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Abstract

Background: Egg allergy is one of the most common food allergies in young child. While oral immunotherapy (OIT) is not routinely recommended in current guidelines, it has been considered as a potential alternative treatment strategy. Studies on OIT for food allergies have been explored; however, no studies with control group have been reported in Japan.

Methods: The first oral food challenge test (OFC) was performed on participants before treatment to ensure the diagnosis and evaluate the threshold dose for egg by the way of double-blind, placebo-controlled food challenge (DBPCFC). Participants were randomly assigned by computerized algorithm to receive OIT with egg (OIT group) or to eliminate egg (EE group). A second OFC was performed in both groups **approximately 6 months** after therapy. Blood samples were collected and egg white -specific IgE and IgG4 were measured before and after the treatment period.

Results: Eight of the 14 (57%) patients in the OIT group passed 4 g of dry egg powder whereas none of the 16 patients in the EE group did. All 14 patients in the OIT group showed increased threshold for egg powder in the second OFC compared to the baseline. There was no significant change in egg white-specific IgE levels during therapy. After therapy, egg white-specific IgG4 levels increased significantly in the OIT group, but not in the EE group.

Conclusion: OIT is effective in increasing the threshold for allergens and inducing desensitization **also among Japanese egg allergy patients similar to North American and European patients.**

Key words: children, egg allergy, oral immunotherapy, randomized controlled trial, IgG4

Introduction

Egg is one of the leading causes for childhood food allergy. In Japan, the prevalence for IgE-mediated egg allergy is 1-4% in young children.¹ In the U.S., that is about 2.6% by 2.5 years of age.² The management strategy described in current local guidelines and other countries is an avoidance of egg intake. However, such practice relies heavily on the discipline of the patients and their caregivers, and may limit quality of life.^{3,4} To that end, oral immunotherapy (OIT) has been explored as a new treatment strategy.⁵ Several lines of evidence have shown that OIT is successful in desensitizing patients who are allergic to cow's milk, peanuts and egg.⁶⁻¹⁵ Recent randomized controlled studies (RCT) have also demonstrated that a high proportion of the patients with egg allergy acquired

desensitization through OIT.¹⁶⁻¹⁹ However, these studies focused mainly on patients in Europe and the U.S. In Japan, while there have been clinical trials, most did not have control groups.²⁰ Here, we performed the first RCT of OIT for egg-allergic patients in Japan.

Materials and Methods

Study patients

Participants were recruited from patients who had been followed with scheduled visits to the outpatient unit at the National Center for Child Health and Development in Tokyo. The inclusion criteria were: 1) age 3 to 15 years; 2) complete elimination of eggs from the diet; 3) egg-specific IgE levels ≥ 0.7 U_A/mL (CAP system, Phadia, Sweden); 4) positive immediate allergic reactions to DBPCFC for eggs performed after admission to the hospital; and 5) desire of patients and caregivers to join this study.

According to the ethical committee's advice, for safety reasons, patients were excluded from the study if they experienced severe anaphylaxis such as hypotension or dyspnea elicited by egg challenge test. The study was performed from June 2005 to March 2008. Before the start of therapy, informed consent was obtained from patients' parents after they had been adequately informed of the potential side effects. The study was approved by our Ethics Committee, and registered with the UMIN Clinical trials registry. (Clinical trial registry number, UMIN000001268.)

Eligible patients were randomly assigned to 2 groups: the oral immunotherapy (OIT) group and the egg elimination (EE) group. Randomization was performed using a computerized system. Patients in the OIT group received oral egg immunotherapy according to the treatment protocol provided below, and patients in the EE group continued with complete elimination of eggs from their diet. A second food challenge test and blood sampling were planned **approximately 6 months** after the first diagnostic **OFC** in both groups.

Oral food challenge test

A DBPCFC, considered the gold standard for the diagnosis of food allergy, was used for all candidates. A pack of 4 g dry hen-egg powder (equivalent to approximately 1/4 raw egg; protein content, 1.7 g) was prepared for this test in coordination with the Research Institute of Q.P. Corporation in Tokyo. In sodium dodecylsulfate-polyacrylamide gel electrophoresis, the migration pattern of the extract from this dry powder was similar to that of the extract from raw egg²¹. Strawberry puree (orange or grape juice was used if patients could not eat strawberries) was used as a vehicle and mixed with dried powdered egg when applied, and a mixture of strawberry and 5 g of sucrose was used as placebo. A maximum of 5 titration steps (0.2 g, 0.4 g, 0.8 g, 1.6 g, 1.0 g) of dry hen-egg powder were given at 15-minute intervals. During the food challenge test, patients were carefully monitored for allergic reactions. The

procedure was stopped if they showed objective symptoms (erythema, urticaria, angioedema, rhinorrhea, repeated sneezing, exacerbated nasal congestion, increasing cough, wheezing, severe abdominal pain, vomiting, or diarrhea) and appropriate treatment for symptoms was initiated. Oral itching or very mild abdominal pain were judged as negative, and tests were continued. Symptoms were assessed based on the symptom scoring system described in the manual published by the American Academy of Allergy, Asthma, and Immunology, which classifies symptoms into mucocutaneous symptoms, upper respiratory symptoms, lower respiratory symptoms, and gastrointestinal symptoms²². **The result was considered positive when the symptom score was one points and over.** Patients were observed for at least 2 hours after the **OFC**. Before this food challenge test, the use of antihistamines or leukotriene receptor antagonists was prohibited for 72 hours, and the use of inhaled steroids for 24 hours.

Blood sampling

Blood samples were collected to measure egg white-specific IgE antibodies and egg white-specific IgG4 antibodies at the first **and second challenge test for both groups**. Specific IgE antibodies were measured using the CAP system (Phadia, Japan). Specific IgG4 antibodies were measured using an enzyme immunoassay (SRL, Tokyo, Japan).

Immunotherapy protocol

The dried powdered egg used for the food challenge test was used as an antigenic food material to induce tolerance. Patients in the OIT group ingested the dry powder every day.

The starting dose was 0.1 mg of dried powdered egg and doses of 0.2 mg, 0.3mg, 0.6 mg, 1 mg, 1.5 mg, 2 mg, 3 mg, 4 mg, 6 mg, 10 mg, 15 mg, 30 mg, 50 mg, 70 mg, 100 mg, 150 mg, 200 mg, 300 mg, 500 mg, 700 mg, 1 g, 1.2 g, 1.5 g, 2 g, 3 g, and 4 g followed. The dose was

increased at 3- to 4-day intervals. **Our dose escalation protocol was designed based on the method published by Patriarca et al.⁶** As a rule, therapy was administered at home. When

any allergic symptom developed, the dose level was reduced on the following day to the previous one and was subsequently raised again. If recurrent episodes of allergic symptoms

developed, therapy was continued in combination with antihistamines. If fever or

gastrointestinal symptoms, such as vomiting and diarrhea, occurred during treatment, therapy

was withheld until the symptoms improved. Once a dose of 4 g, which is equivalent to the

total dose in the **OFC**, was achieved, patients continued on that dose daily until the second

OFC. OIT was discontinued in patients who experienced anaphylaxis with hypotension

during therapy. **Adherence to intake of dried powdered egg was assessed at each visit to**

our outpatient unit.

Outcome measures

The **OFC** was repeated to assess the onset of allergic symptoms at **approximately 6 months** after the initiation of therapy in the OIT group and at **6 months** after the first challenge test in the EE group. The primary outcome was the percentage of patients who were able to tolerate 4 g of powdered egg without allergic symptoms in the second **OFC**. Secondary outcomes were used to compare the cumulative tolerated dose in the first **OFC** with that in the second **OFC** and the changes in egg white-specific IgE and IgG4 levels.

Statistical analysis

The upper limit of determination for specific IgE was 100 U_A/mL, and the lower and upper limits of determination for specific IgG4 were 25 U/mL and 800 U/mL, respectively. The Mann-Whitney U test or the chi-square test was used to test for proportions in groups. Differences in the percentage of patients developing tolerance between the OIT and EE groups, as determined by assessment of efficacy, were analyzed with the Fisher exact test using the intent-to-treat analysis. Changes in specific IgE and IgG4 levels and changes of tolerated doses in the **OFC** before and after initiation of therapy were analyzed with the Wilcoxon signed-rank test. Statistical significance was set at P (two-sided) <0.05.

Results

Study participants

Thirty-six patients (25 boys and 11 girls; mean age, 5.8 years) were included in the study.

Among them, five had bronchial asthma, 10 had atopic dermatitis and 15 had both. The patients were randomly assigned to each group. At the first OFC, there were no significant difference between the two groups in age, sex, serum egg white-specific IgE, egg white-specific IgG4 levels except in **cumulative tolerated doses** before intervention (Table 2 1).

First OFC

Assessment of the onset of allergic symptoms in this test resulted in cutaneous symptoms in 28 patients (78%), gastrointestinal symptoms in 34 (94%), respiratory symptoms in 16 (44%), and multi-organ symptoms in 30 (83%).

Clinical responses during therapy

Seventeen of the 18 patients in the OIT group experienced allergic symptoms during OIT; four had vomiting, five had diarrhea, six had urticaria or angioedema, and three had respiratory symptoms including coughing and wheezing. Of the three patients with

respiratory symptoms, one developed persistent asthma. Eight patients had oral cavity itchiness and six patients had mild abdominal pain; however, no patient required epinephrine injection. Three patients withdrew from OIT; a three-year old boy had recurrent asthma symptoms (onset of persistent type asthma), a 12-year-old boy refused to orally ingest any amount of egg (without any symptom), and a four-year-old boy experienced anaphylaxis twice (Fig. 1). Of the remaining 15 patients, 10 attained the final daily dose of 4 g egg powder. The other five patients reached a final daily dose of egg powder of 2 g, 1.5 g, 30 mg, 70 mg and 70 mg, respectively. In the EE group, no accidental ingestion of egg occurred during the elimination diet.

Second OFC

One patient in the OIT refused to participate in the second OFC. **In the EE group, two didn't have second OFC**; one was because of an onset of coxitis simplex and another was due to the inconvenience on the caregiver's part. Finally, 14 patients in the OIT group and 16 in the EE group took part in the second OFC .

OIT patients and EE patients both underwent a second OFC in median of 6.5 months (range 5-8 months) and 6.0 months (range 6-8 months), respectively (p=0.40). No patient in the OIT group stopped oral immunotherapy before second OFC.

Eight patients in the OIT group passed 4 g of dry egg powder whereas none in the EE group did ($p<0.01$) (Fig. 1). All patients in the OIT group had a higher tolerance of egg powder compared to the baseline. The median cumulative tolerated dose in the second OFC was 4,000 mg in the OIT group, which was significantly higher than the baseline (4,000 mg vs. 600 mg; $p<0.01$). There was no difference between the median **cumulative tolerated doses** in the second OFC and the baseline in the EE group (200 mg vs. 200 mg; $p=0.38$) (Table 3 2). **Though we randomly divided the patients into two groups, significant difference in subject characteristics was already observed before treatment, in that cumulative tolerated dose was less than 200mg in 7 patients in EE group compared to only 1 in OIT group. Thus, we reevaluated the rate of patients with cumulative tolerated dose equal to or over 200mg in both groups, who did not show allergic reactions in the second test. Number of patients who fulfilled the requirement and underwent the second OFC was 13 in OIT group (median cumulative tolerated dose; 600mg) and 9 in EE group (median cumulative tolerated dose; 600mg) ($p=0.27$). Eight among 13 patients in OIT group and 0 among 9 patients in EE group were able to take 4 g of dried powdered egg without any allergic reactions, respectively ($p<0.01$).**

Egg white-specific IgE

In the OIT group, there were no significant changes in egg white-specific IgE levels between the baseline and the results of the second OFC, although a decrease of IgE levels in the second OFC was noted (median: 14.2 U_A/mL vs. 12.4 U_A/mL; $p=0.08$). In the EE group, egg white-specific IgE levels significantly decreased at the time of the second OFC compared to the baseline (median: 36.4 U_A/mL vs. 30.6 U_A/mL; $p<0.05$) (Fig. 2A).

Egg white-specific IgG4 levels

In the OIT group, egg white-specific IgG4 levels increased significantly during the second OFC compared to the baseline (median: 13 U/mL at baseline to 94 U/mL; $p<0.01$). In the EE group, egg white-specific IgG4 levels did not increase at the time of the second OFC compared to the baseline (median: 13 U/mL at baseline to 13 U/mL; $p=1.00$) (Fig. 2B).

Discussion

In this study, the **cumulative tolerated doses** of egg powder after OIT in the OIT group was significantly increased compared to the baseline. Eight patients reached 4,000 mg, five reached 3,000mg, and one patient reached 1,400mg. We believe that these results were attributed to OIT, and not by the natural acquisition of egg tolerance as they were not observed in the EE group.

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Only a few studies in Europe and the U.S. have been published about RCT of OIT for egg allergy patients and there is no report in Asian countries. **In these studies, desensitization rate in OIT was 36~93% and spontaneous remission rate in placebo or elimination group was 0~35%.^{8,16-19}** Our study showed that OIT induced 8 of 14 patients (57%) of **desensitization to the target dose of egg in the intervention group.**

Japan is one of the strictest country in the level requirement for food labelling. As for egg-containing food, egg proteins equal to or greater than 10mg/kg must be labeled²³.

Moreover, egg albumin level of influenza vaccines manufactured in Japan is less than 0.8ng/ml,²⁴ which is much lower than the criteria of World Health Organization which is less than 10 μ g/ml.²⁵ Therefore a genetic factor and an excessive avoidance of egg allergen exposure in Japan, intentionally or unintentionally, may lead to the differences in effectiveness and safety of OIT compared with the studies in the U.S. and Europe. The fact that we obtained similar results to the previous studies nonetheless, show that the OIT may also be applicable to Japanese egg allergy patients likewise.

Several studies have shown that allergic reactions are common during OIT. In some cases of severe allergic reactions, epinephrine injections were needed.²⁶ **In our study, although 94% (17/18) of the patients in the OIT group experienced allergic symptoms such as skin reactions, digestive symptoms and respiratory symptoms and the most of these symptoms were mild, one patient had 2 anaphylactic reactions and withdrew from OIT.**

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Anaphylactic reactions are an inevitable problem during OIT, and all patients need to

carry self-injectable epinephrine and have round-the-clock medical contact systems.

Buchanan et al.¹⁵ treated 7 patients daily with 300 mg of egg protein for 24 months

without an anaphylactic episode. After treatment, 4 of 7 patients were able to ingest 8 g of

egg protein without any allergic reaction. Future studies should focus on ways to induce

tolerance with lower doses of egg.

Though 10 patients in OIT group became able to take 4g of dried powdered eggs while

under treatment, allergic reactions occurred in 2 of them during the second OFC. The

patients' physical condition or overlooking of mild symptoms could be the cause of this

result. The discrepancy of the results during treatment and the second OFC implies that

we need to reevaluate with additional OFC after treatment in order to evaluate the effect

of OIT.

At the second OIT, egg white-specific IgG4 levels increased significantly but not egg

white-specific IgE levels in the OIT group. In the EE group, there were no significant

changes in egg white-specific IgG4 levels but there was a decrease in egg white-specific IgE

levels. It has been suggested that in the early phase of immunotherapy, antigen-specific IgG4

levels increase with no decrease in antigen-specific IgE levels, and that regulatory T cells

may contribute to the suppression of allergic diseases by promoting the production of IgG4

and suppression of IgE.^{27, 28, 29} Our results appear to be compatible with these features;

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however, a period of less than six months might be too short to observe the significant decline of specific IgE in the OIT group. Therefore, longer follow-up on IgE and IgG4 measurements is required for further evaluation.

There are some limitations in our study. **First, we haven't evaluated sustained unresponsiveness because we didn't discontinue egg intake before the second OFC. Since Burks et al.¹⁶ reported that more than half the patients who were desensitized by egg OIT had some allergic reaction with one month of discontinuation of egg taking, there is a possibility of similar consequence in our patients.**

Second, we had an elimination group but not a placebo group as a control. Psychological changes might have affected our results. Third, we observed the efficacy of OIT only for a short period of a half year. While previous studies have shown that desensitization was induced after OIT therapy, its long-term efficacy remains unknown.³⁰⁻³³ Therefore, a longer observation period is needed. Fourth, our study is single-centered study. To exclude center-based bias, a multi-centered study is required. **Fifth, there was significant difference between two groups in cumulative tolerated doses before intervention nevertheless randomization was performed. Although significant difference was observed in the additional analysis after excluding the patients whose cumulative tolerated dose was less than 200mg, we could not evaluate the effectiveness of OIT among severe egg allergy patients.**

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In conclusion, we performed the first RCT of OIT for egg-allergic patients in Japan.

Among those who received OIT, more than half acquired desensitization. Notably, all acquired increased threshold for egg consumption and egg white-specific IgG4 levels increased significantly. Our results suggested that OIT was effective for the management of egg-allergic patients **also among Japanese egg allergy patients similar to North American and European patients..**

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Disclosure statement

The authors declare no conflict of interest.

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Authorship contributions

M.A. and H.Y. equally contributed to this work.

M.A., M.N., I.N., A.A., M.E., T.T. and Y.O designed the study; M.A., M.N., I.N. and Y.O. performed the study; M.A., H.Y., and Y.O analyzed results; M.A., H.Y., and Y.O wrote the manuscript.

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Figure legends

Fig. 1. Conduct of the study

The 36 patients were randomly assigned to receive OIT for egg or elimination of egg.

Three patients withdrew from OIT. One patient in the OIT group and 2 patients in the EE group refused to participate in the second oral food challenge. Finally, 14 patients in the OIT group and 16 in the EE group took part in the second oral food challenge. Eight patients in the OIT group passed 4 g of dry egg powder whereas none in the EE group did ($p<0.01$).

OIT: oral immunotherapy EE: egg elimination

DBPCFC: double-blind, placebo-controlled food challenge

Fig. 2. Changes in egg-white specific IgE levels (A) and IgG4 levels (B) by OIT

In the EE group, the mean egg white-specific IgE levels showed significant changes ($P<0.05$). In the oral immunotherapy group, there were no significant changes in mean egg white-specific IgE levels ($P=0.08$) (A). In the egg elimination group, egg white-specific IgG4 levels of 11 patients were under 25 U/mL both at baseline and second OFC. In the oral

immunotherapy group, the mean egg white-specific IgG4 levels showed significant changes (P<0.01). There were no significant changes in the egg elimination group (P=1.00) (B).

OIT: oral immunotherapy EE: egg elimination OFC: oral food challenge

Table 1. Basic characteristics of participants at entry

	Oral Immuno-therapy Group (n=18)	Egg Elimination Group(n=18)	P value
Male sex, no.	13	12	P=0.72*
Age (y), median(IQR)	5.0 (4 to 8)	5.0 (4 to 6.8)	P=0.73**
Egg white Specific IgE (UA/mL), median(IQR)	19.5 (7.7 to 46.2)	32.4 (11.1 to 96.6)	P=0.29**
Egg white Specific IgG4 (U/mL), median(IQR)	13 (13 to 13)	13 (13 to 13)	P=0.72**
Results of the first DBPCFC	Positive (all participants)	Positive (all participants)	
Cumulative tolerated dose of first DBOFC(mg), median(IQR)	600 (600 to 1400)	200 (<200 to 600)	P<0.01**

*chi-square test.

**Mann-Whitney U test.

DBPCFC: double-blind, placebo-controlled food challenge

Table 2. Cumulative tolerated doses of the first and the second DBPCFC

Oral Immunotherapy group (OIT group)				Egg Elimination group (EE group)		
	Patient Age (y)	Cumulative tolerated dose of first DBPCFC (mg)	Cumulative tolerated dose of second DBPCFC (mg)	Patient Age (y)	Cumulative tolerated dose of first DBPCFC (mg)	Cumulative tolerated dose of second DBPCFC (mg)
	6	1400	4000	5	< 200	<200
	5	<200	3000	6	600	600
	4	600	4000	9	<200	3000
	8	600	4000	10	200	<200
	4	1400	3000	5	<200	200
	6	600	4000	4	600	600
	5	3000	4000	5	<200	1400
	8	1400	4000	5	200	200
	11	600	4000	3	600	600
	5	1400	3000	5	<200	<200
	5	600	4000	11	<200	<200
	8	600	1400	4	1400	<200
	4	200	3000	3	600	200
	4	600	3000	6	<200	<200
				5	1400	600
				3	600	3000
Median	5	600	4000	5	200	200
P value	<0.01			N.S.		

DBPCFC, double-blind, placebo-controlled food challenge

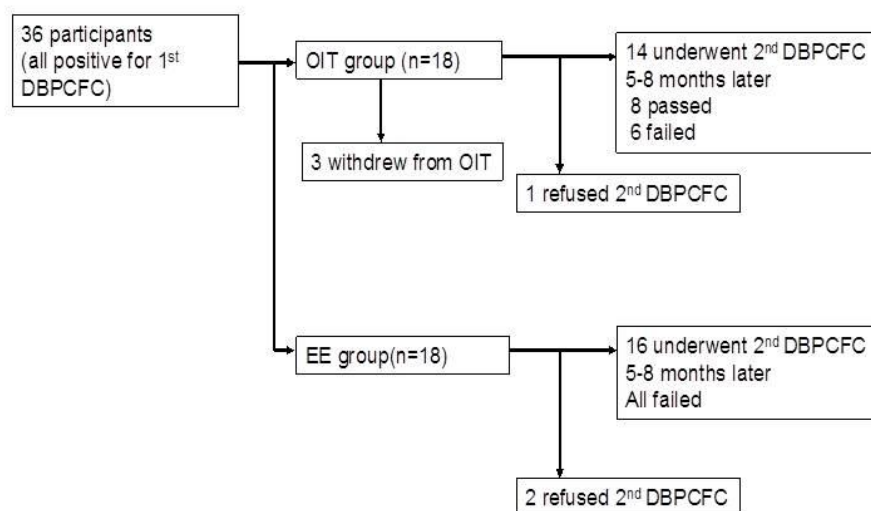


Fig.1 Conduct of the study

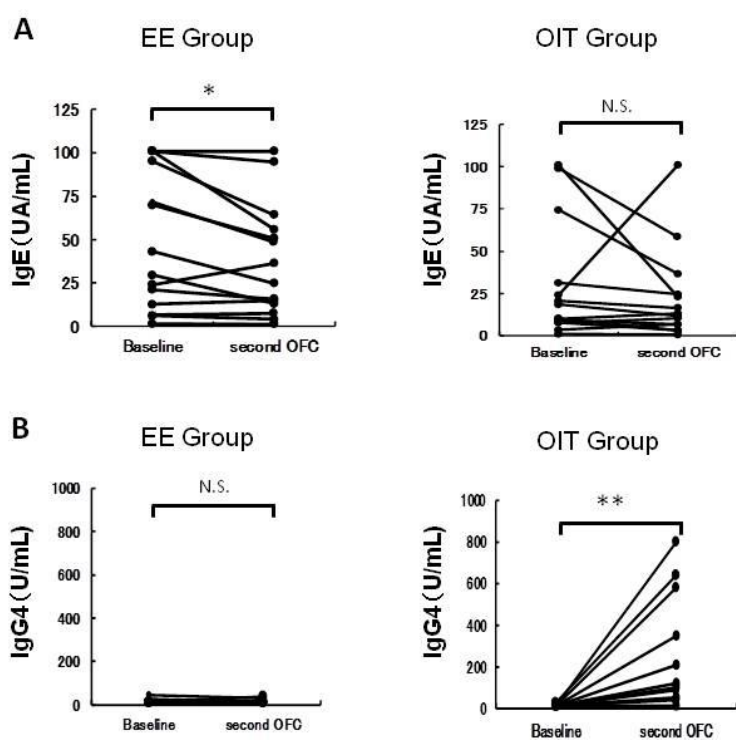


Fig. 2 Changes in egg-white specific IgE levels (A) and IgG4 levels (B) by OIT
 $*p<0.05$; $**p<0.01$; N.S., no significance.