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TRYPTASE AND HISTAMINE MAY SUPPORT ORAL FOOD CHALLENGE IN THE DIAGNOSIS OF ALLERGY

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Allergen-specific immunoglobulin E (IgE) reactions lead to acute degranulation of mast cells and basophils and release of stored mediators, particularly tryptase and histamine, which can be measured *in vitro* after reactions. The aim of this study was to investigate the utility of serum tryptase and plasma histamine during oral food challenge (OFC) in 103 children with suspected food allergy, in order to support the diagnosis of a IgE-mediated reaction. Blood samples for serum tryptase and plasma histamine were collected before the OFC and after the onset of allergic symptoms or after 60 minutes from test completion. Serum tryptase and plasma histamine were measured by a fluoroenzyme immunoassay (ImmunoCAP; ThermoFisher, Uppsala, Sweden) according to the manufacturer's instructions. A correlation between serum tryptase and plasma histamine distributions was observed after OFC ($p = 0,0035$). A correlation was also observed for both serum tryptase and plasma histamine before and after OFC ($p < 0,0001$). Subjects with positive response to OFC had significantly higher values ($p = 0,0375$) of serum tryptase compared to subjects with negative response. The plasma histamine distribution showed a significant difference between measurements before and after OFC, both in the complete population ($p < 0,0001$), and considering the response (negative OFC: $p < 0,0001$; positive OFC: $p = 0,0181$). The diagnostic work-up of IgE-mediated food allergy may include determination of serum tryptase and plasma histamine, in order to support the results of OFC. These markers are strongly related to the same IgE-mediated mechanism and, as they can be both easily measured, can confirm the allergic nature of a reaction in the real-life setting of food allergy.

The oral food challenge (OFC) actually represents the gold standard investigation for the objective diagnosis of IgE-mediated allergy (1). OFC must be performed in a specialist setting with emergency support immediately available, due to the risks of severe reactions, including anaphylaxis (1).

Immediate allergic reactions proceed through allergen-specific immunoglobulin E (IgE) binding, leading to acute degranulation of mast cells and basophils and release of stored mediators, particularly tryptase and histamine, which can be measured *in vitro* after reactions (2). In this setting, serum total tryptase levels should be measured from 15 minutes to 3 hours after symptom onset, while

plasma histamine peaks at 10 minutes after symptom onset and disappears in 60 minutes (3).

Serum tryptase has been proposed for use as a marker of anaphylaxis, and it has proven useful for validating drug and insect sting-induced anaphylactic reactions, but not yet for food-induced anaphylaxis (4); however, a supportive diagnostic role has been recently proposed for food-induced anaphylactic reactions in children (5). Moreover, serum basal tryptase levels has been recently showed to be predictive of moderate to severe anaphylaxis in children with food allergy (6).

The role of histamine in immediate allergic reactions is well known, where it acts as the most

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important mediator which produces the majority of signs and symptoms in these reactions. Some studies suggest that levels of plasma histamine are correlated with the severity of the reaction (7). During an allergic reaction, histamine is released from the mast cells and basophils in parallel with tryptase (8).

The aim of this study was to investigate the utility of serum tryptase and plasma histamine during OFC in order to support the diagnosis of food allergy in children.

MATERIALS AND METHODS

Study Population

One hundred and three patients (35 females, 68 males; mean age: 4,5631 years, age range: 0-16 years) were evaluated for food allergy at the Foundation IRCCS Policlinico San Matteo of Pavia from August 2011 to May 2014. Some patients had sensitizations to inhalants, food, egg or milk. In particular: 36 were mono-sensitized to one of these allergens (5,83% to inhalants, 14,56% to food, 6,8% to egg and 7,77% to milk) and 55 poli-sensitized to two or more allergens (37,86% to inhalants and food, 5,83% to egg and milk, 2,91% to inhalants and milk and 3,88% to inhalants and egg). Only 12 patients did not present any sensitization.

Some patients also presented underlying diseases such as asthma, rhinitis, wheezing, atopic dermatitis, urticaria, skin rash, or failure to thrive. In particular, 55 subjects presented with one underlying disease and 17 had more than one. The remaining 31 patients did not show any disease. Subjects with underlying diseases have been divided into 3 categories: respiratory (16 patients), cutaneous (47 patients) and mixed (9 patients).

Procedures and Measurements

An open OFC was performed for all patients, after obtaining informed consent from parents. OFC was performed for milk (43 patients), egg (51 patients), tree nuts (8 patients) and rice (1 patient). In order to avoid severe reactions, patients received the food in titrated doses, which range from 3 mg to 3 g, and at set intervals, according to validated guidelines (1). OFCs were stopped as soon as objective clinical

reactions were observed (positive response) or the last dose was consumed without clinical symptoms (negative response).

Basal levels of specific IgE were available for all patients. Specific serum IgE levels were measured by immunofluorometric assay (ImmunoCAP; ThermoFisher, Uppsala, Sweden) and expressed in kU/L. Specific IgE levels higher than 0.35 kU/L were considered positive. Blood samples for serum tryptase and plasma histamine were collected before the OFC and after the onset of allergic symptoms or after 60 minutes from test completion. Serum tryptase and plasma histamine were measured by a fluoroenzyme immunoassay (ImmunoCAP; ThermoFisher, Uppsala, Sweden) according to the manufacturer's instructions. The cut-off for tryptase is 3,8 µg/l (pathologic value > 11,4 µg/l), while for histamine is 1 ng/ml.

Statistical analysis

Statistical analysis was performed using the statistical software package Medcalc 9 (Frank Schoonjans, BE). Medians and percentiles (25th and 75th, IQR) were used as descriptive statistics, due to non-normal distribution of the variables. The correlations between tryptase and histamine and between the distributions of each parameter before and after OFC have been evaluated. Only one outlier in histamine distribution has been detected and excluded from the analysis. This value for histamine before OFC was 7,5 times the standard deviation and for histamine after OFC it was 7,78 times the standard deviation. The non-parametric Mann-Whitney's test was used to compare samples resulting positive or negative to OFC. The non-parametric Wilcoxon's test for paired samples was used to compare values before and after OFC. In addition the non-parametric Kruskal Wallis test for analysis of variance was used to compare more than two samples according to sensitization and pathologies. Chi-square's test was used to study the relationship between sensitization and OFC. In addition logistic and linear multiple regression analysis was performed, respectively, to correlate and predict the response to OFC, and to predict histamine and tryptase after OFC. The stepwise strategy was adopted to select significant

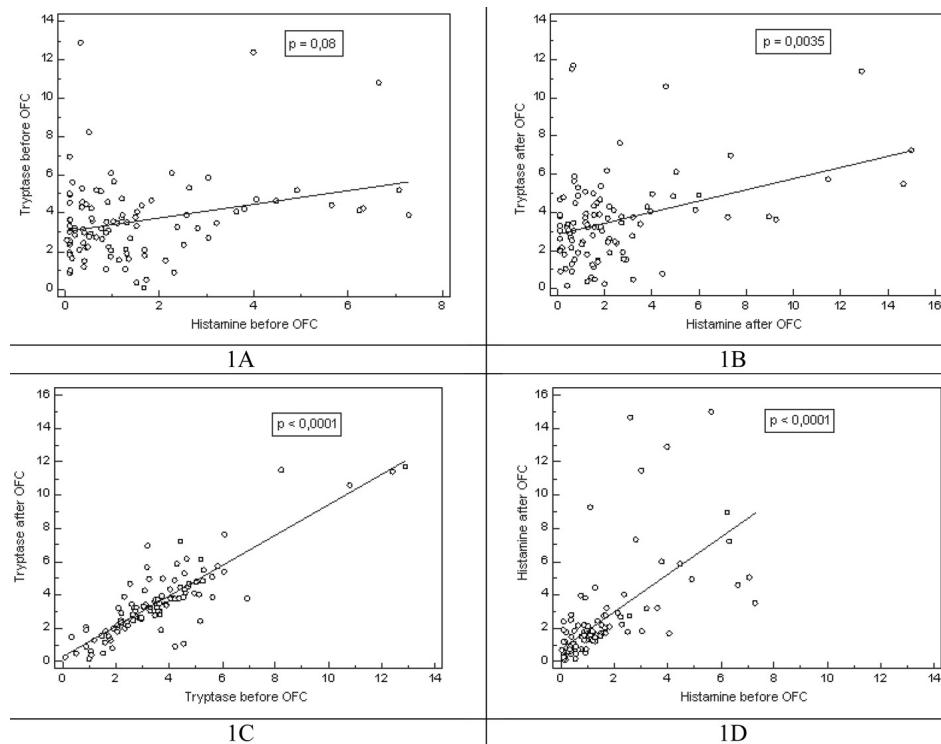


Fig. 1. Correlation between distributions was evaluated: correlation between tryptase and histamine before OFC (Fig. 1A) and after OFC (Fig. 1B). There was a correlation between tryptase before and after OFC (Figure 1C) and between histamine before and after OFC (Fig. 1D).

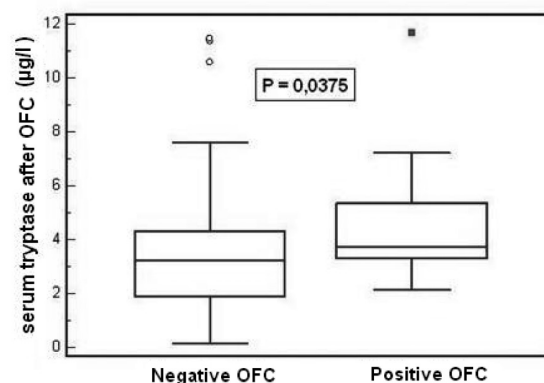


Fig. 2. Tryptase measured after OFC. Values corresponding to a positive response were higher than negative ones.

independent predictors. Values of $P \leq 0.05$ were considered to indicate statistical significance.

RESULTS

After OFCs, mild allergic reactions (positive response) were documented in 15 patients; no symptoms after exposure of the challenged food (negative response) were observed in 88 patients. In

particular, OFC for milk was positive for 4 patients; 2 of these were also positive to the major milk allergen (mean = 0,62 kU/L). OFC for egg was positive for 9 patients and 8 of them were positive to the major egg allergen (mean = 5,246 kU/L). All patients with positive response developed symptoms during the challenge or immediately after the test completion; in addition, no anaphylaxis was observed. In this group, 10 patients presented with cutaneous

Table I. Comparison of Tryptase and Histamine values between negative and positive response groups, stratified according to pre- and post-OFC measures (Mann Whitney Test).

Tryptase	Pre OFC				Post OFC		
	n	median	25 th -75 th	p	median	25 th -75 th	p
Negative OFC	88	3.23	2.095 – 4.54	0.3286	3.22	1.88 – 4.315	0.0375
Positive OFC	15	3.50	2.74 – 4.38		3.74	3.29 – 5.36	
Histamine	Pre OFC				Post OFC		
	n	median	25 th -75 th	p	median	25 th -75 th	p
Negative OFC	88	0.87	0.36 – 1.68	0.5306	1.55	0.645 – 2.57	0.2822
Positive OFC	15	1.31	0.307 – 2.77		1.57	0.67 – 7.305	

Table II. Comparison of Tryptase and Histamine values before and after OFC, stratified according to OFC response (Wilcoxon Test for paired data).

Tryptase	Pre-OFC			Negative OFC			Positive OFC		
	n	median	p	n	median	p	n	median	p
Pre-OFC	103	3.31	0.2301	88	3.23	0.0992	15	3.50	0.4543
Post-OFC	103	3.24		88	3.22		15	3.74	
Histamine	Pre-OFC			Negative OFC			Positive OFC		
	n	median	p	n	median	p	n	median	p
Pre-OFC	103	0.92	< 0.0001	88	0.875	< 0.0001	15	1.31	0.0181
Post-OFC	103	1.55		88	1.55		15	1.57	

involvement (urticaria and angioedema), while 5 patients had gastrointestinal symptoms (vomiting and abdominal pain).

Serum tryptase and plasma histamine distributions were evaluated before and after OFC across the complete population, and then considering responses to OFC. Serum tryptase showed the following values for median and percentiles: 3,31 µg/l (IQR: 2,21 - 4,51) before OFC and 3,24 µg/l (IQR: 2,065 - 4,432) after OFC; plasma histamine distributions showed the following values: 0,92µg/l (IQR: 0,35 – 1,71) before OFC and 1,55µg/l (IQR: 0,67 – 2,72) after OFC. A correlation between serum

tryptase and plasma histamine distributions was observed after OFC ($p = 0,0035$), but not before ($p = 0,08$). Considering distributions of each parameter, a correlation was observed for both serum tryptase and plasma histamine before and after OFC ($p < 0,0001$) (Fig. 1).

Then, distributions were evaluated considering the response to OFC. Subjects with positive response had significantly higher values ($p = 0,0375$) of serum tryptase compared to subjects with negative response (Fig.2). For the other distributions, no statistically significant difference was observed (Table I).

The plasma histamine distribution showed a

Table III. Logistic regression model for the response to OFC (positive or negative).

Dependent variable: response to OFC				
Independent variables	Coefficient	Std. error	t	p
Constant	0.0336			
Tryptase after OFC	0.03168	0,0155	2.044	0.0436
Coefficient of determination $R^2 = 0,0397$				
Significance level: $P = 0.044$				

Histamine and tryptase before OFC, histamine after OFC, age, sex, sensitizations, pathologies and specific IgE were not included in the final model because they did not achieve statistical significance as predictors.

Table IV. Multiple regression model to predict histamine and tryptase values measured after OFC.

Dependent variable: Tryptase After OFC				
Independent variables	Coefficient	Std. error	t	p
Constant	-0.6556			
Tryptase before OFC	0.9421	0.05214	18.068	<0.0001
Pathologies	0.5853	0.2391	2.448	0.0161
Specific IgE	0.5862	0.2357	2.487	0.0146
Coefficient of determination $R^2 = 0.7757$				
Significance level: $P < 0.001$				

Histamine before OFC. Age, sex and sensitization are not included in the model because they are not predictors.

Dependent variable: Histamine After OFC				
Independent variables	Coefficient	Std. error	t	p
Constant	0.1821			
Histamine pre-OFC	1.5495	0.09510	16.293	<0.0001
Coefficient of determination $R^2 = 0.7244$				
Significance level: $p < 0,001$				

Tryptase before OFC, age, sex, sensitization, pathologies and specific IgE were not included in the final model because they did not achieve statistical significance as predictors.

significant difference between measurements before and after OFC, both in the complete population ($p < 0,0001$), and considering the response (Negative OFC: $p < 0,0001$; Positive OFC: $p = 0,0181$) (Table II).

The study population was also divided, according to sensitization, into 3 groups: no sensitized, mono-sensitized and poli-sensitized. We analyzed how the sensitization was distributed according to the response to OFC. All patients with a positive response were sensitized, but also the majority of patients

with negative OFC were sensitized. No statistical significance was observed ($p = 0,13$).

The study population was also divided, considering the presence or the absence of underlying diseases, into 3 groups: patients without any disease, with only one disease and with two or more diseases. Only for the serum tryptase evaluated before OFC, a statistical difference was observed ($p = 0.0188$).

Multiple regression analyses were performed with two different aims. The first one is purely descriptive of the response, so that independent variables were

both pre- and post-test variables. The second one is a predictive aim, so that only pre-test variables were considered as predictors. In both models we also considered age, sex, the presence or absence of sensitization and underlying diseases, and the result (positive or negative) of specific IgE as independent variables. In the descriptive multivariate regression, our results showed that only serum tryptase after OFC was statistically significant to determine OFC response (Table III). In the predictive model, our results showed that none of the variables were predictors for the response to OFC.

Another multiple regression analysis was performed to predict serum tryptase and plasma histamine after OFC from their basal values, the presence or the absence of sensitization and underlying disease, age, sex and the result of specific IgEs (positive or negative). For both markers, their distributions evaluated before OFC were statistically significant. For serum tryptase regression model, also the presence or the absence of underlying diseases and the result of specific IgEs were statistically significant (Table IV).

In addition, we considered the subgroups of underlying diseases as another predictor. None of diseases' subgroups were predictors for serum tryptase after OFC, while for histamine the cutaneous group has resulted significant ($p = 0,0127$).

DISCUSSION

To date, in our knowledge, this is the first study monitoring both serum tryptase and plasma histamine in the diagnostic work-up of food allergy in children. A similar previous study focalized on serum tryptase and eosinophil cationic protein as additional diagnostic tools in adult patients with clinical hypersensitivity to *Anisakis simplex*, without any significant result (9).

Monitoring markers of inflammation during allergic reactions may help to identify the different cell types involved and clarify the mechanism of these reactions. In particular, the determination of serum tryptase and plasma histamine is useful as they allow the participation of mast cells and/or basophils in the reaction to be confirmed and provide an orientation

as to the probable mechanism involved (8).

Our study shows that both serum tryptase and plasma histamine production is strongly related to the same IgE-mediated mechanism and, as they can be both easily measured, can confirm the allergic nature of a reaction in the real-life setting of food allergy.

Moreover, the values of these two markers after OFC may be affected by its response. In particular, significantly higher values of serum tryptase after OFC are observed in patients with positive response, compared to those with negative ones, even if these values are far below the established pathologic cut-off. No anaphylactic reactions were observed in the positive response group indeed. This result is apparently in contrast with previous studies, which reported a lack of tryptase elevation in food-induced anaphylaxis, which was explained as due to slow onset of reactions or because mucosal mast cells and basophils, the major players in food-induced anaphylaxis, contain less to no tryptase as compared with skin mast cells (10, 11). Serial measurements of tryptase levels have been reported to increase the sensitivity and specificity of the test (4); however, this approach has not yet been studied in the pediatric population.

Besides, a recent study detected a strong association of elevated basal levels of serum tryptase with anaphylaxis and severity of reactions in children with food allergy (6); this is consistent with the results of previous studies on patients with venom allergy (12). These findings increased the importance of serum basal tryptase in screening patients with allergy to determine groups at high-risk of reactions, including patients with mast cell activation syndrome (6).

In our study, higher values of plasma histamine after OFC were also observed in patients with positive response, compared to those with negative ones, even if this difference is not significant.

The presence of sensitizations and underlying diseases do not influence distributions. Subjects without any disease had higher values of serum tryptase than subjects with one or more diseases. This result could be explained by a possible use in symptomatic subjects of anti-allergic drugs, such as corticosteroids that reduce serum tryptase values.

The response to OFC seems not to be predicted

by any of the variables considered. Instead, both markers' basal levels could be predictive of the values after OFC; in addition, underlying diseases and specific IgE are predictors for tryptase after OFC. Then, in the diagnostic work-up of food allergy, testing specific IgE may have an added value in patients undergoing OFC.

The strength of our study was monitoring serum tryptase and plasma histamine to support the results of OFCs in children with suspected food allergy, which has never been analyzed before. However, the small number of positive responses could be considered a limitation for this study; further efforts are needed to investigate for a possible different outcome considering a bigger population.

CONCLUSION

The diagnostic work-up of IgE-mediated food allergy may include determination of serum tryptase and plasma histamine, in order to support the results of OFCs. These markers are strongly related and, as they can be both easily measured, can confirm the allergic nature of a reaction in the real-life setting of food allergy.

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