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Comparison of Diagnostic Tests with Oral Food Challenge in a Clinical Trial for Adult Patients with Sesame Anaphylaxis

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ABSTRACT

Sesame food allergy (SFA); especially anaphylaxis, is a life-threatening condition. The accurate diagnosis of SFA is done by skin prick test (SPT), skin prick to prick (SPP) or specific IgE (sIgE) and is confirmed by oral food challenge (OFC). Since there are few studies evaluating and comparing the utility of these methods for diagnosis of sesame anaphylaxis in adult patients, we aimed to compare OFC with diagnostic tests, including SPT, SPP, and sesames IgE; using ImmunoCAP considering the sensitivity and specificity issues in patients with sesame anaphylaxis.

Twenty patients with sesame anaphylaxis were diagnosed based on OFC. Then SPT, SPP, and sIgE were evaluated.

Sixteen patients had positive OFC; while 4 patients had negative results. Out of 16 OFC^+ patients, 7 patients were SPT^+, 15 patients were SPP+, and 2 patients had detectable sIgE. A positive SPT indicated 44% sensitivity and 50% specificity. A positive SPP showed 87.5% sensitivity and 75% specificity. A positive ImmunoCAP test demonstrated 12.5% sensitivity and 75% specificity. The AUC of SPP was significant for the diagnosis of sesame anaphylaxis (p=0.038).

In conclusion, when the OFC is not possible, the SPP test with natural sesame seed may be applicable in patients with a convincing history instead of the artificial or commercial extracts of sesame used for SPT. Positive SPP is a good alternative diagnostic method for patients with sesame anaphylaxis. Also, the poor sensitivity of SPT and sIgE may indicate the poor discriminative capability of these tests.

Keywords: Anaphylaxis; Oral food challenge; Sesame; Skin prick test; Skin prick to prick

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INTRODUCTION

Sesame food allergy (SFA) has been increased during the past 5 decades.[1] It has been estimated that 0.1%-0.2% of the population suffers from SFA. Geographical circumstances and various environmental exposures affect the number of patients with SFA.[2] Two studies showed that sesame is an important cause of food allergy in the Iranian population.[3,4] Sesame seed is frequently used in the food industry, including Halva, bread, sweets, and sauces containing Tahini. Furthermore, it is also used in pharmacy and cosmetics.[1,2]

The clinical symptoms of the patients with SFA range from local symptoms such asurticaria[5] to systemic reactions such as anaphylaxis, which the latter is a life-threatening condition.[4] Thus, the accurate diagnosis of SFA is based on a convincing history accompanied by the demonstration of sensitization by skin prick test (SPT), skin prick to prick (SPP) or specific IgE (sIgE) and confirmed by oral food challenge (OFC).[2] In fact, double-blinded placebo-controlled OFC (DBPCFC) is a gold standard diagnostic test for food allergies but it is expensive and potentially risky.[6,7] Therefore, it is rarely used in routine practice other than in research settings.[2] Previous studies, largely conducted in children, reported that neither SPT nor sIgE is able to appropriately predict true allergy as much as OFC.[8,9] This is of particular importance because anaphylaxis has also been observed in patients having negative results for both SPT and sIgE concentrations.[5,9] According to Simons et al., diagnostic criteria for anaphylaxis were comprised of immediate reactions (minutes to several hours) occurred in either skin or mucosal regions or both surfaces (e.g., flushing, itching, swollen lips/tongue/uvula and generalized urticaria), accompanied by at least one of the following observations: decreased blood pressure, respiratory compromise, and persistent gastrointestinal symptoms.[10] The severity of the reactions (based on both history and objective OFC reactions) was graded from the clinical records according to Ring and Messmer's classification.[11]

Since there are few studies evaluating and comparing the utility of these methods for diagnosis of sesame anaphylaxis in adult patients; here, we assessed the utility of most important diagnostic methods for food allergies, including SPT,SPP, and sIgE in patients suffering from adult-onset sesame anaphylaxis, confirmed by a history of sesame anaphylaxis and positive OFC to find whether there is an alternative diagnostic method for OFC in these patients in order to probably reduce the number of required OFCs in these patients.

MATERIALS AND METHODS

Patient Selection

At first, among 50 patients suspected of SFA, 20 patients having clinical anaphylaxis criteria were selected. All of these 20 patients performed OFC, SPT, SPP, and Immunocap tests. Based on the results, the patients were divided into two groups of OFC-positive and OFC-negative individuals and subsequently, the results were compared. These patients were recruited to this clinical trial study (IRCT20181002041210N1) from January 2018 to February 2019 to the referral allergy clinic of Rasool-e-Akram Hospital (Tehran, Iran). This study was approved by the Ethics Committee of Iran University of Medical Sciences (IR.IUMS.FMD.REC1396.9511568001). They were initially diagnosed based on OFC. Then SPT with commercial extract, SPP with sesame paste (Tahini), and sIgE by ImmunoCAP were performed.

Oral Food Challenge (OFC)

OFC with Tahini (a toasted oil-based sesame seed paste) was performed according to the recommendations of the Europrevall French protocol by Dano et al. with the following semilog serial increments every 15-30 minutes as follow:0.003,0.03, 0.3, 3, 30, 100, 300, 1000, and 3000 mg of sesame protein(cumulative dose of 4.4 grams of sesame protein).[12] OFC was carried out in a highly controlled setting under experienced supervision and rescue medications(antihistamine, glucocorticoid, inhaled beta-agonists, epinephrine, etc.) were available. A challenge was scored as positive or negative when objective symptoms were present or absent following the ingestion of tahini, respectively.

Skin Prick Test (SPT)

SPTs were performed with a commercially prepared standard extract purchased from Greer Laboratories (Lenoir, NC, USA). Negative and positive control tests included normal saline and

histamine, respectively. A positive SPT was defined as a wheal diameter ≥3mm larger than the negative control. All patients underwent SPT with sesame and most important cross-reactive foods, including soy and other nuts (peanut, walnut, etc.) or any possible allergen.

Skin Prick to Prick (SPP)

SPP test (which is an SPT that uses Tahini instead of a commercial extract) was also performed for all the patients with the same method of SPT. In addition, Tahini was used for SPP as a tasty and convenient preparation, which was reliably used in previous studies.[13]

Specific IgE (SIgE)

Serum samples were analyzed for sesame sIgE; using the ImmunoCAP fluorescence enzyme immunoassay (Phadia, Uppsala, Sweden) method for all patients. The detection limit of the assay was 0.35 kU/L.A. The positive ImmunoCAP test was defined as≥0.35 kU/L.

RESULTS

In this study, 13 males (65%) and 7 females (35%) participated. The median age was 37.5 years (ranged from 18 to 55), the mean±SD age was 37.4±10.77, and the male: female ratio was 1.85. Allergic comorbidities among 20 participants, included asthma in one case (5%), urticaria in one case (5%), allergic rhinitis (AR) in 7 cases (35%), and atopic dermatitis (AD) in 2 (10%). Also, 11(55%) cases have a history of allergic diseases in their families. The median(IQR) cumulative dose was 133 mg (ranged from 0.3 to 433 mg)in the OFC+ patients. Symptoms associated with a positive OFC were cutaneous (100%), cardiovascular (46.1%), respiratory (31.2%), and gastrointestinal (25%) reactions.

All patients had a positive history of anaphylactic reactions after exposure to sesame-containing foods (25% of the patients had a history of SFA). Among 20 patients with a positive history of anaphylaxis to sesame containing foods, OFC was positive in 16 patients; while 4 patients had negative results. Detailed allergic reactions during OFC are listed in Table 1. Overall, 31.25% (5/16) of patients showed grade 1 reactions, 30.75% (6/16) grade 2 reactions, 25% (4/16) grade 3 reactions, and 6.2% (1/16) grade 4 reactions. Epinephrine was administered in6 patients (37.5%) during OFC (Table1).

Seven (43%) patients out of sixteen OFC+ patients were positive for the SPT test; while 2out of 4 OFC- patients (50%) were positive. No significant difference was observed in SPT results between OFC+ patients and OFC- patients (Figure 1, Table 2). A positive SPT demonstrated 44% sensitivity, 50% specificity, 77.8% positive predictive value (PPV), and 18.2% negative predictive value (NPV) (Table 2). The Receiver operating characteristic (ROC) curve analysis of SPT wheal size revealed an area under the curve (AUC) (CI 95%) of 0.5 (0.18-0.82). The AUC of SPT was not significant for the diagnosis of SFA (p=0.99) (Figure 2).

Fifteen (93%) patients out of sixteen OFC+ patients were positive for the SPP test, while 1 out of 4 OFC- patients were positive. There was a significant difference in SPP results between OFC+ patients and OFC- patients (p<0.01) (Figure 1, Table 2). A positive SPP showed 87.5% sensitivity, 75% specificity, 93.3% PPV, and 60% NPV (Table 2). The ROC curve analysis for SPP demonstrated an AUC (CI 95%) of 0.84 (0.58-0.99). The AUC of SPP was significant for the diagnosis of SFA (p=0.038). For varying SPP cut-offs, sensitivity was highest at a lower cut-off value and specificity increased at higher values (Figure 2).

Two (12.5%) cases out of 16 OFC+ patients were positive for sIgE; using the ImmunoCAP method, which was0.65 and 0.85 KU/L. In addition, 14 (87.5%) patients were undetectable. Whereas 1 out of 4 OFC- patients were poorly positive, which was 2 KU/L. A positive ImmunoCAP test demonstrated 12.5% sensitivity, 75% specificity, 66.7% PPV, and 17.6 NPV (Table 2). The ROC curve analysis for sesame sIgE revealed an AUC (CI 95%) of 0.44 (0.1-0.78) (Figure 2). The AUC of sIgE was not significant for the diagnosis of SFA (p=0.7).

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Table1. Demographic and clinical features of 20 patients with positive and negative OFC

No	OFC	Gender	Age	Age onset	Allergic comorbidity	Allergic Family history	Index reaction grading	Positive SPT for other allergens	OFC cumulative dose (mg sesame Protein)	OFC Reaction Grading	Treatment during OFC
1	Positive	M	55	30	-	-	III	-	0.3	I	Glucocorticoid
2	Positive	M	37	25	AR	AD	III	-	133	III	Epinephrine
3	Positive	M	54	24	-	-	III	Almond	33	I	Glucocorticoid
4	Positive	M	44	15	-	Sesame allergy	IV	-	433	IV	Epinephrine
5	Positive	M	45	32	-	-	III	-	33	III	Epinephrine
6	Positive	M	41	31	-	-	II	-	133	II	Glucocorticoid
7	Positive	F	50	26	AD, AR, Urticaria	AR	III	Mite	13	I	Antihistamine
8	Positive	M	32	23	-	-	III	Vanilla, fish	33	II	Glucocorticoid
9	Positive	F	28	23	AR	Sesame allergy, asthma	III	Aeroallergens	133	III	Epinephrine
10	Positive	F	25	18	AR	-	II	Soy	133	II	Glucocorticoid
11	Positive	M	38	22	-	AD	II	-	133	II	Epinephrine
12	Positive	M	40	37	AR	-	II	-	133	II	Glucocorticoid
13	Positive	F	33	18	-	Sesame allergy	II	-	433	II	Glucocorticoid
14	Positive	F	34	28	-	-	III	-	433	I	Antihistamine
15	Positive	M	38	25	-	Sesame allergy	II	-	433	I	Antihistamine
16	Positive	M	31	20	-	Sesame allergy	III	-	133	III	Epinephrine
17	Negative	M	55	50	AR	-	II	-	4400	-	-
18	Negative	F	24	19	AR	Nut allergy	II	Walnut	4400	-	-
19	Negative	M	26	25	-	Nut oral allergy	II	-	4400	-	-
20	Negative	F	18	16	Asthma, AD	Asthma	I	Egg, soy, peanut	4400	-	-

Abbreviation: AD: Atopic Dermatitis, AR: Allergic Rhinitis, F: Female, M: male.

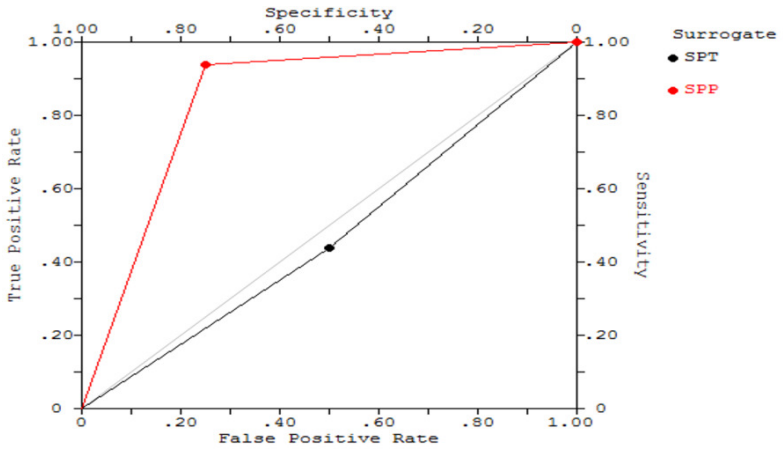

Figure 2. The ROC curve of SPT, SPP, and sIgE

Table 2. The utility of OFC and Comparison with SPT, SPP & specific IgE

Studied Cases	OFC	SPT (wheal)	SPP (wheal)	specific IgE
Case1	Positive	Negative (2 mm)	Positive (10 mm)	Negative (<0.1 KU/L)
Case2	Positive	Negative (0 mm)	Positive (8 mm)	Negative (<0.1 KU/L)
Case3	Positive	Positive (3 mm)	Positive (10 mm)	Negative (<0.1 KU/L)
Case4	Positive	Positive (3 mm)	Positive (5 mm)	Negative (<0.1 KU/L)
Case5	Positive	Positive (3 mm)	Positive (8 mm)	Negative (<0.1 KU/L)
Case6	Positive	Negative (2 mm)	Positive (5 mm)	Negative (<0.1 KU/L)
Case7	Positive	Positive (5 mm)	Positive (8 mm)	Negative (<0.1 KU/L)
Case8	Positive	Positive (6 mm)	Positive (10 mm)	Negative (<0.1 KU/L)
Case9	Positive	Positive (3 mm)	Positive (5 mm)	Positive 0.65 KU/L)
Case10	Positive	Positive (6 mm)	Positive (3 mm)	Negative (<0.1 KU/L)
Case11	Positive	Negative (0 mm)	Positive (4 mm)	Negative (<0.1 KU/L)
Case12	Positive	Negative (0 mm)	Positive (5 mm)	Positive (0.85 KU/L)
Case13	Positive	Negative (0 mm)	Positive (4 mm)	Negative (<0.1 KU/L)
Case14	Positive	Negative (0 mm)	Positive (3mm)	Negative (<0.1 KU/L)
Case15	Positive	Negative (0 mm)	Positive (4 mm)	Negative (<0.1 KU/L)
Case16	Positive	Negative (0 mm)	Negative (0 mm)	Negative (<0.1 KU/L)
Case17	Negative	Negative (0 mm)	Negative (0 mm)	Negative (<0.1 KU/L)
Case18	Negative	Negative (0 mm)	Negative (0 mm)	Negative (<0.1 KU/L)
Case19	Negative	Positive (3 mm)	Negative (0 mm)	Negative (<0.1 KU/L)
Case20	Negative	Positive (5 mm)	Positive (3 mm)	Positive (2KU/L)
Statistically significant VS OFC		0.9	0.032	0.9
Sensitivity (%)		44	87.5	12.5
Specificity (%)		50	75	75
Positive Predicted Value (%)		77.8	93.3	66.7
Negative Predicted Value (%)		18.2	60	17.6
Likelihood Ratio		0.87	3.5	-
Odds Ratio		-	21	-
ROC Curve Analysis				
AUC (95% CI)		0.5 (0.18-0.82)	0.84 (0.58-0.99)	0.44 (0.1-0.78)
p value		0.99	0.038	0.7

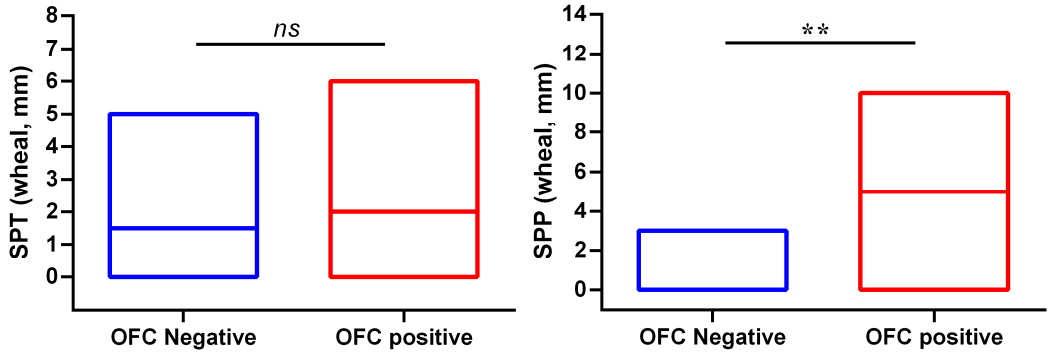

Figure 1. Comparison of SPT (left) or SPP (right) results between OFC+ patients and OFC- patients

DISCUSSION

SFA is a global problem of public health, particularly in the Middle East;[1] however, the prevalence of SFA is not well-known in developing countries such as Iran. Most patients are diagnosed during infancy and early childhood.[2] Although patients with adult-onset anaphylaxis to sesame has not been frequently evaluated to date, Nabavi et al. In 2017, showed that sesame is one of the three food allergies leading to anaphylaxis in adults in Iranian population.[5] Also, Dalal et al.[2] and Derby et al.,[14] similarly reported the probability of developing anaphylaxis to sesame in adulthood in a de novo manner. All cases initially presented sesame anaphylaxis for the first time in adulthood after years of sesame consumption.[2,14] Therefore, the accurate diagnosis with the best safe and cost-benefit diagnostic methods in addition to the novel therapeutic approaches are very important in the optimal management of the patients with sesame anaphylaxis.

The family history of SFA was seen in 5 patients, 25% of patients, which may indicate a possible family history (genetic) of SFA, similar to peanut allergy. Although there are various risk factors contributing to the development of food allergies, genetic predisposing factors and family history should be considered.[15] The ratio of male to female in this study was 1.8 to 1, which may indicate a higher prevalence of SFA in men than women, but due to the small size of the statistical population, this conclusion cannot be definitively generalized to the community. This finding was also confirmed in a study by Fazlollahi et al., in 2007, that showed that the prevalence of SFA in Iran is higher in males.[3]However, in the study of Li et al., there was no significant difference in age and sex in adult patients with SFA from 2010 to 2016.[16]

This study is one of the first presentations of SFA assessments exclusively conducted in adult-onset sesame anaphylaxis in the Iranian population in order to compare SPT, SPP, and S-IgE with OFC test, as a gold standard test, in patients with adult-onset sesame anaphylaxis. The results showed that 93% of the OFC+ cases were positive for SPP, 43% for SPT, and 12% for sIgE. Furthermore, the AUC of SPP was significant for the diagnosis of SFA. To date, the diagnosis of SFA is based on clinical history in addition to testing for sesame sensitization and OFC to confirm the diagnosis. Other methods for the establishment of food allergy diagnosis are comprised of SPT,[8,17-19] measurement of sIgE[17,18] specific component testing against a recombinant allergen,[6] and BAT.[18,20] The SPT sounds to be more informative in comparison to sIgE in children and adults; however, this finding in adults remains to be clarified.[17,21]Although OFC is a gold standard test for diagnosing the food allergy, there are some difficulties in doing it e.g.cost, attempt, and dangers. Hence, it is useful to evaluate other diagnostic methods, especially for the patients in which an OFC is not applicable.

We showed that 93% of the OFC+ patients had positive results for SPP, which is in agreement with the results of the Della-Torre et al. study. They introduced a case with SFA, which had a positive SPP to sesame with SPT and sIgE negative.[13] In another study conducted by Barbarroja-Escudero et al., out of 10

patients with a history of severe SFA and SPT negative, 9 patients had positive results for SPP with sesame seeds. This is probably due to the lack of lipophilic antigens in commercial sesame seeds as a consequence of removing the lipids during the preparation of extracts. It was also shown that serum IgEs were associated with oleosin, a soluble allergenic protein in sesame fat, which was degraded in experimental extracts.[22]However, in a recent study conducted by Li et al. on SFA, there was no difference in the results of all SPT and sIgE tests between the positive and negative OFC groups. Thus they also suggested that low sensitivity of the tests with synthetic extracts may be related to the lack of clinically relevant major allergens used in the extracts with patients' pathogenic allergens similar to our study.[16] Nevertheless, the reason for the lack of diagnostic capabilities of the SPP test with sesame seeds in the mentioned study in comparison to our work is probably the retrospective nature of the study and the patients did not show a strong history of IgE-related sensitivity after consuming sesame products. Leduc et al. identified hydrophobic oleosins (Ses i 4 and Ses i 5) as major sesame allergens and sensitization to oleosins seem to be associated with more severe systemic reactions.[23] The poor performance of SPT with commercial extracts may be attributed to the destruction of oleosins. However, SPT remains a critical diagnostic method because it is a relatively straightforward and inexpensive technique.

These findings are in line with data previously published in several studies that showed neither SPT nor sIgE had a significant positive predictive value in children.[5,8] In this regard, although Permaul et al.[8] and Zavalkoff et al.[22] reported a high sensitivity for serum sIgE, Permaul et al. did not support the use of an undetectable sesame sIgE to rule out SFA similar to our findings. In addition, after using OFC, they reported that 29% of sesame-allergic patients had sIgE lower than the detection threshold, while our result showed 88% were undetectable.[8] Moreover, the findings of this research are consistent with the findings of Li et al. that reported SPT and sIgE results are not predictive of SFA in adults.[16] In contrast, other studies have not strongly supported the poor diagnostic utility of sesame SPT and sIgE, e.g. Cohen et al. who showed a convincing history of an immediate reaction along with a positive SPT was found to have a positive predictive value in 14 of 16 (88%) cases.[5] As one of the most interesting results of our study, 93% of the OFC+ patients with anaphylaxis to sesame had positive SPP test results using Tahini and SPP was negative in 75%(3/4) OFC negative patients as well. At least two studies proposed sesame SPP as a reliable method to diagnose SFA in patients with negative SPT and serum specific IgE.[13,22] The accurately determining the SFA is difficult because of the probability of false-negative results of skin tests and sIgE. Furthermore, the possibility of a false-positive diagnosis caused by cross-reactivity with other allergens is another limitation to previous studies. In contrast, the strength of this study is the confirmation of the diagnosis of all patients based on OFC for inclusion in the study. Moreover, owing to the fact that the present study was conducted in less than a one-year period and only the patients with a severe type of IgE-mediated reactions (anaphylaxis) were included, so the number of the participants was limited and it is suggested future studies are needed in a larger scale. Also, regarding the poor results of the sIgE in SFA diagnosis, as well as the importance and severity of SFA, Component-resolved diagnostics[6,22,23] is considered necessary to identify major allergens related to clinical settings in this geographic area in order to prepare appropriate extracts containing pathogenic allergens. Finally, the use of new diagnostic methods such as basophil activating test (BAT)[18,20] is also promising in future studies.

According to the findings of this study, positive SPP with natural sesame seed (Tahini in this study) in adult patients with an establishing history of anaphylaxis in comparison to the OFC may be a good alternative method for SFA diagnosis and this test may obviate the need for OFC in most patients with SFA. In other words, when the OFC is not possible, with respect to the dangers and cost of doing it, the SPP test with natural sesame seed may be applicable in patients with a convincing history instead of the artificial or commercial extracts of sesame used for SPT. Furthermore, the SPT and sIgE alone are not probably good methods for the diagnosis of SFA, highlighting the poor discriminative value of these tests.

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