs (NSAIDs) were done. Serum-specific IgE test and CAST by the enzyme-linked immunosorbent assay (ELISA) test were also performed. The specificity of CAST for drug allergy was 84.4% and the sensitivity was 48.9%. The specificities of SPT, patch test and specific IgE in the allergic group were 91.1%, 91.1% and 73.3%, while their sensitivities were 51.1%, 33.3%, and 62.2%, respectively. In spite of the sub-optimal sensitivity and high specificity of CAST test in the diagnosis of drug allergy, this test is an important diagnostic tool. It can be used especially in the cases with immune-mediated type of allergy, when other diagnostic tests are not dependable, or unavailable.

KEYWORDS: drug allergy, cellular antigen stimulation test, penicillin, NSAIDs.

INTRODUCTION

There is no doubt that drug hypersensitivity reactions are complex diseases, which can be either immune-mediated (drug allergy) or nonimmune-mediated with potentially serious consequences [1]. The diagnosis of drug allergies and pseudoallergies is difficult and is mainly based on detailed clinical history, and skin testing for certain drugs. Although provocation tests as drug challenge tests have the best sensitivity and are the gold standard for diagnosis, they are potentially harmful and ethically limited especially for those with severe reactions [2]. Therefore, the availability of validated diagnostic tests that renders provocation tests to limited use is mandatory. Skin tests and specific IgE tests are seldom available, or relevant for the diagnosis of immediate type drug allergies except Beta-lactam allergy [3]. Some *in vitro* cellular tests which determine the reactivity of blood cells, particularly basophils, to various allergens, have also been available for many years. In the 1980s, basophil degranulation was examined microscopically [4] but has been largely disused since. An alternative cellular test that is based mainly on the determination of sulfidoleukotrienes (LTC4, LTD4, LTE4) released

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by basophils pretreated with IL3 and stimulated by allergens *in vitro*, has been suggested. The cellular antigen stimulation test (CAST) has been used in allergy diagnosis for inhalation allergies, food allergy, allergies to insect venoms, occupational allergens as well as different drugs [5]. The value of CAST as a diagnostic test should be evaluated, particularly when other *in vivo* or *in vitro* diagnostic tests are not reliable, as in cases of drug or food allergies, as well as in non-IgE-mediated hypersensitivity reactions. For this purpose, this study aimed to assess the efficacy of CAST as a diagnostic test in patients with a history of drug allergy mainly penicillin and non-steroidal anti-inflammatory drugs (NSAIDs).

METHODS

Forty-five patients with a history of drug allergy either for penicillin or non-steroidal anti-inflammatory drugs were recruited from the Allergy and Clinical Immunology outpatient clinics at Ain Shams University hospitals. Another 45 non-drug allergic healthy individuals served as the control group. The clinical reactions as reported by the patients after drug ingestion were urticaria and/or angioedema, exanthema, rhinitis, asthma symptoms and anaphylaxis. Skin prick test, patch test, specific IgE test and CAST were performed. Any medications e.g. antihistamines, steroids, antidepressants were avoided 7-10 days prior to testing for all patients. Patients on immunotherapy for any other atopic disorders were excluded. There was a period ranging from 4 to 6 weeks between the allergic reaction to blood sampling. An informed consent was obtained from all participants, and the study was approved by the Research Ethics Committee of Ain Shams University.

Diagnostic tests

Skin prick test (SPT)

To perform the skin prick test, the commercially available forms of the drugs were used to prepare fresh sequential dilutions using physiologic saline (10^{-2}, 10^{-1}).

SPT was performed by applying the diluted drug preparation on the volar surface of the forearm after sterilization of the skin. Drops were applied approximately 2-3 cm apart, followed by gentle pricking through each drop using a sterilized lancet. In addition, 0.1% histamine in phosphate-buffered saline and physiologic saline were used as positive and negative controls, respectively. SPT result was read within 20 minutes as an immediate response, and considered positive if mean wheal diameter was 3 mm (or more) greater than the negative control [5]. The SPT panel used for drug allergy was ampicillin, amoxicillin, ibuprofen, and sodium diclofenac.

Patch test

Preparation of the drugs in certain concentrations was done; the concentrations of ampicillin, amoxicillin and ibuprofen were 10% in petrolatum and that of sodium diclofenac was 1% in petrolatum. A few drops of the drug preparation in aluminium chambers were put on the upper back of the patients. We used hypoallergenic type of adhesive tape to keep the chambers in place for 48 hours. Patients were asked to keep their backs dry, with no baths, showers or unnecessary sweating. The patches were removed after 2 days. A suitable marker was used to mark the test sites on the back. These marks had to be visible two days later (4 days after application). Readings of results were evaluated from 48 to 96 hours and were interpreted as follows: Negative, irritant reaction, equivocal/uncertain, weak positive (+), strong positive (++), extreme reaction (+++).

Irritant reactions: sweat rash, follicular pustules and burn-like reactions.

Uncertain reactions: pink area under the test chamber.

Weak positives: slightly elevated pink, or red plaques.

Strong positives: papulovesicles.

Extreme reactions: blisters, or ulcers [6].

Specific IgE test

Quantification of drug-specific IgE (sIgE) with IgE immunoassays relies upon the detection of a drug-(hapten)- carrier-antibody complex. Specific IgE immunoassays for ampicillin, amoxicillin, ibuprofen and sodium diclofenac were performed according to manufacturer's instructions. Results of sIgE assays have been expressed as kUA/L units, and a cutoff value of 0.35 kUA/L for positive results was determined [7].

CAST

The CAST (BÜHLMANN LABORATORIES, Switzerland) was intended for the quantitative determination of sulfidoleukotrienes (sLT) produced by isolated leukocytes upon contact with specific antigens. CAST measured both IgE-mediated and non-IgE-mediated leukotriene release using ELISA. In short, this test started with leukocyte isolation then cell stimulation followed finally by leukotriene determination. At first, sufficient blood was collected into ethylenediamine tetraacetic acid (EDTA) venipuncture tubes. Erythrocyte sedimentation occurred after dextran addition to the patient's blood. After removal of thrombocytes, the pellet of leukocytes was resuspended using an IL3-containing stimulation buffer. Cell stimulation was done for 40 minutes at 37 °C with an anti-IgE receptor antibody (stimulation control) or with no antibody (background) or 'allergen' in different concentrations, then, the supernatant was either frozen at -20 °C until measurement or immediately tested for sLT concentration by ELISA. The ELISA was performed using pre-coated microtitre plates. 16 wells per assay were used for the standard curve and controls, 2 wells per patient for background, 2 wells per patient for stimulation control and 2 wells for each allergen. For each well, enzyme label (alkaline phosphatase) and antibody were added, then incubated, and after a washing step, substrate solution (para-nitrophenyl-phosphate) was added, incubated and stopped with 2N NaOH. Color absorbance was measured at 405 nm in a microtiter plate reader. Leukotriene release was reported in picograms (pg)/ml. The technical cut-off values were all above 40 pg/ml.

Statistical methods

IBM© SPSS© Statistics version 23 (IBM© Corp., Armonk, NY, USA) and XLSTAT Version 2014.5.03 (Addinsoft©, NY, USA) were used to analyze data. The Shapiro-Wilk test was used to examine the normality of numerical data distribution. Normally distributed numerical variables were presented as mean ± SD and categorical variables as ratio (%) or number. The diagnostic value of a test was examined by construction of a 2-by-2 contingency table and calculation of the following indices: sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV).

RESULTS

This case control study included 45 drug allergic patients, 26 (57.8%) females and 19 (42.2%) males with their mean age 20 ± 4 years, as well as 45 healthy controls, 23 (51.1%) females and 22 (48.9%) males with mean age 22.2 ± 2.6 years. The clinical reactions as reported by the patients after drug ingestion were urticaria and/or angioedema 20 (44.4%), exanthema 14 (31.1%), rhinitis 6 (13.3%), asthma symptoms 2 (4.4%) and anaphylaxis 3 (6.6%). Among drug allergic patients, 23 (51.1%) reported history of penicillin allergy (10 (22.2%) for Ampicillin and 13 (28.9%) for Amoxicillin), while 22 (48.9%) patients had NSAIDs allergy; 11 (24.4%) for Ibuprofen and 11 (24.4%) for Diclofenac.

Positive results of SPT, patch test and specific IgE test were found in 23 (51.1%), 15 (33.3%) and 28 (62.2%) of all drug allergic patients, respectively, while the positivity of CAST result was 22 (48.9%) among drug allergic patients in comparison to the control group (7 (15.6%)). The overall sensitivities of SPT, specific IgE and patch tests were 51.1%, 62.2% and 33.3%, respectively, while their overall specificities were 91.1%, 73.3% and 91.1%, respectively.

With regard to the diagnostic value of CAST in the drug allergic group, the overall sensitivity was 48.9% and specificity was 84.4%, with positive predictive values reaching up to 75.9% (Table 1). Moreover, the sensitivity of CAST in the diagnosis of penicillin allergy was 43.5%, and for NSAIDs allergy was 54.5% (Table 2).

Further analysis of the above-mentioned diagnostic tests among drug allergic patients revealed that SPT was found positive in only 9 (39.1%) penicillin allergic patients and in 14 (63.6%) NSAIDs allergic patients. For patch test, positive results were observed in 9 (39.1%) penicillin allergic patients and only 6 (27.3%) NSAIDs allergic patients. However, specific IgE showed higher positive results for both groups: 18 (78.3%) and 10 (45.5%) for penicillin and NSAIDs allergic cases, respectively. Finally, CAST results were positive in 10 (43.5%) penicillin allergic patients and 12 (54.5%) NSAIDs allergic patients (Table 2). In penicillin allergic patients, specific IgE showed the highest sensitivity values (78.3%) of all other

Table 1. Overall sensitivity, specificity, positive predictive value and negative predicative values of CAST in the diagnosis of drug allergy.

	Drug allergic patients	**Control group**
CAST positive	22/45	7/45
Sensitivity	48.9%	
Specificity	84.4%	
Positive predictive value (PPV)	75.9%	
Negative predictive value (NPV)	62.3%	

All data are expressed as No and %
CAST; cellular antigen stimulation test

Table 2. Sensitivity, specificity and positivity of SPT, Patch test, Specific IgE and CAST tests in each of the penicillin and NSAIDs allergic patients.

	Drug allergic patients (n = 45)	
	Penicillin allergy (n = 23)	**NSAIDs allergy (n = 22)**
SPT		
Positive	9 (39.1%)	14 (63.6%)
Sensitivity	39.1%	63.6%
Specificity	91.3%	90.9%
Patch test		
Positive	9 (39.1%)	6 (27.3%)
Sensitivity	39.1%	27.3%
Specificity	86.9%	95.4%
Specific IgE		
Positive	18 (78.3%)	10 (45.5%)
Sensitivity	78.3%	45.4%
Specificity	73.9%	72.7%
CAST		
Positive	10 (43.5%)	12 (54.5%)
Sensitivity	43.5%	54.5%
Specificity	82.6%	86.3%

All data are expressed as No and %
SPT; skin prick test, CAST; cellular antigen stimulation test, IgE; immunoglobulin E

tests used while SPT showed more sensitivity (63.6%) than others in the diagnosis of NSAIDs allergy (Table 2).

Sensitivity of CAST increased up to 60.9% when used in combination with specific IgE in diagnosis of penicillin drug allergy. However, combination of CAST with other assays failed to improve the diagnostic sensitivity (Table 3).

DISCUSSION

Drug allergy causes bad effects on the patient's quality of life. Additionally, the diagnosis of drug allergy is challenging [8]. Unfortunately, reliable tests for the *in vitro* diagnosis of drug allergy do not exist; so the researchers are working on improving existing practices and creating new ones. CAST test antigen stimulation was developed a few

Table 3. Sensitivity and specificity of CAST combined with SPT, Patch test and Specific IgE test.

	Penicillin allergic patients n = 23	NSAIDS allergic patients n = 22
CAST and SPT		
Sensitivity	41.3%	59%
Specificity	86.9%	88.6%
CAST and Patch test		
Sensitivity	41.3%	40.9%
Specificity	84.7%	90.8%
CAST and Specific IgE test		
Sensitivity	60.9%	49.9%
Specificity	78.2%	79.5%

All data are expressed as No and %
SPT; skin prick test, CAST; cellular antigen stimulation test, IgE; immunoglobulin

years ago for identification of both IgE and non-IgE-mediated hypersensitivity reactions. We aimed to evaluate the diagnostic value of CAST test in either IgE-mediated, or non IgE-mediated hypersensitivity drug reactions. The overall diagnostic sensitivity of CAST test is 48.9%, which is nearly similar to that of SPT and specific IgE test (51.1% and 62.2%, respectively) in the current study.

For the immediate type of beta-lactams allergy (e.g. amoxicillin and ampicillin), the CAST sensitivity (43.5%) is almost concordant with the sensitivity of SPT (39.1%) but much lower than the sensitivity of specific IgE (78.3%). This agrees with previous studies where CAST sensitivity ranged between 30 and 5%, depending upon the type of the Beta-lactam allergen used in each study (the drug itself/plurivalent drug-polylysine/the drug-protein conjugate) [2, 9, 10]. On the other hand, specificity usually is higher and reaches up to 80% or more. Moreover, the CAST test has been found to be positive in many cases of Beta-lactam allergy that have been confirmed by positive provocation challenge in spite of negative skin, and specific IgE tests [11].

In the case of allergic reactions to NSAIDs, no available diagnostic tests are proved to be reliable. The CAST was supposed to be efficient with NSAIDS, according to previous studies [12, 13]. In the present study, the sensitivities of CAST test, SPT and specific IgE test for NSAIDS (Ibuprofen and Diclofenac) are nearly similar (54.5%, 63.6%, 45.4%, respectively). As compared to published studies about efficacy of CAST test in NSAIDs allergy, one study done by *Pierzchalska et al*., [14] showed that CAST had no role in diagnosis. Another study by Lebel *et al*., in 2001 [2], showed that despite a high specificity of 88% and low sensitivity of only 21%, the test was considered to be of no diagnostic value. The rest of the studies reported variable sensitivities between 60 and 71% and specificities between 97 and 100 [15]. Heterogeneity in the allergens used, especially the dose, may possibly explain the variability in the results and their interpretation.

For better evaluation of CAST test in non-immediate type drug allergy, patch test was used in the present study, and we observed that sensitivity of patch test is 39.1% for Beta-lactam allergic patients and 27.3% for the NSAIDs allergic group, which are much lower than the sensitivity of CAST test (43.5% and 54.5% for penicillin and NSAID allergy, respectively). In contrast to our findings, Bircher *et al*., [16] concluded that CAST assay is mostly negative in delayed type drug reactions such as in morbilliform exanthem.

Finally, we attempted to determine whether the combination with other diagnostic tests may improve the efficiency of CAST tests, and we noticed that results did not improve the diagnostic power except for the combination of CAST and specific IgE in cases of penicillin allergy.

CONCLUSION

CAST test can be helpful in the diagnosis of drug allergy, especially in the cases with immune-mediated

type of allergy, when other diagnostic tests are not dependable, or unavailable.

CONFLICT OF INTEREST STATEMENT

There are no conflicts of interest.

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