ALI POURVALI[1] (iD), SABA ARSHI[2] (iD), MOHAMMAD NABAVI[2] (iD), MOHAMMAD HASAN BEMANIAN[2] (iD),
SIMA SHOKRI[2] (iD), SHOLEH KHAJOEI[3] (iD), FARHAD SEIF[4,5] (iD), MORTEZA FALLAHPOUR[2] (iD)

Sustained unresponsiveness development in wheat oral immunotherapy: predictive factors and flexible regimen in the maintenance phase

[1]Department of Pediatrics, Amir Kabir Hospital, Arak University of Medical Sciences, Arak, Iran
[2]Department of Allergy, Rasoul Akram Hospital, Iran University of Medical Sciences, Tehran, Iran
[3]Clinical Research Development Center, Imam Khomeini Hospital, Jiroft University of Medical Sciences, Jiroft, Iran
[4]Department of Photodynamic Therapy, Medical Laser Research Center, Academic Center for Education, Culture, and Research (ACECR), Tehran, Iran
[5]Department of Immunology and Allergy, Academic Center for Education, Culture, and Research (ACECR), Tehran, Iran

Corresponding authors
Saba Arshi; Morteza Fallahpour
Department of Allergy
Rasoul Akram Hospital
Iran University of Medical Sciences
Niayesh Street
Tehran 14496-14535, Iran
ORCIDs: 0000-0002-7277-3243;
0000-0002-5148-8312
E-mails: arshi.s@iums.ac.ir;
fallahpour.m@iums.ac.ir

IMPACT STATEMENT
There is no approved assay for predicting the SU development during OIT, however, our study showed a 50% reduction in sIgE may be a valuable predictor of SU achievement.

Summary

Background. *Immunotherapy may induce sustained unresponsiveness (SU) in which the patient can tolerate the allergen without any severe symptoms after discontinuing immunotherapy. The present study evaluated serum and cutaneous markers for predicting SU in patients with wheat anaphylaxis who underwent oral immunotherapy. We investigated the effectiveness of a flexible regimen of 5 to 10 g wheat protein (WP) in the maintenance phase of oral immunotherapy (OIT).* *Methods.* *This study was conducted on 19 patients with wheat anaphylaxis who underwent OIT. The results of the skin prick test (SPT), besides specific serum IgE (sIgE) and IgG4 (sIgG4) to WP, were evaluated before the desensitization. The maintenance dose started from the preferred dose of 5 to 10 g WP after the build-up phase, if the patient could tolerate it. All patients were recruited 7 to 9 months after undergoing this flexible regimen, and the results of SPT and sIgE, and sIgG4 levels were obtained once more. The patients underwent oral food challenge (OFC) after a 3-4-week avoidance to evaluate SU.* *Results.* *There was an association between mean IgE reduction and SU ($p < 0.0006$), while no association was observed between the mean increase in specific IgG4 ($p = 0.1$), and the mean wheal diameter decrease ($p = 0.29$). A 50% reduction in sIgE was associated with SU. Thirteen patients were considered to have a SU. There was no association between the flexible regimen and the desensitization rate.* *Conclusions.* *The reduction of 50% sIgE is a predictive factor for SU in patients with IgE-mediated wheat allergy.*

Introduction

Sustained unresponsiveness (SU) after oral immunotherapy (OIT) is defined as the ability to consume the desirable allergen after a period of 2 to 10 weeks of allergen avoidance in a patient with a history of anaphylaxis or type I hypersensitivity reactions to a food allergen (1, 2). So far, there is no data about the time required to achieve beneficial and long-lasting immune responses and unresponsiveness. The only gold-standard criterion for a definitive diagnosis of unresponsiveness is the discontinuation of the maintenance phase and implementation of oral food challenge (OFC) (3). From the viewpoint of allergic reactions, immunotherapy should be restarted at a low dose in cases of positive food challenges and a lack of tolerance. It cannot be immediately initiated at the previous maintenance dose. Overall, both challenges are time-consuming and risky and may result in anaphylactic reactions (4).

The prevalence of food allergy varies in different geographic areas (5). Overall, 0.3-1% of children are affected by wheat allergy (6). Anaphylactic reactions after exposure to the allergen commonly occur within minutes, up to several hours. Common symptoms include a range of skin symptoms, gastrointestinal disorders, respiratory disease, and sometimes anaphylaxis.

Oral immunotherapy relies on the consumption of gradually increasing doses of allergens to induce unresponsiveness (7). In all cases, there are two phases of desensitization: build-up and maintenance phase. In the build-up phase, patients gradually receive an increasing dosage of the specific allergens to reach the target dose, offering a rush schedule that accelerates the build-up phase to reach the target dose in several days, and a conventional schedule that takes several weeks to several months (8, 9). Although the purpose of immunotherapy differs from patient to patient, the main purpose is to provide tolerance with no severe type I hypersensitivity reaction.

It is worth noting that, after inducing wheat immunotherapy, permanent tolerance is not fully achieved in short term and allergic reactions may occur, especially after fever, illness, and exercise (10, 11). Re-challenge is needed for the evaluation of SU and tolerance. If the challenge is negative, the person develops tolerance or SU, while if the challenge is positive, the person remains sensitive. Various changes are observed in sIgE, sIgG especially sIgG4, cytokine levels and basophil surface marker during and after immunotherapy. These changes may be related to developing SU and tolerance (12-14); however, some of these factors are research-based, inaccessible, or cost-effective.

In wheat-sensitive individuals, specific IgE levels to wheat protein (WP) may decrease, stabilize, or more likely increase during the immunotherapy build-up phase and decrease at the end of OIT (15); however, there is no study to correlate these changes with predicting the development of SU to determine a threshold for this prediction. This study aimed to evaluate serum and cutaneous indicators of predicting SU in patients with anaphylaxis or type I hypersensitivity to wheat who underwent OIT. In most studies, the maintenance dose is fixed (16-18); however, there are numerous wheat flour products in Iran, and it is difficult for parents to maintain a fixed and accurate dose for a long time during the maintenance phase and accidental ingestion is common. Therefore, this study investigated the effectiveness of a flexible regimen of 5 to 10 g W) in the maintenance phase of oral immunotherapy (OIT).

Materials and methods

In this prospective interventional study, pre-and post-treatment evaluations were performed on 19 patients with a history of anaphylaxis reactions to wheat referred to Rasoul-e-Akram Hospital. Considering that no cut-off SPT wheal size and sIgE preclude OFC and in order to establish the lowest starting dose of OIT, open OFC was performed in the patients. The OFC was started with the initial dose of 10 mg and completed with a total dose of 5 g of WP. Objective or severe subjective signs and symptoms were noted in all 19 patients and the OFC was considered positive. These patients underwent OIT after performing skin prick test (SPT), assessing the specific wheat IgE and IgG4 levels.

In the build-up phase, we asked patients to use the Semolina flour and spaghetti, during which the intake was gradually increased up to a maximum of 5 g WP as the target dose. This amount is equivalent to roughly four medium slices of traditional Iranian bread (50 g) or 80 g of boiled spaghetti. Subsequently, the patients who tolerated 50 g of bread were tested for consuming another 50 g (including 0.5, 2, 5, 7.5, 10, 12.5, and 12.5 g, every 15 to 20 min) as the maintenance phase. If any positive anaphylaxis reaction (generalized urticaria, respiratory or gastrointestinal and/or cardiovascular symptoms) was observed at any stage, the challenge was discontinued, the results were recorded, and immunotherapy was continued with the predetermined maintenance dose (50 g of bread). Otherwise, if the patient could tolerate 100 g of bread, the fixed-dose limitation was removed, and the patient could consume the preferred dose of at least 50 g up to 100 g of bread or 80 to 160 g of boiled spaghetti daily.

All 19 patients were recruited 7-9 months later and the SPT, specific wheat IgE and IgG4 levels, to WP were assessed. Finally, the patients underwent a single-blind, placebo-controlled oral food challenge (SBPCFC) after a 3-4-week avoidance to evaluate SU. Specific wheat IgE and IgG4 levels were measured quantitatively using the ImmunoCAP system (Thermo Fisher-Phadia, Uppsala, Sweden). Wheal diameter of SPT (Stallergenes Greer, USA) was measured by the mean largest and smallest diameters, and the wheal diameters more than 3 mm considered positive. This study was approved by the

Ethics Committee of Iran University of Medical Sciences (IR.IUMS.FMD.REC.1398.099). Written informed consent was obtained from the subjects prior to study initiation. These patients were recruited for this clinical trial study (IRCT20190612043872N1) in February 2019. The sample size for this study was calculated, considering this is a pilot study. This study was designed without a control group.

Statistical analysis

Statistical analyses were carried out using the Statistical Package for the Social Science (SPSS) version 20.0 (SPSS Inc., Chicago, Illinois, USA). The results were expressed as mean and standard deviation (mean ± SD) for the quantitative variables and as percentages for the qualitative variables. A comparison between quantitative variables was performed with the paired t-test and P-values less than 0.05 were considered statistically significant.

Results

A total of 19 patients with wheat allergy (6 females, 13 males) were included in this study with a mean age of 7.42 ± 3.6 years.

At the end of the study, 13 patients showed SU and were able to discontinue daily use of the regimen. The mean duration of OIT was 15.30 ± 0.94 months in these 13 patients. Demographic information and laboratory findings are summarized in **tables I**, **II**. The mean sIgE was 122.988 Ku/L before immunotherapy and it decreased to 16.4 Ku/L and 91.4 Ku/L in patients who achieved SU and the ones who did not, respectively. The results showed that there was a significant association between the mean IgE reduction and SU ($p < 0.0006$). The mean sIgE decreased to 65.68 Ku/L with 100% specificity and sensitivity in the patients who achieved SU (**table III**). The mean sIgG4 prior to OIT was 46.94 Ku/L, which increased to 99.6 Ku/L in the patient who developed SU, while the mean sIgG4 was 55.8 Ku/L in those who did not develop SU. There was no significant association between the mean increase in specific IgG4 and SU ($p = 0.1$). Nevertheless, data analysis revealed a significant association between the mean reduction of sIgE/sIgG4 and SU ($p = 0.0069$).

Furthermore, the mean size of wheal in the prick test was 10.9 mm; however, it decreased to 5 mm and 8.1 mm after OIT in the patients who developed and did not develop SU, respectively.

Table I - *Demographic characteristics of the patients.*

Patients	Onset of OIT (year)	Buildup time (week)	Tolerance of flexible MD	Sustained unresponsiveness	OIT time (month)
1	16.6	26	Yes	Yes	14
2	9	26	Yes	Yes	14.5
3	5.3	24	No	No	17
4	3.3	27	No	Yes	16
5	4.6	27	Yes	Yes	16
6	6.6	25	Yes	Yes	15
7	12.2	27	Yes	Yes	16
8	8.1	28	No	Yes	16.5
9	4.2	28	No	No	15
10	2.9	29	No	No	15.5
11	4	27	Yes	Yes	15.5
12	6.2	28	Yes	Yes	16.5
13	6.6	27	Yes	Yes	15
14	10.6	28	Yes	Yes	14
15	7.8	28	Yes	Yes	16
16	12	25	Yes	Yes	14
17	4.11	29	Yes	No	15
18	10.5	28	Yes	No	15
19	2.4	30	No	No	17

OIT: oral immunotherapy; MD: maintenance dose.

Table II - Laboratory findings of the patients before OIT (first time) and the end of the study (second time).

Patients	SPT first time (mm)	SPT second time (mm)	SIgE first time (Ku/L)	SIgE second time (Ku/L)	SIgG4 first time (Ku/L)	SIgG4 second time (Ku/L)
1	11	4	96.22	8.24	110	131
2	16	8	140	31.12	101	108
3	8	3	184	84.4	118	120
4	4	2	108	0.83	6	11
5	10	7	100	34.6	0.3	18
6	6	4	101	24.6	7.43	132
7	9	2	89	18.2	11.2	101
8	11	6	98	9.42	117	151
9	15	10	220	82.2	100	109
10	16	13	110	78.86	2.1	5.2
11	9	3	89.98	0.31	4.2	107
12	12	6	100	21.8	6.24	98.3
13	13	5	122	11.28	100	201
14	14	8	99	34.22	99.1	221
15	10	6	142	18.24	100	102
16	8	5	92.40	0.35	0.1	104
17	16	6	114	88.9	9.2	22.1
18	11	10	160	54.45	0	18.24
19	8	7	182	160	0	2.5
P-value	0.29		< 0.0006		0.1	

OIT: oral immunotherapy; SPT: Skin Prick Test; sIgE: specific IgE; sIgG4: specific IgG4.

No significant association was found between the wheal diameter of prick test and final unresponsiveness (p = 0.29). Thirteen patients out of 19 patients could tolerate a two-fold dose and non-fixed regimen in the maintenance phase and 6 cases failed to tolerate 10 g WP and had to continue the previous dose after build-up phase. Out of 13 patients who achieved SU, 11 patients could tolerate the flexible regimen of 50 to 100 g of bread (p = 0.027). Moreover, there was no significant difference between the desensitization rate and flexible regimen (p = 0.44).

Discussion

In the present study, we evaluated the indicators for SU prediction in patients with wheat anaphylaxis who underwent OIT, as well as the ways to speed up the successful immunotherapy process. The important point is that specific IgG1 and IgG4 levels increase during wheat OIT, while this increase is slight in the build-up phase, which is considerable during the maintenance phase (15). The diameter of the wheal in SPT is also decreased in patients after the immunotherapy (3). In our study, the level

Table III - Sensitivity and specificity of sIgE level in association with SU.

Cutpoint sIgE (IU/ml)	Sensitivity (%)	Specificity (%)	Correctly classified (%)
31.14	100	33.33	78.95
65.68	100	100	100
92.05	38.46	100	57.89

of sIgE decreased, and although IgG4 levels increased after the immunotherapy, this increase was not significant.

As Sampson *et al.* showed, the specific IgE (sIgE) above 100 KU/L is associated with a 100% positive predicted value (PPV) for food sensitivity to wheat which eliminates the need for OFC, and the sIgE less than 0.35 KU/l is associated with a very rare chance of an allergic reaction in these individuals (19). This means that there is a possibility of a threshold for SU and consequently the termination of immunotherapy. In another study, Shek *et al.* demonstrated that a 50% reduction of IgE in egg and milk developed a good tolerance (20).

Overall, in the present study changes of wheal diameter and sIgG4 before and after OIT did not predict SU, and this might be due to the low sample size; however, we have demonstrated that there is a relationship between the degree of decrease in wheat sIgE concentrations and sIgE/sIgG4 ratio after OIT and the likelihood of developing SU. The mean sIgE was reduced to nearly half, exactly from the first mean of 122.988 Ku/L to the second mean of 65.63 Ku/L, in patients who achieved SU with the sensitivity and specificity of 100%. On the other hand, the clinician may assess the SU whenever the wheat sIgE levels fell to 50% and eliminate time wasted, risks and expense.

Del Rio *et al.* indicated that their patients could tolerate more than two-fold routine maintenance dose of wheat (13 g WP) without serious systemic reactions, while most studies use, on average, 5.2 g of WP as a maintenance dose (15). The desensitization rate decreased when we used very low-dose OIT (2, 3, 21). One possible explanation is that persistent high-dose exposure to a special antigen preferentially stimulates IL-10 production that suppresses the immune system, but intermittent exposure to another antigen stimulates IL-4 production, which triggers or augments allergic reactions (22).

We investigated the various maintenance doses between the minimum of 5 g to the maximum of 10 g of WP according to the patient's preference. Thirteen out of 19 patients could tolerate a two-fold dose; thus, the limitation of fixed-dose consumption was removed. Of 13 patients who achieved SU, 11 patients received the flexible-dose OIT, which indicates that the immunotherapy with a flexible regimen develops the patient's satisfaction; however, desensitization rate was not affected by this regimen compared with a fixed-dose regimen. This may be due to using minimum doses of WP in a daily diet. The advantage of this study is the participants' high satisfaction with the flexible regimen. Furthermore, the results of this study can be applied to different allergens such as peanut, which is more common in other geographical areas.

The possibility of estimating the appropriate time for assessing SU regarding the reduction of nearly half of the sIgE compared to its initial value to terminate OIT is another strength of this study. However, some limitations of the present study should be acknowledged. The small sample size of this research limited its generalizability. Thus, it is recommended for future studies to enroll a larger sample size to confirm the results of this study. Aside from that, most patients report dissatisfaction with avoiding allergens for more than four weeks to assess SU; however, a 2-week avoidance has been used to assess SU in some trials (1, 2).

This study investigated flexible regimens (a regimen between 5 to 10 g wheat protein if the patient can tolerate it, depending on their preference) rather than fixed ones during the maintenance phase. The current study cannot determine the exact time of achieving SU. However, reduction in sIgE and SIgE/sIgG4 may predict tolerance and unresponsiveness time. In the current study, more than 50% reduction in sIgE has been associated with SU; however, there is no significant association between IgG increase and wheel diameter reduction in the prick test. Moreover, changing a fixed-dose regimen to a flexible regimen between the minimum dose and a two-fold dose (if the patient tolerates it) may increase the patient's satisfaction and the chance of achieving SU. Altogether, this protocol may be effective and safe while decreasing severe anaphylactic reactions.

Fundings

Contributions

AP, MF: conceptualization, writing – original draft. SA, MN, MHB: administrative, technical, and material support. AP, SSh, ShKh: statistical analysis and data interpretation. MF: supervision. FS: writing – review and editing (for important intellectual contents). All authors: final approval.

Conflict of interests

The authors declare that they have no conflict of interests.

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