

Oral Wheat Immunotherapy: Long-Term Follow-Up in Children with Wheat Anaphylaxis

Delara Babaie^a Motohiro Ebisawa^b Habib Soheili^c Ramin Ghasemi^d
Fariborz Zandieh^e Mitra Sahragard^f Hamideh Seifi^a Mazdak Fallahi^a
Majid Khoshmirsafa^g Sepideh Darougar^h Mehrnaz Mesdaghi^a

^aDepartment of Allergy and Clinical Immunology, Mofid Children's Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran; ^bClinical Research Center for Allergy and Rheumatology, National Hospital Organization, Sagami-hara National Hospital, Sagami-hara, Japan; ^cDepartment of Allergy and Immunology, Medical Center Hospital, Tehran University of Medical Sciences, Tehran, Iran; ^dAllergy Clinic, Isabne-Maryam Hospital, Isfahan University of Medical Sciences, Isfahan, Iran; ^eDepartment of Pediatric Allergy and Immunology, Bahrami Children Hospital, Tehran University of Medical Sciences, Tehran, Iran; ^fDepartment of Pediatrics, Shahid Beheshti University of Medical Sciences, Tehran, Iran; ^gImmunology Department, School of Medicine, Iran University of Medical Sciences, Tehran, Iran; ^hDepartment of Pediatrics, Tehran Medical Sciences Branch, Islamic Azad University, Tehran, Iran

Keywords

Anaphylaxis · Desensitization · Diet · Food allergy · Hypersensitivity

Abstract

Introduction: There has been substantial increase in food allergies in recent decades. The management of severe food allergy often includes strict avoidance and medical therapies. However, oral immunotherapy (OIT) is a promising treatment option for these patients, which is still being investigated. **Methods:** The study recruited children from 2 years onward with a history of wheat anaphylaxis who had been referred to the Mofid Children Hospital. Wheat allergy was confirmed by a double-blind placebo-controlled food challenge. OIT was started to reach 5.28 g of wheat protein supplied in 60 g of bread. Besides immunologic measurements, a second and third oral food challenge (OFC) was performed after 3 months and 1 year of maintenance therapy to evaluate the long-term efficacy of wheat OIT (WOIT). **Results:** Seventeen patients completed the 3-month main-

tenance phase; 8 of them demonstrated negative OFCs. All of the 9 with positive OFCs were asked to continue the daily consumption of 60 g of bread for another year. Three patients with positive OFCs were followed for 1 more year and were asked to continue eating 60 g of bread every other day. The serum level of wheat sIgE was significantly increased at the end of the buildup phase ($p = 0.026$) and dramatically dropped at the end of the maintenance phase ($p = 0.022$). **Conclusion:** To conclude, WOIT is an effective and safe modality of treatment if it is administered under strict supervision.

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Introduction

Food allergy is the most prevalent allergic disorder among infants with an increasing incidence worldwide [1], which has recently peaked at 10% [2, 3]. Approxi-

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Table 1. The amount and duration of bread ingestion in the DBPCFC in patients with wheat allergy

Time, 24 h	0	15	30	45	60
Single dose					
Cake	1/14*	1/14	1/7	2/7	3/7
Cumulative dose					
Cake	1/14	1/7	2/7	4/7	1
Wheat Protein, g	0.08	0.17	0.35	0.70	1.3

DBPCFC, double-blind placebo-controlled food challenge.
* Portion of bread used in the DBPCFC.

mately 5% of children have severe food allergies, which may result in life-threatening anaphylaxis [2, 4]. Over 80% of food allergies are due to the consumption of milk, egg, soy, wheat, peanut, tree nuts, fish, and shellfish, which can vary depending on geographical location and feeding pattern [3].

Strict avoidance and medical therapy are the main management strategies for patients with food allergies. Currently, oral immunotherapy (OIT) is an alternative treatment approach with promising results [5]. OIT was first reported as early as 1908 in the *Lancet* [6] and was not widely accepted until much later [7]. OIT refers to the sequential administration of increasing amounts of an allergen to diminish the allergic responses on future exposures [8]. The ultimate goal of this approach is to induce tolerance [9] so that any accidental or intentional re-exposures would not induce an untoward reaction. Immune tolerance is the substantial basis of unresponsiveness which can be induced through oral, topical, and inhaled routes [10]. Peripherally induced antigen-specific CD4⁺CD25⁺Foxp3⁺ Tregs, T-cell anergy, and IgA/IgG play important roles in the development of oral tolerance [11].

Worldwide, wheat is a widely consumed grain [12]; therefore, gluten-related disorders are common. Wheat allergy is usually caused by an IgE-mediated reaction with a generally favorable prognosis in pediatric patients [13]. Although OIT has a century-long history, preliminary studies indicate that it is only recently being used as a therapeutic approach in children with wheat allergies [14–16]. Hence, each patient should undergo a careful assessment of the risk/benefit ratio. While the efficacy and safety of this mode of treatment have yet to be proved for all food allergens, this study provides further evidences on the efficacy and safety of OIT in patients with wheat anaphylaxis who received wheat OIT and were followed for a considerable period of time.

Patients and Methods

Study Design

Patient Selection

Children from 2 years onward with a history of wheat anaphylaxis were recruited to the study. All patients underwent a skin prick test (SPT) to wheat flour extract (Greer, USA). Wheals ≥ 3 mm larger than the negative control were considered positive. Wheat allergy in these patients was confirmed by using a double-blind placebo-controlled food challenge (DBPCFC).

Inclusion and Exclusion Criteria

Children 2 years and older with history of anaphylaxis to wheat, positive SPT to wheat extract, and positive double-blind placebo control test were eligible. Those with severe and uncontrolled asthma, significant systemic diseases, poor compliance, or unwillingness to participate in the study were excluded.

Double-Blind Placebo-Controlled Food Challenge

In the double-blind placebo-controlled food challenge, patients received 35 g of cake that consisted of a mixture of rice and corn flour as a placebo or the same amount of cake containing 1.3 g of wheat protein without premedication on different days. Table 1 illustrates how 14 pieces of 2.5 g cake were given to patients at 15-min intervals. The challenge was considered positive when symptoms such as generalized urticaria or respiratory and gastrointestinal symptoms were elicited.

OIT Protocol

The study was conducted at the Mofid Children Hospital, Tehran, Iran, between November 2015 and October 2019. Visits and OIT were done at the outpatient allergy clinic and food challenge ward. Those with adverse reactions remained in the ward for 4–6 h or were transferred to the emergency room if needed. The study consisted of a buildup phase followed by a maintenance phase. The starting dose of OIT was determined based on the threshold of reaction in the oral food challenge (OFC) which is shown in Table 2. Reactions to challenges were classified as mild, moderate, and severe [17, 18], and medications were administered accordingly. For patients with moderate allergic reactions, OIT was initiated with the same or a slightly higher cumulative dose, whereas in those with severe symptoms, the starting dose was reduced to the previous level (Table 2). OIT was carried out to reach a wheat protein content of 5.28 g per 60 g of bread. The protein content of wheat flour (Tarkhine Co., Tehran, Iran) was determined by the Nutrition Institute of Shahid Beheshti University of Medical Sciences. All patients were instructed to prepare cake and bread using the same flour during buildup and maintenance phases. All patients continued to consume 60 g of bread every day for 3 months after the buildup phase (maintenance phase). Another OFC was performed after 2 weeks of wheat avoidance at the end of the maintenance phase. The OFC was also repeated after a 1-year maintenance phase. Desensitized patients were followed for 1 more year to ensure whether they have achieved sustained unresponsiveness (SU).

Buildup Phase

The starting dose of OIT in each patient was calculated according to OFC results. Antihistamines were held for at least 5 days prior to the OFC. The challenge procedure started with a piece of cake weighing 35 g containing 1.3 g of wheat protein and continued

Table 2. Wheat OFC to determine the threshold and the starting dose of OIT

Time, 24 h		0	15	30	45	60
OFC	Single dose, g					
	Cake	2.5	2.5	5	10	15
	Cumulative dose					
	Cake	2.5	5	10	20	35
	Protein, g	0.08	0.17	0.35	0.70	1.3
Level of threshold eliciting systemic reactions in OFC		A1	2	3	6	B3
Starting level of WOIT		A1*	A2(A1)	A3(A2)	A5 (B1) [‡]	B3(B2)

WOIT, wheat oral immunotherapy; OFC, oral food challenge; OIT, oral immunotherapy.

* In case of systemic reactions, we started from 1 step before the eliciting step. In the case of severe reactions or unacceptable doses, we started from half dose of A level. [‡]The protein content of A5 is equal to B1. In the case of mild reactions, we moved to B1. In the case of moderate reactions, we started from cake (A5).

Table 3. OIT steps in the buildup phase

Level*		1	2	3	4	5
A	Cake, 250 g	2.5	5	10	20	28
	Protein, 8.8 g	0.08	0.17	0.31	0.62	0.88
B	Bread, 100 g	16	20	25	30	
	Protein, 5.28 g	0.88	1.05	1.32	1.58	
C	Bread, 100 g	18	25	40	50	60
	Protein, 8.8 g	1.58	2.2	3.52	4.4	5.28

OIT, oral immunotherapy. * Level A consists of 250 g of cake which contains 100 g of wheat flour (8.8 wheat protein). Level B: 100 g of bread which was made by mixed flour containing 5.28 g of wheat protein in 100 g of flour. Level C: 100 g of bread with 8.8 g of wheat protein.

at 15-min intervals (Table 2) until an obvious allergic symptom was provoked. At this point, the OFC was stopped, and the cumulative tolerated dose was defined as the threshold in which the patient revealed allergic symptoms. The provoked symptoms were classified as mild, moderate, and severe and treated accordingly [19].

Table 3 is a summary of buildup phase applied for wheat OIT in this study. The buildup phase consisted of 3 levels: A, B, and C. The patients in level A consumed the respective quantity of cake that was made from 100 g of wheat flour (Tarkhine Co., Iran) by the same formula for all of them and weighing 250 g. In level B, patients started bread which was prepared with a mixture of wheat and rice flour and contained 5.28 g of wheat protein in 100 g; in due course, it was changed to full-wheat flour bread (8.8 g of wheat protein in 100 g flour) at level C. Tolerance was defined as the ability to consume 5.2 g of wheat protein on a daily basis without experiencing any symptoms.

Patients attended the clinic twice a week for up-dosing during the buildup phase. In this phase, the duration varied based on the starting dose (which could be anywhere between level A and B1 [Table 3]) and the severity and number of reactions that occurred

during this phase. Doses were adjusted by trained specialists and were prepared by experienced nurses according to the established protocol. Any reactions were monitored for at least 4 h, and in the case of severe reactions, patients were admitted. Symptomless patients were sent home after an hour of close supervision. The daily home dosing was continued until the next escalation visit. Patients or caregivers were taught how to measure out the determined quantities of cake or bread using their own scales.

When they reached the change level, the first step was to add the same amount of wheat protein but in a new bread form. In those who developed reactions during the buildup phase, the dose was either repeated or returned to the previous step, depending on the severity of the reactions. We provided half-dose steps in cases of severe reactions.

Maintenance Phase

After completing the buildup phase, all patients were required to consume 60 g of bread a day for 3 months as the maintenance dose. They also had to follow a wheat elimination diet.

Caregivers were asked to fill a questionnaire about the time of consumption of bread, complications following wheat consumption, and use of prescribed rescue medications. Exercise was prohibited 2 h before and after the wheat ingestion to ensure safety. An action plan, as well as a rescue kit containing 2 EpiPen auto-injectors, corticosteroids, and antihistamines, was provided for each patient to manage any severe adverse reactions. They had to keep the kits for at least 1 year after SU.

Food Challenge

At the end of the maintenance phase, patients underwent the OFC after a 2-week period of complete wheat avoidance. Those who passed the test successfully were considered to have a sustained unresponsive state, allowing them to eat wheat products without restriction. Those with positive OFCs were asked to continue consuming 5.2 g of wheat protein on a daily basis for 1 year. At the end of the year, another OFC was performed after a 2-week complete wheat avoidance. Those who passed the OFC this time were deemed tolerant. Those who still had positive OFCs were asked to continue eating a minimum of 60 g of wheat every day for another year, and wheat avoidance and the OFC were applied again.

Immunologic Investigations

Wheat-Specific IgE Concentrations and SPT

A SPT to wheat, rye, oat, and barley (Greer Co., USA) was performed before OIT, at the end of the buildup phase and after the 3-month maintenance phase. Serum-specific IgE (sIgE) to wheat using the ImmunoCAP100 system was measured before OIT, after the completion of the buildup phase and at the end of the maintenance phase (3 months).

Statistical Analysis

Statistical analysis was performed with SPSS version 23.0 (SPSS, Inc., Chicago, IL, USA). Data are expressed as mean $\pm$ SD. Differences between before OIT, end of the buildup phase, and end of the maintenance phase were analyzed with paired *t*-tests and the one-way analysis of variance test (repeated measure). Data were presented using Prism 6.01 software (GraphPad, San Diego, CA, USA), and a value of $p < 0.05$ was considered statistically significant.

Ethical Consideration

The study was conducted according to the principles of the Declaration of Helsinki and approved by the Iranian Registry of Clinical Trials with the reference code of IRCT: 2013081214305 N2. The study design and likely risks were fully explained to the patients' caregivers. Informed consent was obtained from all patients' guardians.

Results

Twenty-five children aged 2 years and over with a history of wheat anaphylaxis participated in the study. The patients were recruited to the trial based on the inclusion criteria (Fig. 1).

The first dropout was in the buildup phase and due to food aversion. The second patient was excluded from the study because of safety concerns due to a number of al-

lergic reactions at home and poor family observation. Eighteen patients entered the maintenance phase of the study and were all asked to continue eating 60 g of bread made of the mentioned flour for the next 3 months. During the second month of the maintenance phase, patient number 4 (Table 4) developed abdominal pain and vomiting. The symptoms persisted for 2 weeks. Therefore, OIT was discontinued, and she was referred to a pediatric gastroenterologist for further evaluation. Her condition was regarded as an adverse reaction to OIT, and she was followed. Endoscopic evaluation of the patient revealed severe esophagitis, but the number of eosinophils did not show significant increase in biopsy specimens. Her symptoms were totally relieved after 10 days of wheat elimination and PPI treatment. However, she was excluded from the study. Table 4 represents the demographics of 19 patients.

The mean $\pm$ SD age of the patients was 7.1 ± 4.61 years (ranging from 2.0 to 17.0 years). Most of the patients (14 vs. 5) were male. Nine patients (47%) had physician-diagnosed asthma, and 4 had a history of hyper-reactive airway disease. Of the 9 patients (47%) with concomitant food sensitizations in their SPT results, 5 had aeroallergen sensitization as well.

Eight of the 17 participants who completed the 3-month maintenance phase showed negative OFCs without experiencing any adverse effects. Nine had positive OFCs, whose threshold of reaction had increased. All of them continued the daily consumption of 60 g of bread for 1 year. Toward the end, 6 patients exhibited negative OFCs, while 3 showed positive OFCs with mild reactions. The 3 remaining desensitized patients were monitored for one more year and asked to consume 60 g of bread every other day. At the end of the second year, their OFC revealed negative results.

Among 407 doses of OIT administered in the clinic during the buildup phase, 29 (7.2%) allergic reactions occurred, of which 19 were mild, 8 were moderate, and 2 were severe (Table 5). The reactions were managed according to the severity.

SPT and Wheat-sIgE Level

The baseline mean $\pm$ SD wheal diameter of the SPT for wheat flour extract was 9.8 ± 4.22 mm. The mean wheal diameter of the SPT was 6.1 ± 3.13 mm at the end of the buildup phase, whereas it was 4.3 ± 2.43 mm at the end of the maintenance phase (Fig. 2). There was a statistically significant decrease in the wheal diameter from baseline to the end of the protocol ($p = 0.02$). The mean duration of the buildup phase was 77 days (33–147 days). At the

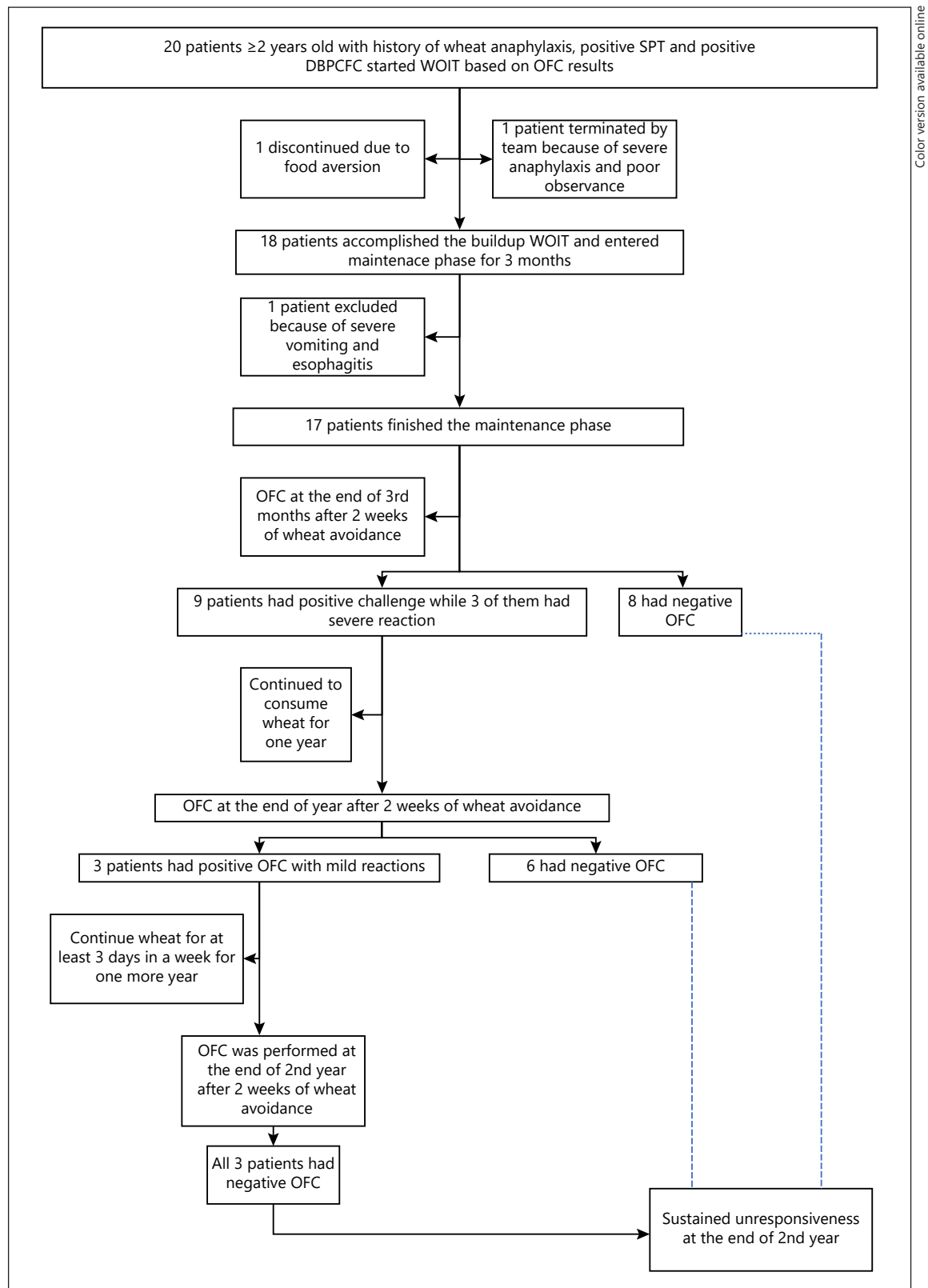


Fig. 1. Progression of patients in the various phases of WOIT, OFC results, and reasons for discontinuation. WOIT, wheat oral immunotherapy; OFC, oral food challenge.

Table 4. Demographics of the patients with wheat anaphylaxis and results of OIT and OFC for each patient

Pt. N	Age	Sex	Concurrent allergy	SPT F/A*	OFC	Symptom in OFC	Starting dose	Final dose	OFC 3rd mon	Symptom	Treatment	OFC 1st y	Treatment	OFC 2nd y
1	5	M	No	No	0.7	Anaphylaxis	0.35	60	Positive	Mild (Abd. Pain)	Anti-histamine	Neg		
2	6	M	Asthma	F+	0.35	Anaphylaxis	0.17	60	Neg	Cough	Epi	Neg		
3	2.4	F	No	No	0.35	Delayed anaphylaxis	0.17	60	Positive	Cough urticaria	Epi	Neg		
4	4	F	HRAD	F+/A+	0.7	Itching and cough	0.7	60	Neg	Excluded from study because of severe vomiting and esophagitis in maintenance phase				
5	6	F	HRAD	F+/A+	0.35	Cough	0.35	60	Positive	Cough	Anti-histamine	Neg		
6	3	M	No	No	0.35	Anaphylaxis	0.17	60	Positive	Mild itching	Anti-histamine	Neg		
7	7	M	Asthma	No	0.7	Urticaria	0.7	60	Neg		Anti-histamine	Neg		
8	3.5	M	Asthma	No	0.7	Cough	0.7	60	Positive	Cough sneeze	Epi	Positive	Anti-histamine	Neg
9	12	F	Asthma	No	0.08	Itching and abd. pain	0.08	60	Positive	Itching	Anti-histamine	Neg		
10	2	M	HRAD	No	0.08	Vomiting	0.08	60	Neg					
11	12	M	Asthma	No	0.17	Anaphylaxis	0.08	30	Neg	Terminated by physician because of severe reactions and poor observance				
12	6	M	Asthma	F+/	1.3	Anaphylaxis	0.8	60	Positive	Wheeze urticaria	Epi	Positive	Anti-histamine	Neg
13	3	M	HRAD	No	0.08	Vomiting	0.08	60	Neg					
14	7	M	Asthma	No	0.35	Anaphylaxis	0.17	60	Neg					
15	4.5	F	No	F+	0.35	Urticaria	0.35	60	Neg					
16	12	M	No	F+/A+	0.35	Anaphylaxis	0.17	60	Neg					
17	4.5	M	AD/asthma	F+	0.08	Abd pain	0.08	60	Positive	Abd pain urticaria	Epi	Neg		
18	16	M	AD/asthma	F+/A+	0.08	Anaphylaxis	0.05	60	Positive	Abd pain	Anti-histamine	Positive	Anti-histamine	Neg
19	17	M	No	F+/A+	0.17	Anaphylaxis	0.08	60	Neg					

OIT, oral immunotherapy; OFC, oral food challenge; SPT, skin prick test. * Aeroallergen and food sensitization.

Table 5. Adverse reactions and treatments during the buildup phase

Adverse reactions and treatments	Buildup phase (patients number = 18)
Total number of OIT doses in the clinic	402
Adverse reactions, total, <i>n</i> (%)	29 (7.2)
Severity of symptoms, <i>n</i> (%)	
Mild	19 (4.7)
Moderate	8 (1.9)
Severe	2 (0.4)
Organ-specific symptoms, <i>n</i> (%)	
Skin	18 (4.4)
Respiratory	13 (3.2)
Mucosal	2 (0.4)
Gastrointestinal tract	9 (2.2)
Cardiovascular	0 (0.0)
Medication, <i>n</i> (%)	
Anti-histamine	23 (5.7)
Steroid	16 (3.9)
B2-inhalation	8 (1.9)
Adrenaline IM	3 (0.4)
OIT, oral immunotherapy.	

end of the buildup phase, the serum level of wheat sIgE increased ($p = 0.026$). A statistically significant increase in serum wheat sIgE was observed at the end of the buildup phase ($p = 0.026$). The serum level of wheat sIgE dropped dramatically at the end of the maintenance phase which was statistically significant ($p = 0.022$) as well (Fig. 3). The comparison of the serum baseline wheat sIgE with sIgE after OIT accomplishment (at the end of the 3-month maintenance phase) also showed significant difference ($p = 0.028$).

Long-Term Follow-Up

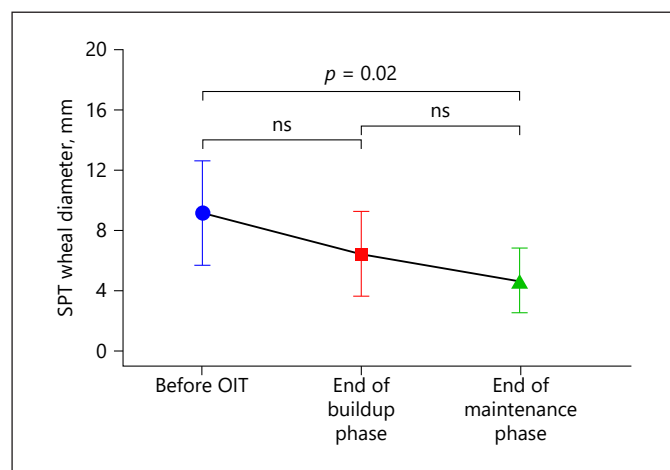
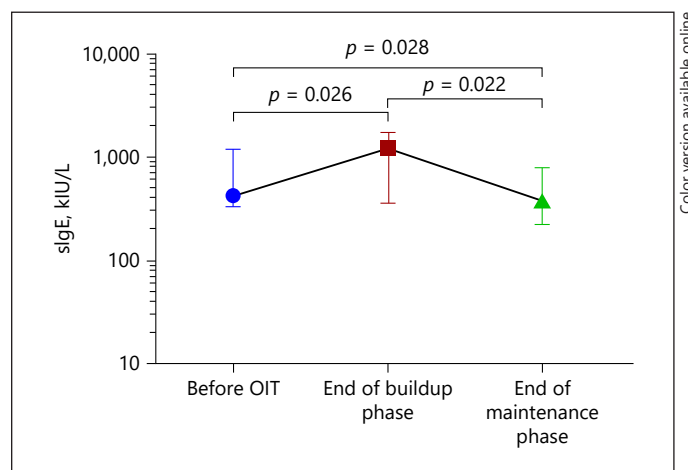
All patients who accomplished the protocol were followed for 24 months. Seven patients (36.8%) had occasional symptoms in the first year, mainly triggered by exercise. None of them were severe, and only 4 of them required short acting β -2 agonist inhalation. In 29% of the tolerant patients, respiratory symptoms were provoked by exercise in the second year Table 6.

Discussion

This study evaluated the efficacy and safety of wheat immunotherapy in 20 children with previous history of anaphylaxis to wheat, of whom 1 patient could not con-

Table 6. Number, severity, and triggers of the reactions and their management in 2-year follow-up after wheat OIT

Patient ID	Number of symptoms in the 1st year	Symptoms/trigger	Treatment	Number of symptoms in the 2nd year	Symptoms/trigger	Treatment
01	2	Wheeze/unknown	No	0		
02	0			0		
03	0			0		
04	Excluded from the study					
05	3	Wheeze/exercise	Salbutamol	1	Wheeze/exercise	No
06	0			0		
07	3	Dyspnea/exercise	Salbutamol	2	Dyspnea/exercise	Salbutamol
08	0			0		
09	0			0		
10	0			0		
11	Excluded from the study					
12	0			0		
13	2	Wheeze/exercise	No	0	Wheeze/exercise	No
14	1	Wheeze/exercise	No	1	Wheeze/exercise	No
15	0			0		
16	0			0		
17	0			0		
18	2	Dyspnea/exercise	Salbutamol	2	Dyspnea, wheeze/ exercise	Salbutamol
19	1	Wheeze/exercise	Salbutamol	1	Wheeze/exercise	Salbutamol

**Fig. 2.** Figure 2 compares the wheal size of the patients before OIT, at the end of the buildup phase, and at the end of the maintenance phase. As can be seen the wheal size has decreased after the maintenance phase compared to the beginning of the procedure. OIT, oral immunotherapy.**Fig. 3.** Figure 3 compares the levels of sIgE to wheat before OIT, at the end of the buildup phase, and at the end of the maintenance phase. sIgE values measured after OIT were significantly decreased when comparing those measured before OIT with those measured at the end of the study. However, values during the buildup phase show significant rise as was expected. sIgE, specific IgE; OIT, oral immunotherapy.

tinue the study because of food aversion. Similar findings to food aversion were previously explained in animal models by changes in taste preference due to high levels of allergen sIgE antibodies [20]. The reason why the second patient was dropped from the study was the safety

concerns. Another patient developed severe vomiting and epigastric pain during the maintenance phase. Since OIT has been suggested as a trigger for eosinophilic esophagitis in 2.7% of patients [19, 21], with the majority of cases reported in milk [22], peanut, and egg OITs

[19]; the patient underwent an endoscopic evaluation. Biopsy specimens were analyzed, and the number of eosinophils was not more than 15/HPF. She was excluded from the study even though she did not meet the eosinophilic esophagitis criteria. She was responsive to wheat elimination diet and proton pump inhibitor treatment. Clinical results of the remaining patients showed that the treatment was effective in life-threatening food allergy events, particularly if the patients accidentally ingested allergens.

The ultimate goal of OIT is to induce SU and prevent fatal reactions after accidental exposure [23]. The study by Jones et al. [9] demonstrated that longer the duration of OIT, the more sustained responses the patients would experience (27.5% after 2 years of OIT vs. 50% after 4 years of OIT). In the present study, participants underwent an OFC after 3 months of maintenance therapy, and it was repeated in 12 and 24 months, if necessary. Although the threshold of the reactions had been increased, the reaction was severe in 3 out of 9 positive patients in the first challenge, which turned to a mild, self-limiting reaction at the second challenge.

According to several studies, 5–36% of patients may fail OIT and be withdrawn during treatment for varied reasons [7]. In the present study one of our participants failed to continue the buildup phase, so the rate of failure was 5%. Patients undergoing OIT may experience breakthrough reactions to maintenance doses despite their initial tolerance. Cofactors such as intercurrent infections, exercise, menstruation, fatigue, and ingestion of allergens on an empty stomach may explain these reactions [24, 25]. We had struggles with male teenage patients who had regular gym training, which affected the timing and quantity of daily dose. Even after a 2-week period of SU, long-term follow-ups of the patients who underwent OIT showed that regular gym workout could induce moderate to severe allergic symptoms [26]. Studies have shown a 60% tolerance induction rate in wheat OIT (WOIT) [14] and up to 61% for hen's egg and 27% for cow's milk in rush OIT [27]. The results of our study are in agreement with the results of these 2 studies, showing that WOIT appears to have better therapeutic outcomes than cow's milk OIT in patients with a history of anaphylaxis. The rate of adverse reactions in this study was approximately 7.2%. In 2 instances in this study, intramuscular adrenaline was required in emergency management of the patients, which is similar to other reports [6, 9, 14]. In line with other studies exercise was the most common triggering factor among the patients (7 cases: 36.8%) who became

symptomatic after reaching 2 weeks of SU [26]. The respiratory symptoms triggered by exercise were mild and treated with short acting β -2 agonists in 4 (21%). Hence, patients and caregivers should be warned about the triggering cofactors, especially exercise, even after achieving a 2-week SU.

sIgE levels significantly increased in the buildup phase as expected during subcutaneous immunotherapy [28]. However, a considerable decrease in sIgE was detected after the procedure was completed. Furthermore, the measurement of serum IgG4 concentration, the quantification of regulatory T cells, and the basophil activation test may be employed to monitor the response to OIT, particularly in patients without a favorable response to such therapies.

The first limitation of the study was the small sample size. In addition, feeding children with certain amounts of specific foods on a daily basis was fairly challenging. The possibility of severe adverse reactions also made the parents doubtful of continuing the study. Nagakura et al. [29] managed to overcome this last limitation by administering a minimum dose of 400 mg, particularly to younger children.

Nevertheless, we believe that our results accurately reflect the efficacy of OIT in long-term follow-up of patients. Similar to the previous studies [9], OIT induced desensitization and tolerance in our patients.

To conclude, WOIT is an effective and safe treatment modality when administered under strict supervision. Certain individuals may not be able to tolerate the side effects and complete the course. Our results demonstrate that WOIT induces desensitization and long-term immune tolerance without further complications or serious sequelae.

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Statement of Ethics

This study protocol was approved by the Iranian Registry of Clinical Trials with the reference code of IRCT: 2013081214305 N2. Written Informed consent was obtained from the patients' legal guardians.

Conflict of Interest Statement

The authors have no conflicts of interest to declare

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Author Contributions

D.B. and M.E. contributed to the study concept and design. D.B., H. Soheili, R.G., F.Z., Z.C., M.S., H. Seifi, M.F., M.K., and S.D. contributed to acquisition of data. M.M. contributed to technical analysis. D.B. and S.D. drafted the manuscript. D.B., M.E., S.D., and M.M. critically revised the manuscript. D.B. supervised the study.

Data Availability Statement

Data are available, and they can be found upon asking from the first author.

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