

Characterization and Stability of specific IgE to White Egg's, Gliadin's and Peanut's Proteins among Children

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ABSTRACT

The aim of the present study was to characterize allergen-specific immunoglobulin E (IgE) among children in Fez region. Eighty one children were recruited from the Hospital University Center of Fez. All of them had completed a questionnaire before taking sera. The sera were used to measure total IgE and specific data to proteins of egg's white (EWP), peanuts (PP) and gliadins (G). In last part, we studied the reactivity of specific IgE to native and to heat- and acid-treated allergens.

Allergen-specific IgE measurement indicated more positive values for gliadins (46.9% up to 2IU/ml) than egg white's (29.6%) and peanut's proteins (22.2%). According to predictive values published by Sampson (2001), 14.3% of children are sensitive to egg white's proteins, 4.1% to gliadins and 2.7% to peanut's proteins. The allergenic potential of EWP and gliadins among children were partially diminished by heat and acid treatment.

Allergen-specific IgE measurement indicates that children from Fez region are more sensitive to EWP than peanut's proteins and gliadins. Treatments of these food proteins indicated that recognition by children IgE can be reduced by thermal or acid treatment of these allergens.

Key words: Children; Egg white; Gliadins; Peanuts; Physical-Chemical; Specific IgE; Treatment

INTRODUCTION

Food allergy corresponds to clinical manifestations related to sensitivity to food proteins, most often IgE mediated. The determination of IgE is a valuable marker for the diagnosis of patients to establish allergic diseases.

A number of studies have shown that food allergy is taking an important part in common life. Most of allergists believe that the prevalence of food allergy is rising.¹ Many reasons are behind the increase of the food allergy in industrialized countries, for instance food diversification and the evolution of related technologies. Allergy is considered as a very important public health problem and is estimated by WHO as the fourth disease in the world.

The prevalence of food allergy in the general population is estimated between 6 and 8% for

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children.^{2,3} Egg, wheat and peanut allergy is the most common IgE- mediated food allergies for children.⁴⁻⁷ Prevalence of allergy to these foods differs from one country to another. Among children, it is estimated between 1% and 14.5%.⁸⁻¹⁰ In Morocco, this subject has not been explored by researchers, because of the lack of epidemiological data. A recent and unique work showed the prevalence of food allergy in children at 3% based on skin tests in the region of Marrakech.¹¹

This study assessed the serum specific IgE to food allergens including (egg white, gliadins and peanuts) for children from center of Morocco in order to estimate their sensitivity profile. Numerous works have shown modification of food allergenicity by various physical and chemical treatments.¹²⁻¹⁴ In this study, we have explored, under temperature and acid treatments, the modification of food proteins and their recognition by children specific IgE.

PATIENTS AND METHODS

Patients

In this study eligible children were eighty-one children (37 boys, 44 girls) recruited from the Hospital-University Center of Fez and from Meknes hospitals. Forty-seven were from Meknes, three girls and one boy were from Taounate city, one boy from Taza city and the rest from Fez. They were recruited from February 2008 to December 2008 and only one girl presented urticaria without any reported allergy. Referring to the questionnaire, children and their parents didn't report any food intolerance.

Patients ranged in age from 1 month to 15 years with a median age of 7.3 years (table 1) and a sex ratio of 0.84 (Male/female). Children under 7 years represented 50.6% (n=41). Adolescents (> 10 years) represented 33.3% with an average age of 12.5 years.

As part of the study protocol, a questionnaire was fulfilled. Subjects were asked whether they had allergic reactions to food, and if so, the type of reaction was recorded in detail for ten possible food allergens.

Serum Collection

After formal consent of the children's parents, a blood sample of 1 ml was collected in a tube, which did not contain anti-coagulant. After centrifugation at 3000 per minute in 5 min, the serum was taken and then conserved at -20° C.

Gliadin Extraction

For gliadin extraction, flour (100 mg) was sequentially extracted according to a modified Osborne procedure.^{15,16} The albumin was extracted firstly with deionized water. Then globulin was separated from pellet by 0.5 M of NaCl. Finally, gliadins were isolated from restant pellet after solubilisation in 70% ethanol. For every extraction, mixture was vortexed every 10 min then centrifuged 5 min at 2000 rpm. This operation was repeated three times for the three proteins. Supernatant, containing gliadins, was pooled, diluted at 1:10 in Phosphate buffered saline

Egg White Extraction

Whole hen-egg white was homogenized by stirring for 5 min and then suspended at 1:10 in NaCl 0.9% before centrifugation to eliminate insoluble materials. Supernatant was then diluted at 1:5 in NaCl 0.9% and stored at -20°C.

Peanut Proteins Extraction

Proteins of peanuts were extracted according to Brown method.¹⁷ Peanuts were defatted using chloroform. Once this preparation filtered, the powder was dried at 50°C for one hour. Then mixed with 0.5 M NaCl in PBS and centrifuged 15 min at 3000 rpm. Finally, the supernatant was filtered and stored at -20°C until use.

Specific and Total IgE Measurement

The total and specific IgE concentrations were measured for gliadins, egg-white proteins, and peanut's proteins.

Total serum IgE was determinate by ELISA. 100µl per well of sera were deposited in 96 microplates then incubated overnight at 4°C. After washing, 200µl of 0.25% Bovine serum albumin were added to every well before incubation with anti-IgE peroxydase conjugate for 60 min at 37°C. Anti-IgE fixed was revealed by adding for 15 min 50µl of 0.05% OPD (O-Phenylenediamine). Reaction was stopped, by adding 50µl of 50mM HCl. Then, absorbance was measured at 450 nm.

Specific IgE was determinate using same procedure for total IgE except that microplates were pre-coated with specific food proteins. Pre-coating was performed by adding 100µl of 1mg/ml of food proteins per well and incubating microplates overnight at 4°C. Microplates were then washed and stored at -20°C until

Allergenicity of White Egg, Gliadin and Peanut in Children

use. Specific IgE was measured without previous patients sensitization or oral challenge.

Effect of Temperature and Acid Treatments on the Sensitivity of Specific IgE

Study of the effect of temperature and acid treatments on allergenicity of the proteins of egg white, gliadin and peanut were performed as indicated before.¹⁸ Briefly, food proteins solutions were heated at 100°C 30 min. then 100µl was added to Microplates. For acid treatment, food protein's solutions were adjusted to pH 2 by HCl addition. The objective was to study the variation of the children IgE to these treated proteins. In the same conditions, we compared results obtained to the IgE binding measured with non-treated proteins. This later constitutes control values for treated groups.

Ethics

The ethics committee of the University Hospital Center of Fez approved this study.

Statistical Analysis

Statistics analysis was based on the student's t-test taking $P < 0.05$ as the limit of significant value.

RESULTS

Sera were analyzed for total IgE and food allergen-specific IgE antibodies. Sera of thirty-one children evaluated for total IgE indicated an average of 39.8IU/ml. Values ranged from 2.5IU/ml to 128.8IU/ml. This higher value was recorded for a girl aged three years. Thirty nine percent of total IgE values were between 30 and 80IU/ml. These high values were recorded for twelve boys and thirteen girls (Table 2).

Table 1. Description of the study population

	Children	Boys	Girls
n (%)	81 (100%)	37 (45.7%)	44 (54.3%)
< 5 years	29 (35.8%)	16 (43.2%)	13 (29.5%)
n (%)			
Average age (Years)	7.3	6.5	7.9

Table 2. Distribution of total IgE measured in 70 children

Total IgE (IU/ml)	Children	Boys	Girls
>80	2 (3.2%)	0	2 (5.5%)
30-80	25 (39.7%)	12 (44.4%)	13 (36.1%)
2-30	36 (57.1%)	14 (51.9%)	22 (61.1%)

For specific IgE values (Table 3), egg white proteins represented the major allergen to which 29.6 % of children showed positive IgE levels. Eight children (five girls and three boys) represented IgE values up to 7 IU/ml and the higher value was recorded in a boy (8 years) serum with 49.2 IU/ml.

For other allergens, 46.9% of sera analyzed were positive for gliadins in two girls (9 and 15 years) and one boy (6 years) presenting a specific IgE higher than 25 IU/ml. Higher value was recorded for a girl of 9 years with 51.9 IU/ml. Five children (three girls and two boys) possessed values up to 15 IU/ml. For peanut's proteins, 22.2% of specific IgE were positive in two girls (6 and 8 years) showing a higher IgE value of 17.8 and 19.8IU/ml respectively.

In order to study the effect of temperature and pH on the allergenicity of two allergens, gliadin and EWP, we have selected 6 and 7 children with higher specific IgE against EWP and gliadin respectively (Table 4).

Table 3. Specific IgE measured in children sera

	Children	Boys	Girls
Specific Gliadin	10.5 ± 1.7 (n=38)	9.5 ± 2.1 (n=19)	11.5 ± 2.7 (n=19)
IgE	(51.9 IU/ml)	(40.5IU/ml)	(51.9 IU/ml)
Specific Egg-white	9.0 ± 2.0 (n=24)	11.6 ± 4.3 (n=11)	7.1 ± 1.4 (n=13)
IgE	(49.2 IU/ml)	(49.2 IU/ml)	(15.5 IU/ml)
Specific peanut	7.5 ± 1.3 (n=18)	5.3 ± 1.7 (n=8)	9.3 ± 1.8 (n=10)
IgE	(19.8 IU/ml)	(13.9 IU/ml)	(19.8 IU/ml)

Values indicate means (± SEM) of positive specific-IgE (n) with maximum values observed in boys and girls.

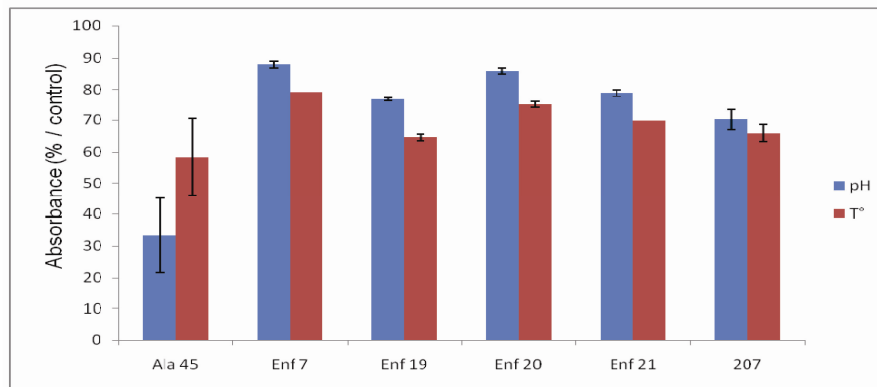


Figure 1. Effect of heat and acid treatments of egg white proteins (EWP) on IgE binding. Absorbance is represented in percentage of data obtained with control (conducted with Egg native proteins)

Table 4. Specific IgE to gliadins and egg white proteins

N° Patient	IgE anti-gliadin (IU/ml)	N° Patient	IgE anti-EWP (IU/ml)
Ala43	34.1	Ala45	39.4
Ala44	20.5	Enf7	15.2
Ala52	83.7	Enf19	10.6
Enf 301	16	Enf20	14.3
Enf 91	28.7	Enf21	8.8
Enf 2	13.2	207	15.5
Enf 11	10.5		

Sera of these children were used to study the production by children specific IgE of processed food

proteins. This recognition was assessed by ELISA using food proteins heated at 100°C or incubated at pH2 for 30 min. Regarding EWP (Fig.1), the findings showed that six children showed a decrease in the recognition of EWP treated by acid from 10% to 66% with an average of 27.9% of diminution. Where EWP was heated at 100°C for 30 min, we observed a decrease of IgE binding of 31.2% varying from 22% to 43%.

For gliadins, heat and acid treatments (Fig. 2) showed that, except of the child “Enf301”, all other children showed a decrease in IgE binding. 43% of children showed a diminution superior to 30% in both treatments. In contrast, we have noticed a small increase of recognition under the treatment of temperature and acid in one child.

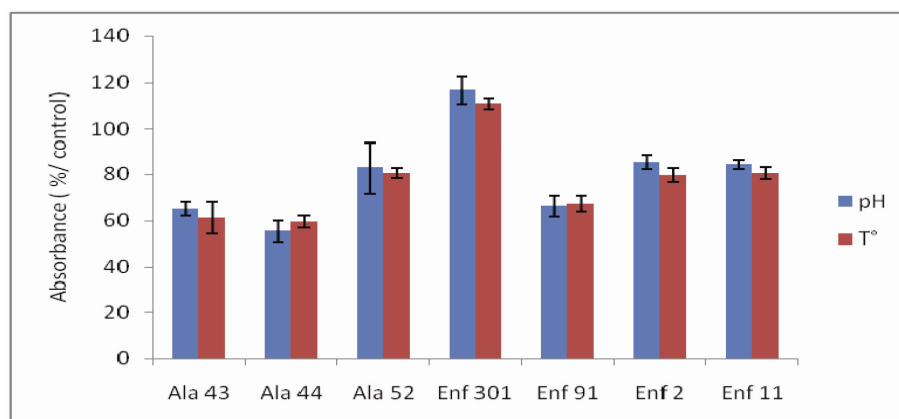


Figure 2. Effect of heat and acid treatments of gliadins on IgE binding. Absorbance is represented in percentage of data obtained with control (conducted with native gliadins)

DISCUSSION

The aim of this study was to evaluate food sensitivity in Moroccan children and particularly in Fez-Meknes region. The children were recruited from UHC of Fez and Meknes hospitals. They had not been challenged or sensitized by allergens. They were questioned and their sera analyzed for total and specific IgE for gliadins, egg white and peanut's proteins by ELISA.

Results of total IgE measurements showed that 39.1% of the children presented values up to 30 IU/ml. These levels indicate that these children were suffering from sensitivity probably not related to only food since we observed only 7.5% of food sensitivity (Table 3). These IgE levels were probably related to respiratory allergies.

Specific IgE analysis for three allergens: gliadins, egg white and peanuts showed that 70.4 % presented positive IgE values. A higher level was recorded for gliadins with 46.9 %. From these positive results, three children presented a value up to 25 IU/ml. According to predictive allergy value published by Sampson,¹⁹ these data indicate that those persons were sensitive to gliadins supposing that the sensitivity to gliadins was about 4.1 %.

For egg white proteins, positive results recorded were 29.6%. According to Sampson^{2,19} a level of 7IU/ml of specific IgE to egg white is a predictive value for allergy. This conclusion has been supported, later, by Rancé et al.²⁰ According to data, we found that 14.3 % of children were sensitive to egg white proteins. Our results are in accordance with the study published by Ghadi,¹¹ concerning sensitization of atopic children in Marrakech from Morocco. These authors observed that from the food allergens used, egg white was the major allergen to which children were more sensitive.

The results of the latest allergen studied, peanuts, showed 22.2% of positive values with two girls who were sensitive to peanuts since specific IgE was higher than predictive value, which is of 15IU/ml.^{2,19,21} This indicates that sensitivity of children to peanuts was about 2.7%. The children sera were equally served for the evaluation, by ELISA, of the IgE reactivity to egg white proteins and gliadins under physical and chemical treatments. The findings showed that 66% of children sera showed a decrease of at least 30% of IgE binding on proteins treated with a maximum of 43%. This decrease in IgE-binding on heated egg white was

observed previously by Anet et al., (1985) who showed a decrease of about 58%²² and Uriso²³ and Huang²⁴ who indicated a decrease of 55.3% of IgE binding. Concerning gliadins, we showed a decrease of allergenicity under thermal treatment which agrees with the study of Varjonen and al.,²⁵ who indicated that IgE-binding capacity of the allergenic proteins decreased as heat-processing temperature and heating time increased.

The effect of acidity on the allergenicity of EWP and gliadins indicated that most of children sera presented a decrease in the recognition of proteins. This indicates that acidic denaturation of protein, like heating, is sufficient to reduce food allergenicity.

In conclusion, from specific IgE measurements, we could suppose that children from Fez-Meknes region are more sensitive to egg white than gliadins and peanut's proteins. The allergenic potential of EWP and gliadins is partially diminished by treatment of these proteins by acid or by heating.

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