Fecal Calprotectin for Prediction of the Oral Challenge Test Response in Children with Food Hypersensitivity Skin Symptoms

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Abstract

The **aim** of the study was to assess the use of quick tests for fecal calprotectin detection in children with skin symptoms of the Food Hypersensitivity for both diagnostic of the food intolerance and prediction of the oral challenge test response. **Materials and methods**. 76 children with skin symptoms suspected of the food allergy were recruited prospectively (mean age was 25,3 [11,5; 41,8] months). The diagnosis of food allergy was made using EAACI criterion in case of FA reproducible occurrence on exposure to the suspected food. Serum and stool samples were taken at the first visit of the patients with skin clinical symptoms. Stool samples were analyzed for calprotectin, serum – for level of the common IgE and specific IgE antibodies to the common for Ukraine food allergens (milk, egg). **Results**. FC was detected in coprofiltrate in 42 children (55%). They statistically more often had increased common IgE independently from age, but correlative link between them was absent (r=0,15, p=0,22). More than one third of the patients suspected cow milk food allergy and one fourth – allergy to eggs. Other one third of the patients couldn't detect any clear causative food that was associated with food allergy symptoms appearance. FC level had no correlation with specific IgE levels (p>0,05). Oral challenge test (OCT) was done in 68 patients. 8 children were

not tested with OFC because of the clear anamnesis or positive sIgE levels. All of them had detected FC in the stool (n=8).

Conclusions. FC level in stool did not influence on the result of the OCT and had no association with type of the tested product. FC should not be used for prediction of the OCT result and was not associated with product for OFC test.

Keywords: fecal calprotectin, food intolerance, children, oral challenge test

Introduction

Food hypersensitivity in children can have different phenotypes and one of them is skin symptoms. Less is known about diagnostics of the non-immune mechanisms of the food hypersensitivity in children. Possible etiologies include intolerances: enzymatic, toxic, pharmacologic [1]. But in practice it is rather difficult to detect the clear cause of the skin symptoms, especially if this reaction has delayed mechanisms and different triggers.

Intestinal barrier is the first line that has being in contact with food commonly, and allergens particularly in children. It is presented as the complex of the structures. And except anatomical layers, biochemical substances and symbiotic microorganisms are not less important [2]. Pathological permeability of the intestinal barrier in food allergic patients has being studying. Its' defects lead to the interstitial inflammation due to the mucosal membrane contact with big amount of bacteria and chemical substances. Due to this activated neutrophils and other lymphocytes, submucosal mast cells produce cytokines. Faecal calprotectin is recognized as one of the main markers of the gut mucosa inflammation. It was shown that Faecal calprotectin (FC) have been suggested as promising biomarker that can be used to distinguish between clinically active and inactive irritable bowel disease [3]. On the other hand it may be increased in different organic diseases, such as bacterial or viral gastroenteritis, diverticulitis, nonsteroidal enteropathy, colorectal carcinoma, and even after pelvic radiation [4]. These diseases are very rare in children. Moreover FC level doesn't change in celiac disease [5]. That makes Fecal calprotectin level perspective biomarker of the inflammation of the gut mucosa.

The **aim** of the study was to assess the use of quick tests for Fecal calprotectin detection in children with skin symptoms of the Food Hypersensitivity for both diagnostic of the food intolerance and prediction of the oral challenge test response.

Materials and methods

76 children from 1 to 161 months old with skin symptoms suspected of the food allergy were recruited prospectively from the patients of the Children multifield

hospital #5, Zaporizhzhya and University clinic, Zaporizhzhya (mean age was 25,3 [11,5; 41,8] months). The diagnosis of food allergy was made using EAACI criterions in case of FA reproducible occurrence on exposure to the suspected food. Patients with food anaphylaxis were not included. Serum and stool samples were taken at the first visit of the patients in the acute period of the clinical symptoms. Stool was taken in the room temperature to the sterile tube, after that stored at -25°C until used. Stool samples were analyzed for calprotectin, serum – for level of the common IgE and specific IgE antibodies to the common for Ukraine food allergens (milk, egg). Calprotectin was measured in faeces by rapid test CalprotectinRAPID TEST (Italy). This test was performed in less than 30 min with methodic given in the instruction. Such test was chosen because it may be more suitable in primary care and earlier this methodic showed a good correlation with the traditional laboratory ELISA tests [6, 7, 8, 9].

Blood samples were collected after fasting in cooling vacutainer and after that it was immediately centrifuged (4°C for 3.000×30 min). After centrifugation serum was blind coded and stored at -25° until used. Serum antibodies levels were measured by ELISA technique. Specific IgE level >0,35 kU/l was considered as positive. Common IgE level grater, than referent to age, was considered as increased.

Oral challenge test (OCT) was performed in accordance with the EAACI recommendations and local Ethical requirements [1]. Challenge test was performed in patients who had negative specific IgE levels. Open oral provocation was used for infants up to 1 y.o. Double blind placebo controlled oral challenge was provided in others. Two main products were used for oral challenge: milk and egg.

All statistical analyses were performed in commercial software Statistica (Statsoft, USA). All continuous variables were tested for a normal distribution using the Shapiro-Wilk's W test. Continuous variables were presented as median inter-quartile range because of the non-normal distribution. Differences from the three and more groups were analyzed using the non-parametric ANOVA (Kruskall-Wallis) test. One-to-one comparisons for unpaired data were done with non-parametric Mann-Whitney's test.

Results

Children were divided into two groups depending on the FC level. FC was detected in coprofiltrate in 42 children (55%). Both groups of the children (FC positive and negative) were comparable and had no valid differences in the age (p=0,55) and state (p=0,87). On the first step anamnesis was assessed in each child.

Table 1.
Characteristics of the anamnesis data of the children from both groups

	Cow milk protein	Egg	Colored fruits and vegetables (red, yellow)	Food containing biological amines	Non chocolate sweets	Not detected
All patients n=76 (%)	34 (44,7%)	15 (19,7%)	14 (18,4%)	21 (27,6%)	10 (13%)	29 (38%)
FC positive n=42 (%)	21 (50%)	10 (23%)	7 (16,6%)	13 (30,9%)	8 (19%)	16 (38%)
FC negative n=34 (%)	13 (38%)	5 (14%)	7 (20,5%)	8 (23,5%)	2 (5,%)	13 (38,2%)

Differences between the groups were not significant

As it can be seen on the table 1, more than one third of the patients suspected cow milk food allergy and one fourth – allergy to eggs. These data correspond to the other author's latest data [1]. Moreover other one third of the patients couldn't detect any clear causative food that was associated with food allergy symptoms appearance. Food that contained biological amines was associated with FA symptoms as in patients with positive FC, as in those who had negative FC.

On the second step specific IgE serum levels to cow milk an egg proteins were detected. In those patients who had positive effect from the elimination diet and clear detected allergen after anamnesis analysis specific IgE serum levels were not done.

Only 3,9% of the studied children had specific IgE antibodies to common cow milk protein, 2,6% - to casein, 5,2% - to a-lactalbumin and 6,6% - to ß-lactoglobulin and Bovine serum albumin. 13,1% of the patients had sIgE to eggs (table 2). And only four children (5,2%) had both antibodies to the CMP and eggs.

Table 2.
Pre-challenge levels of the serum specific IgE in children from the both groups

	Increased common IgE	Common milk protein	Casein	a-lactalbumin	ß-lactoglobulin	Bovine serum albumin	Egg	Not done
All patients n=76	34 (44,7%)	3 (3,9%)	2 (2,6%)	4 (5,2%)	5 (6,6%)	5 (6,6%)	10 (13,1%)	23 (30,2%)
FC positive n=42	21 (50%)*	2 (4,7%)	2 (4,7%)	4 (9,5%)	4 (9,5%)	4 (9,5%)	6 (14,2%)	12 (28,5%)
FC negative n=34	13 (38,2%)	1 (2,9%)	-	-	1 (2,9%)	1 (2,9%)	4 (11,7%)	11 (32,3%)

*-Significant differences between groups p<0,05.

FC level had no correlation as with common IgE level (r=0,15, p=0,22) as with specific IgE levels (p>0,05). But significant difference was found in common IgE levels between FC positive and negative groups. Increased common IgE more frequently was seen in children with FC positive test independently of age.

On the next step Oral challenge test (OCT) was done in 68 patients. 8 children were not tested with OFC. It should be noted that all children that were not done challenge test due to the clear anamnesis or positive sIgE levels had detected FC in the stool (n=8). 6 of them had positive elimination died results and 3 – increased levels of the sIgE to CMP. Further analysis showed that in the most part of the children challenge test was positive with milk 22%, 15,7% of the tested patients had positive OCT with eggs. Only 13,1% of children were tested both milk and egg and only one third of them had positive results. These data corresponds to the recent summarized European data that showed milk and egg as the commonest causes of the food allergy symptoms in children. And that usually children has allergy to one or two products and there is no need in the strict elimination diet [1].

FC level in stool did not influence on the result of the OCT and had no association with type of the tested product. This probably confirms absence of the link between FC and specific IgE levels, but doesn't answer why FC positive patient significantly frequently had increased common IgE levels (Table 3).

Table 3.
Challenge test results depending on the FC detection

	Negative OCT	Positive OCT				Not done
		Milk	Egg	Both milk and egg	Fruits (apple, banana)	
All patients n=76	39 (51,3%)	17 (22,3%)	12 (15,7%)	3 (3,9%)	2 (2,6%)	8 (10,5%)
FC positive n=42	18 (42,8%)	11 (26,1%)	8 (19%)	3 (7,1%)	-	8 (19%)
FC negative n=34	21 (61,7%)	6 (17,6%)	4 (11,7%)	-	2 (5,8%)	-

Differences between the groups were not significant.

Discussion and further perspectives. More than half of the children with acute skin food allergy symptoms had positive FC tests. They statistically more often had increased common IgE independently from age, but correlative link between them was absent (r=0,15, p=0,22). But all children with clear data of the immediate type immune reaction to food (they were not done OFC test) had increased FC levels. This gave us opportunity to suspect some probable FC test predictive value in the OFC test assessment. And suppose subclinical or latent inflammation of the small intestine in children with skin symptoms and no gastrointestinal symptoms of FA because of the FC detected level. That obviously indicates the secondary association between common IgE level and FC in this patients cohort and needs further studies. This suspicion partially was confirmed by Merras-Salmio L. et al. who founded no increase of the FC level in children with detected FC and positive provocation tests results in children with gastrointestinal symptoms of the cow milk allergy [10]. On the other hand previous studies showed association between FC and gut microbiota composition in neonates [11].

Our study showed no association between FC and results of the OCT and product for OCT. Partially this confirms previous data of other authors who indicated no link between type of the feeding and FC. But they showed developmental responses of the digestive tract to different feeding strategies, additional supplements, especially the major role of the intestinal microbiota [12].

This fact makes us to suspect some link between common IgE level and FC in children through the gut microbiome and probable permeability change [10]. Alternatively, anamnesis data revealed that quantity of the children with FA symptoms associated with food containing biological amines has no significant difference between the groups. This fact raises doubts in the link between gut permeability and FC increase. That needs further studies too.

Conclusions

Despite only presence of the skin allergy symptoms in children there might be ongoing asymptomatic low-grade inflammation in the gut mucosa. FC should not be used for prediction of the OCT result, but asymptomatic gut mucosa inflammation was seen in 55% of children with FA symptoms on the skin.

Ethical Declaration: The study was approved by the local ethics committee of State Medical University, Zaporozhye, Ukraine. And this study was carried out in conformity with the Declaration of Helsinki.

Conflict of Interest: The authors declare no conflict of interest.

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