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

Ali Pourvali[1], Saba Arshi[2*], Mohammad Nabavi[2], Mohammad Hasan Bemanian[2], Sima Shokri[2], Sholeh Khajoei[3], Farhad Seif [4,5], Morteza Fallahpour [2*]

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 Immunology and Allergy, Academic Center for Education, Culture, and Research (ACECR), Tehran, Iran
5- Neuroscience Research Center, Iran University of Medical Sciences, Tehran, Iran

Corresponding authors: Saba Arshi and Morteza Fallahpour, **Email**: arshi.s@iums.ac.ir and fallahpour.m@iums.ac.ir, Tel/fax: 00982164352383, Address: Tehran, Sattar Khan St., Niayesh St., Rasoul Akram Hospital.

*These authors have contributed equally as the corresponding author to this work.

Running title: Sustained unresponsiveness in oral wheat immunotherapy

Abstract

Background and aim: 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 .Moreover, 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 result 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 ($p=0.29$). In the present study, a 50% reduction in sIgE was associated with SU. Thirteen patients were considered to have a SU. Moreover, there was no association between the flexible regimen and the desensitization rate.

Conclusion: The results revealed that the reduction of 50% sIgE is a predictive factor for SU in patients with IgE-mediated wheat allergy.

Keywords: Maintenance phase, Permanent tolerance, Anaphylaxis, Wheat desensitization, Sustained unresponsiveness

An impact state: 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.

Ali Pourvali: 0000-0002-1529-8175

Saba Arshi: 0000-0002-7277-3243

Mohammad Nabavi: 0000-0001-6558-0390

Mohammad Hasan Bemanian: 0000-0003-0998-4651

Sima Shokri: 0000-0002-2176-3934

Sholeh Khajoei: 0000-0002-8833-1941

Farhad Seif: 0000-0003-1275-2157

Morteza Fallahpour: 0000-0002-5148-

Introduction

Sustained unresponsiveness (SU) after oral immunotherapy (OIT) in a patient with a history of anaphylaxis or type one hypersensitivity reactions to a food allergen is defined as the ability to consume the desired allergen after period of 2 to 8 weeks of avoidance while natural tolerance not achieve(1).There is no data until now about the time required to achieve beneficial and long-lasting immune responses and unresponsiveness.The only gold standard criteria for definitive diagnosis of unresponsiveness is discontinuation of maintenance phase for at least 2 to 8 weeks and doing oral food challenge (OFC) again (1,2,3).

From the viewpoint of allergic reactions, in case of positive food challenge and the lack of tolerance, immunotherapy should be restarted at a low dose and cannot be immediately initiated at the previous maintenance dose. Overall, both challenges are time-consuming and risky and may result in anaphylactic reactions (2, 3 ,4). The prevalence of food allergy varies in different geographic areas (5) and, the prevalence of wheat allergy has been reported to be between 0.3 and 1 in children(6).Anaphylactic reactions after the exposure to the allergen usually 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 and desensitization (7),In all cases, there are two phases of desensitization: build-up and maintenance phase.In the build-up phase, patients are gradually receiving an increasing dosage of the specific allergens to reach the target dose, offering a rush schedule which accelerates the build-up phase to reach the target dose in several days, and a conventional schedule which takes several weeks to several months (8,9). The purpose of immunotherapy differs from patient to patient.However, the main purpose is to provide a

permanent tolerance with no serious type I hypersensitivity. Albeit, the priority is achieving a possible SU period.

After inducing wheat immunotherapy, especially wheat-dependent exercise-induced anaphylaxis (WDEIA), permanent tolerance is not fully achieved in short term, and the patient may experience mild symptoms but no sign of anaphylaxis is observed (10). Re-challenge is needed for the evaluation of sustained unresponsiveness and tolerance (11). If the challenge is negative, the person develops tolerance or SU, while if the challenge is positive, the person remains sensitive.A number of changes are observed in sIgE,sIgGespecially sIgG4, cytokine levels and basophil surface marker during and after immunotherapy. These changes may be related to developing SU and tolerance (12,13, 14) However, some of these factors are research-based, inaccessible, or cost-effective.

In wheat-sensitive individuals, specific Ig E levels to wheat protein may decrease, stabilize, or more likely increase during the immunotherapy build-up phase (15)

There is no study to correlate these changes with SU prediction and to determine a threshold for this prediction.This study aimed to evaluated serum and cutaneous indicators for predicting SU in patients with anaphylaxis or type I hypersensitivity to wheat who underwent OIT.Moreover, this study investigates the unlimited regimens (a regimen between the minimum of maintenance dose and two-fold, depending on the patient's request) instead of the fixed ones during the maintenance phase, to see whether the patients possibly achieve SU faster.This is a novel study that evaluates such regimen and its effects on immunotherapy.

Materials and Methods

In this study, pre-and post-treatment evaluations were carried out on 19 patients with a history of anaphylaxis reaction to wheat who had been referred to Rasoul-e-Akram Hospital and

underwentOIT after skin prick test (SPT), the assessment of specific serum wheat IgE and IgG4 levels and positive oral food challenge.Thiscross-sectional interventional study was approved by the Ethics Committee of Iran University of Medical Sciences (IR.IUMS.FMD.REC.1398.099).These patients were recruited for this clinical trial study (IRCT20190612043872N1) from February 2019.The sample size for this study was calculated considering this is a pilot study.

The patients passed the build-up phase using semolina flourand spaghetti,which is gradually increased up to a maximum of 50 g (four medium slices) bread as the target dose.Subsequently, the patients who were able to tolerate 50 g of bread, were tested to consume another 50 g (including 0.5, 2, 5, 7.5, 10, 12.5, and 12.5 g every 15 to 20 min) as maintenance phase.If any positive reaction is observed at any stage, the challenge is discontinued, the results are recorded, and the patient continues to consume the predetermined maintenance daily dose of about 50 g of bread.Otherwise, if the patient can tolerate the dose, the fixed-dose limitation is removed and the patient can consume desired dose at least 50 g up to 100 g of fresh bread or pasta on a daily basis. All 19 patients were recruited 7-9 months later and specific wheat IgE and IgG4 levels to total wheat protein were assessed. The SPT was also done for all patients. Finally, the patients underwent single-blind, placebo-controlled oral food challenge (SBPCFC) after 3-4 a week avoidance, to evaluate sustained unresponsiveness. Specific wheat IgE and IgG4 levels were measured quantitatively by ImmunoCAP method and wheal diameter of SPT was measured by the mean largest and smallest diameters.

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 and the mean patients' age was 7.42±3.6 years. Demographic information and laboratory findings are summarized in table1, 2.The results showed that there was a significant association between mean IgE reduction and SU ($p < 0.0006$). The mean sIgE before beginning of immunotherapy was 122.99 and in the patients who achieve SU sIgE was 65.68 with 100% specificity and sensitivity (Table3). While there was no significant association between the mean increase in specific IgG4 and SU ($p=0.1$).

Furthermore, 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 unlimited regimen in the maintenance phase and 6 cases had to continue the previous dose and failed to tolerate unlimited regimen. At the end of thestudy, of 19 patients, 13 patients showed SU and were able to discontinue daily use and the mean duration of SU was 15.44±0.95 months in these 13 patients. Out of 13 patients who achieved SU, 11 patients were able to tolerate an unlimited regimen of 50 to 100 g bread or spaghetti ($p= 0.027$).

Discussion

In this study, we evaluated the indicators of SU prediction in patients with wheat anaphylaxis who were undergoing OIT, as well as the ways to speed up the successful immunotherapy time.The important point is that specific IgG1 and IgG4 levels increase during wheat OIT and while this increase is slight in the build-up phase, it is considerable during the maintenance phase

(15). The diameter of the whealin SPT is also decreased in patients after the immunotherapy (2). In our study, the level of sIgE decreased, and although IgG4 levels increased after the immunotherapy, this increase was not significant.

As Sampsonet al., showed the specific IgE (sIgE) above 100 KU/L is associated with 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 (16), Which means that there is a possibility of a threshold for SU and consequently the termination of immunotherapy.Overall, in our study, wheal diameter and sIgG4 changes did not predict SU and this might be due to low sample size. However, sIgE changes predict this.The sensitivity and specificity of 100% in sIgE was reduced to almost half, exactly from the first mean of 122.988 to 65.63, in patients who achieved tolerance.

Shek et al.'s study demonstrates that a 50% reduction of IgE in egg and milk, develops a good tolerance (17).Nevertheless, greater sample size is needed in our study to observe the effect of IgE reduction in wheat.As the hypothesis that higher cumulative allergen dose in OIT may lead to higher rates of allergy remission (18), we investigated the various maintenance doses between the minimum of 50 g to the maximum of 100 g wheat or spaghetti according to the patient's request. Thirteen out of 19 patients could tolerate two-fold dose and the limitation of fixed-dose consumption was removed.

Of 13 patients who achieved SU, 11 patients were received the variant dose, which indicates that the immunotherapy with a flexible regimen develop the patient's satisfaction along with a greater SU compared with a fixed-dose regimen.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 (19). However, immunotherapy may also depend on the time exposure along with the dose of antigen. At the end of the study, all 6 patients who could not tolerate a two-fold dose at the beginning of maintenance phase,while were challenged with a two-fold maintenance dose and all of them were able to tolerate the new dose. It seems that all patients can tolerate more than usual maintenance dose at a special time during the maintenance phase. In other words, 13 patients finally developed sustained unresponsiveness to clarify the exact mechanisms of treatment effect, it is necessary to study diverse populations with a larger sample size.

Conclusion

The current study cannot determine the exact time ofachieving SU. However, reduction in sIgE may be a predictor of tolerance and unresponsiveness time. In our study, reduction of more than 50% in sIgE has been associated with sustained unresponsiveness; however, there is no significant association between IgG increase and wheel diameter reduction in prick test.Moreover, changing a fixed-dose regimen to a desired regimen between the minimum dose and a two-fold dose (if the patient tolerates) may increase the patient's satisfaction and the chance of achieving sustained unresponsiveness.

Funding/Support

This study received no funding or support.

Conflict of interest

All authors have no conflict of interest.

Ethical considerations

The authors have obtained written informed consent from the subjects prior to study initiation.

References

1. Jones SM, Burks AW, Keet C, Vickery BP, Scurlock AM, Wood RA, Liu AH, Sicherer SH, Henning AK, Lindblad RW, Dawson P. Long-term treatment with egg oral immunotherapy enhances

sustained unresponsiveness that persists after cessation of therapy. Journal of Allergy and Clinical Immunology. 2016 Apr 1;137(4):1117-27.
2. Rekabi M, Arshi S, Bemanian MH, Rekabi V, Rajabi A, Fallahpour M, et al. Evaluation of a new protocol for wheat desensitization in patients with wheat-induced anaphylaxis. Immunotherapy. 2017;9(8):637-45.
3. Vickery BP, Berglund JP, Burk CM, Fine JP, Kim EH, Kim JI, et al. Early oral immunotherapy in peanut-allergic preschool children is safe and highly effective. Journal of Allergy and Clinical Immunology. 2017;139(1):173-81. e8.
4. Salari F, Bemanian MH, Fallahpour M, Tavakol M, Shokri S, Baniadam L, Khoshmirsafa M, Seif F, Nabavi M, Arshi S. Comparison of Diagnostic Tests with Oral Food Challenge in a Clinical Trial for Adult Patients with Sesame Anaphylaxis. Iranian Journal of Allergy, Asthma and Immunology. 2020 Feb 1:27-34.
5. Lyons SA, Clausen M, Knulst AC, Ballmer-Weber BK, Fernandez-Rivas M, Barreales L, Bieli C, Dubakiene R, Fernandez-Perez C, Jedrzejczak-Czechowicz M, Kowalski ML. Prevalence of food sensitization and food allergy in children across Europe. The Journal of Allergy and Clinical Immunology: In Practice. 2020 Sep 1;8(8):2736-46..
6. Kattan J. The prevalence and natural history of food allergy. Current allergy and asthma reports. 2016;16(7):47.
7. Gernez Y, Nowak-Węgrzyn A. Immunotherapy for food allergy: are we there yet?. The Journal of Allergy and Clinical Immunology: In Practice. 2017 Mar 1;5(2):250-72..
8. Pérez-Rangel I, del Río PR, Escudero C, Sánchez-García S, Sánchez-Hernández JJ, Ibáñez MD. Efficacy and safety of high-dose rush oral immunotherapy in persistent egg allergic children: A randomized clinical trial. Annals of Allergy, Asthma & Immunology. 2017 Mar 1;118(3):356-64.
9.Bégin P, Dominguez T, Wilson SP, Bacal L, Mehrotra A, Kausch B, Trela A, Tavassoli M, Hoyte E, O'Riordan G, Blakemore A. Phase 1 results of safety and tolerability in a rush oral immunotherapy protocol to multiple foods using Omalizumab. Allergy, Asthma & Clinical Immunology. 2014 Dec 1;10(1):7.
10. Furuta T, Tanaka K, Tagami K, Matsui T, Sugiura S, Kando N, Kanie Y, Naito M, Izumi H, Tanaka A, Sjölander S. Exercise-induced allergic reactions on desensitization to wheat after rush oral immunotherapy. Allergy. 2020 Jun;75(6):1414-22.
11. Patil SU, Steinbrecher J, Calatroni A, Smith N, Ma A, Ruiter B, Virkud Y, Schneider M, Shreffler WG. Early decrease in basophil sensitivity to Ara h 2 precedes sustained unresponsiveness after peanut oral immunotherapy. Journal of Allergy and Clinical Immunology. 2019 Nov 1;144(5):1310-9.
12. Santos AF, Du Toit G, Douiri A, Radulovic S, Stephens A, Turcanu V, et al. Distinct parameters of the basophil activation test reflect the severity and threshold of allergic reactions to peanut. Journal of Allergy and Clinical Immunology. 2015;135(1):179-86.
13. Hardy LC, Smeekens JM, Kulis MD. Biomarkers in food allergy immunotherapy. Current allergy and asthma reports. 2019 Dec;19(12):1-9.

14. Du Toit G, Sampson HA, Plaut M, Burks AW, Akdis CA, Lack G. Food allergy: Update on prevention and tolerance. Journal of Allergy and Clinical Immunology. 2018;141(1):30-40.
15. del Rio PR, Díaz-Perales A, Sanchez-García S, Escudero C, do Santos P, Catarino M, et al. Oral immunotherapy in children with IgE-mediated wheat allergy: outcome and molecular changes. J Investig Allergol Clin Immunol. 2014;24(4):240-8.
16. Sampson HA. Utility of food-specific IgE concentrations in predicting symptomatic food allergy. Journal of Allergy and Clinical Immunology. 2001;107(5):891-6.
17. Shek LP, Soderstrom L, Ahlstedt S, Beyer K, Sampson HA. Determination of food specific IgE levels over time can predict the development of tolerance in cow's milk and hen's egg allergy. Journal of allergy and clinical immunology. 2004 Aug 1;114(2):387-91.
18. Loh W, Tang ML. Debates in Allergy Medicine: Oral immunotherapy shortens the duration of milk and egg allergy-the con argument. World Allergy Organization Journal. 2018;11(1):1-7.

19. Maggi E, Vultaggio A, Matucci A. T-cell responses during allergen-specific immunotherapy. Current opinion in allergy and clinical immunology. 2012;12(1):1-6.

Table1. 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 2. 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

Table 3. 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