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Comparing Basophil Activation Test and Specific IgE Assay in the Diagnosis of Allergy to Penicillin G and Ibuprofen

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ABSTRACT

The risks associated with in vivo tests in the diagnosis of immediate drug hypersensitivities result in evaluating alternative in vitro tests, such as the Basophil Activation Test (BAT). This pilot study aimed to set up a BAT and compare it with a specific Immunoglobulin E (sIgE) assay for penicillin G and Ibuprofen in patients with immediate hypersensitivity to β-lactams or nonsteroidal anti-inflammatory drugs (NSAIDs).

Eleven subjects with a clear history of immediate hypersensitivity to one of the β-lactams (n=5), the NSAIDs (n=3), or both (n=3) entered this study. BAT and sIgE assays were performed regarding the patient's history.

The most frequent manifestations were angioedema, shortness of breath, urticaria, and nausea. Eight patients had anaphylactic reactions. The results presented a positive BAT for penicillin G and one for Ibuprofen. Moreover, three patients with a history of the β-lactams reaction demonstrated positive sIgE to β-lactams in the ImmunoCAP. Despite a lack of agreement between the positive results of the BAT and sIgE assay, five patients were identified by one of these methods.

Despite positive BAT and sIgE results in two and three patients, respectively, the risks, high cost, and time-consuming nature of drug challenges render these tests valuable for reducing the number of patients who are candidates for a drug challenge.

Keywords: Basophil activation test; β-lactam; Drug hypersensitivity; Nonsteroidal anti-inflammatory drugs; Specific IgE;

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INTRODUCTION

Penicillin, a β-lactam drug, is known to cause adverse reactions in about 10% of patients.[1] Since the discovery of penicillin, a spectrum of immunological and non-immunological adverse reactions has been reported.[2] The use of β-lactam drugs is limited due to the risk of allergic reactions to this group of medications. This limitation leads to administering broad-spectrum antibiotics, thereby escalating the likelihood of antimicrobial resistance and the occurrence of adverse drug reactions.[3,4] Despite frequent reports of penicillin allergy in the community, only a relatively small percentage of these claims are confirmed through proper testing. Therefore, the exact investigation and diagnosis of allergy to penicillin could be important.[3]

The other group of drugs with the most common hypersensitivity reactions is the nonsteroidal anti-inflammatory drugs (NSAIDs).[5] Respiratory hypersensitivity induced by NSAIDs in a general population across 22 European centers has been estimated to range between 0.5% to 4.8%.[6] Among NSAIDs, Ibuprofen is considered the most common medicine that is administered to decrease inflammatory conditions such as fever, inflammation, and pain.[7,8] While reports of ibuprofen hypersensitivity reactions are frequent, few cases are definitively diagnosed.[9] Considering the risks of drug provocation tests and the time-consuming process, the performance and improvement of diagnostic tests is needed.[8]

The Basophil Activation Test (BAT), a flow cytometric functional test, has established its position among allergy tests.[10] The overexpression of CD63 or CD203c on the basophils' surface following exposure to an allergen implies a positive BAT.[10]

Considering the limitations and risks of in vivo tests in the diagnosis of drug allergies, the main purpose of this study was to set up a BAT for drug allergens in Iran. Additionally, we compared the results of the BAT and specific IgE assay to penicillin G and Ibuprofen in patients with a clinical history of immediate hypersensitivities to β-lactam family or NSAIDs.

MATERIALS AND METHODS

Patients

In this pilot cross sectional study, 11 patients with a clear history of type I hypersensitivity reactions to β-lactam antibiotics (n=5) or one of the NSAIDs (n=3) or both (n=3) were referred to the Immunology, Asthma and Allergy Research Institute (IAARI), Tehran University of Medical Sciences during 2019 and 2020. Having one of the symptoms, including flushing, urticaria, angioedema, bronchospasm, and anaphylaxis after consumption of β-lactams and/or an NSAID, was considered the criterion to enter the study. A complete medical history and 5 mL blood sample were taken from each participant.

Basophil Activation Test

Two mL of whole blood with ethylenediaminetetraacetic acid (EDTA) anticoagulant was taken from all subjects. To perform BAT, the Flow CAST kit (BÜHLMANN, Switzerland) was used. The patients did not receive any systemic corticosteroid at least 24 hours before sampling. Four pyrogen-free tubes for flow cytometry measurements were prepared and labeled as follows: background, anti-FcεRI Ab positive control (PC1), fMLP positive control (PC2), and allergen tubes. Initially, 50 µL of the corresponding stimulus was added to each tube for each patient: 50 µL of stimulation Buffer (in background tube), 50 µL of anti-FcεRI Ab as stimulation control (in PC1 tube), 50 µL of stimulation control fMLP (in PC2 tube), and 50 µL of allergen in the last tube.

Thereafter, 100 µL of stimulation buffer was added, followed by adding 50 µl of the patient's whole blood in all tubes. Incubation at 37°C in a water bath for 15 minutes was done after adding 20 µL of staining reagent to the tubes. For lysing, 2 mL prewarmed (18–28°C) lysing reagent was used. After incubation at 18–28°C and centrifugation at 500*g* for 5 minutes, the supernatant was emptied. Then the cell pellet was resuspended with 300 µL of wash buffer. The data were acquired on the FACSCanto II flow cytometer (BD, USA) on the same day. The results of flow cytometry were analyzed by FlowJo software 7.6 (FlowJo LLC, Ashland, OR, USA). Furthermore, for obtaining optimal specificity and sensitivity, cutoff levels are considered for different allergen groups. According to the manufacturer's instructions, the cutoff and stimulation index (SI) used in this study were considered 5% and 2%, respectively.

$$\mathrm{SI} = \frac{\%\mathrm{CD63}_{\mathrm{allergen}}}{\%\mathrm{CD63}_{\mathrm{background}}}$$

Specific IgE Assay

Three mL of blood was collected from each subject to measure specific IgE. After separating the serum, they were frozen at −20°C until analysis. The ImmunoCAP system was used to measure the level of specific IgE for Penicilloyl G, and in some cases, Amoxicilloyl, based on history (ThermoFisher Scientific/Phadia, Uppsala, Sweden) on patients' serum (Leuven, Belgium). Moreover, the specific IgE to ibuprofen was measured in patients' serum by Hytec 288 (Hycor Biomedical Inc., USA).

RESULTS

A total of 11 individuals, 6 males and 5 females, were included in the current study. The mean age was 31.18 years (12–55) years old. All patients showed hypersensitivity reactions to the β-lactams family or NSAIDs in the clinical history. More than 70% of the patients demonstrated their manifestations within 30 minutes, while the others manifested their symptoms within 2 hours. Three patients had a history of respiratory disorders, 1 had a history of shortness of breath in childhood, 4 had seasonal allergies, 1 had food allergies, and 1 patient had asthma. A family history of allergic diseases, including seasonal allergies, asthma, food allergies, drug allergies, and urticaria, was reported by four patients.

Table 1 details the demographic characteristics and clinical symptoms of the patients. As the data in Table 1 showed, skin (angioedema and urticaria), gastrointestinal (nausea), and respiratory (shortness of breath) symptoms were the most common manifestations. Furthermore, 8 patients experienced anaphylactic reactions after consumption of the mentioned medications.

The findings of flow cytometry demonstrated a positive BAT for penicillin G ($CCR3^+/CD63^+$=39.1%) (Figure 1) and a positive BAT for Ibuprofen ($CCR3^+/CD63^+$>5% and SI=9.30) (Table 2). Moreover, three patients with a clinical history of reaction to the β-lactam family in 4 tests showed positive specific IgE to **Penicilloyl G**and/or **Amoxicilloyl**in the ImmunoCAP method (Table 3). In addition, no agreement was observed between the positive results of the BAT and the specific IgE test.

Table 1. The demographic and clinical symptoms of patients after taking β-lactam drugs or NSAIDs

Patient	Age, y	Sex	Culprit drug	Symptoms
P1	27	M	Amoxicillin, co-amoxiclav	Dyspnea, flushing, urticaria (anaphylaxis)
P2	55	M	Amoxicillin ibuprofen	Nausea, wheezing, dyspnea, bronchospasm, hypotension (anaphylaxis)
P3	17	F	Cefixime	Pruritus, urticaria, dyspnea, nausea (anaphylaxis)
P4	25	M	Amoxicillin	Anaphylaxis, angioedema, dyspnea
P5	12	F	Penicillin G + ketorolac, ibuprofen	Flushing, bronchospasm, fainting (anaphylaxis)
P6	30	M	Penicillin G, amoxicillin	Angioedema, nausea, gastrointestinal cramps (anaphylaxis)
P7	39	M	Penicillin G	Dyspnea, fainting, bronchospasm, hypotension (anaphylaxis)
P8	22	F	Penicillin G + ibuprofen	Urticaria, conjunctivitis, angioedema
P9	35	M	Ibuprofen	Face and neck angioedema
P10	35	F	Ibuprofen	Angioedema, dyspnea, flushing
P11	46	F	Ibuprofen, diclofenac, aspirin	Generalized urticaria, angioedema, nausea (anaphylaxis)

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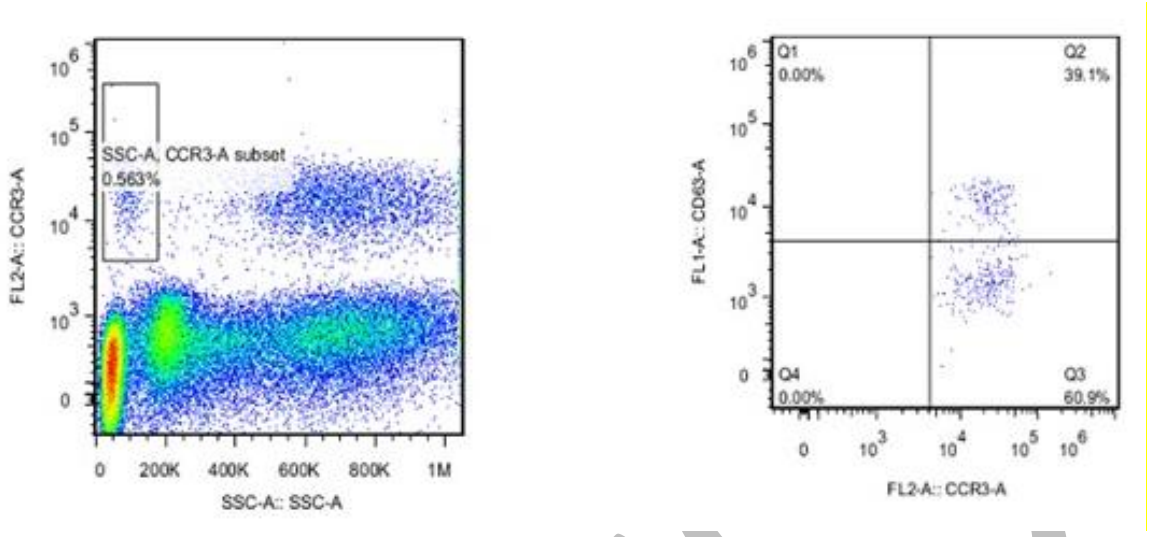

Figure 1. The results of flow cytometric analysis. Q2: The percentage of activated basophils ($CCR3^+/CD63^+$) following penicillin G stimulation.

Table 2. The percentage of activated basophils ($CCR3^+/CD63^+$) in patients with immediate reactions to Penicillin G and Ibuprofen

Patient	Age, y	Basophil activation test (flow cytometry)	Activated basophils (%$CD63^+$)	Considered cutoff (%)	Result
P1	27	Penicillin G	1.80	≥5	Negative
P2	55	Penicillin G	0.88	≥5	Negative
P3	17	Penicillin G	0.43	≥5	Negative
P4	25	Penicillin G	1.88	≥5	Negative
P5	12	Penicillin G	2.15	≥5	Negative
P6	30	Penicillin G	39.1*	≥5	Positive
P7	39	Penicillin G	0.70	≥5	Negative
P8	22	Penicillin G	0.50	≥5	Negative
P8	22	Ibuprofen	0.37	≥5	Negative
P9	35	Ibuprofen	5.23*	≥5	Positive
P5	12	Ibuprofen	1.93	≥5	Negative
P10	35	Ibuprofen	0.32	≥5	Negative
P11	46	Ibuprofen	1.31	≥5	Negative

* Positive

Table 3. The specific IgE concentration to β-lactam and NSAIDs using ImmunoCAP or Hytec 288

	Specific IgE, kIU/L		
Patient	**Penicilloyl G, kU/L**	**Amoxicilloyl, kU/L**	**Ibuprofen, kU/L**
P1	**Negative**	**Negative**	
P2	**0.59***	**1.25***	**Negative**
P3	**0.4***	**Negative**	**-**
P4	**Negative**	**0.85***	
P5	**Negative**	**ND**	**Negative**
P6	**Negative**	**Negative**	
P7	**Negative**	**ND**	
P8	**Negative**	**ND**	**Negative**
P9	**-**	**-**	**Negative**
P10	**-**	**-**	**Negative**
P11	**-**	**-**	**Negative**

*Positive; IgE: immunoglobulin E; ND: not determined; NASID: nonsteroidal anti-inflammatory drug

DISCUSSION

In this study, 2 out of 11 patients showed positive BAT to penicillin G or ibuprofen. Additionally, the specific IgE assay to penicillin G or amoxicillin was positive in 4 tests for 3 patients. Angioedema, shortness of breath, urticaria, and nausea, were the most frequent manifestations in this study. Among the participants, eight individuals reported experiencing anaphylactic shock. These findings are in line with the previous studies.[11,12] Marraccini et al reported urticaria and angioedema as the most common adverse drug reactions.[12] In addition, angioedema, anaphylaxis, and urticaria were identified as the most frequent reactions to penicillin in the Leecyous et al study.[11] In another study, skin symptoms (72%) accounted for the majority of adverse reactions in patients with drug allergies to NSAIDs.[13]

More than 70% of our patients experienced their symptoms within half an hour, while 36% of patients in the Marraccini et al study manifested their symptoms within an hour.[12]

A positive BAT for Ibuprofen was found in a patient with adverse reactions to NSAIDs. In the previous study, a total of 11 out of 18 patients with hypersensitivity reactions to NSAIDs had a positive BAT to Aspirin, whereas none showed a positive BAT to Ibuprofen. Kim et al indicated a specificity and positive predictive value of more than 90%, along with a sensitivity and negative predictive value of about 60% for BAT to NSAIDs.[13] Another study found that 11 out of 19 patients (57.9%) responded positively to drug stimulation by increasing the expression of two markers, CD203c and CD63. A useful method for the diagnosis of anaphylaxis resulting from a drug could be BAT, especially when the hypersensitive response is triggered by a new medication, or when evaluating drug-specific IgE or conducting intradermal testing is unfeasible. BAT may produce false negative results, depending on the type of drug or metabolite being measured. However, by concurrent assessment of CD63 and CD203c, their diagnostic performance can be greatly improved.[14] In addition, the Flow 2 Cast method was demonstrated to have a specificity of 80% and a sensitivity of 55% for antibiotic hypersensitivity in a study conducted by Eberlein et al in Germany.[15]

The mean sensitivity of BAT and immunoassay tests for β-lactams were 51.7% and 50.1%, respectively, while specificities of 89.2% and 81.01% have been reported for them.[16] The results of the specific IgE test using the ImmunoCAP method against the β-lactam were positive in 3 out of 8 patients with a history of allergy to the β-lactam family, while the result of the specific IgE test against the NSAIDs family with the Hycor method was not positive for none of the patients. In the study of Kim et al only one patient out of 8 patients with history of reaction to cephalosporin showed a positive specific IgE test to Cefaclor with the ImmunoCAP method.[14] Moreover, in the study of Leecyous et al the agreement between the specific IgE

test with the immunoCAP and BAT for penicillin was very low (Ƙ=0.25). Additionally, the simultaneous use of both tests slightly increased the diagnostic sensitivity in immediate hypersensitivity reactions to penicillin.[11] In other previous studies, the Kappa coefficient between the BAT test and ImmunoCAP was 0.4 and 0.54,[12,17] which shows the moderate agreement between the 2 assays for β-lactam and thus the difference between different studies.

Although about 80% of our patients had a maximum of 12 months since their reaction to the drug, according to the study of Salas et al to minimize false negative results, it is better to perform the BAT test within 12 months after the initial reaction.[18] A negative BAT can occur for several reasons, one of which is the time interval between the primary reaction and the time of the test.[19] Shortening the interval time between the reaction and performing the test leads to an improvement in the sensitivity of the test.[19] Contrary to these studies, a rare case was reported by YL Au et al regarding a positive BAT to benzylpenicilloylpolylysine (PPL) and positive specific IgE to some β-lactam after about 20 years of avoidance following an anaphylactic reaction to ampicillin. This report suggests performing the BAT and specific IgE assay in patients with drug-induced anaphylaxis regardless of the time interval between the allergic reaction and assay.[20] A reason for this allergic reaction could be the presence of these antibiotics in food products such as chicken[21,22] or cross-reactivity with other beta-lactams.[4] Moreover, a sensitivity of 85.7% and a specificity of 93.3% were detected for BAT in an interval of less than three years between allergic reaction to neuromuscular blocking agents and assay.[23] On the other hand, metabolites of drugs could be involved in -hypersensitivity reactions induced by immune system and can be the main reason of non-reactivity of specific IgE and BAT assays.[19] In addition to the above, the expression of CD63 and CD203C markers could differ based on the drug type. Moreover, some patients (10%) do not respond to positive controls in BAT, resulting in inconclusive BAT results.[24] Furthermore, the results of the specific IgE assay and BAT could be affected by the reduction of specific IgE in serum and time.[24,25] According to Fernandez et al's study, the BAT test becomes negative earlier than the specific IgE assay (RAST). As the results of the survival analysis revealed, after 12 months, only 12.2% of BAT tests and 22% of RAST tests remained positive.[25]

The COVID-19 pandemic had a substantial impact on this study. Patient hesitancy to visit hospitals due to concerns about infection complicated coordination and enrollment efforts. This reluctance may have affected various factors, including patient referral times and the duration between symptom onset and testing, ultimately introducing some limitations to the study.

It is suggested that future studies be conducted with larger sample sizes and differentiating between specific allergens to improve understanding of the role of the BAT in diagnosing drug allergy. Moreover, this method cannot be used alone in cases of concurrent sensitization to drug allergens and should be completed by the gold standard method (Challenge test).

The BAT and specific IgE tests were positive in two and three patients, respectively, but due to the life-threatening and time-consuming nature of the drug challenge and the lack of proper facilities, these tests are beneficial in reducing the number of patients who are candidates for a drug challenge. In other words, a positive specific IgE test or a positive BAT, along with a reliable history of immediate hypersensitivity reactions to drugs, could be helpful in the diagnosis of drug allergy.

STATEMENT OF ETHICS

This study has been approved by the Ethics Committee of Tehran University of Medical Sciences (IR.TUMS.IAARI.REC.1397.010). A written Informed consent was obtained from all patients.

FUNDING

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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DATA AVAILABILITY

The data will be available upon reasonable request from the corresponding author via email.

AI ASSISTANCE DISCLOSURE

We would like to declare that we used Sider (https://sider.ai) to improve some sentences and language editing. After using this tool, the authors reviewed and edited the content as needed and took full responsibility for the content of the publication.

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