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Assessment of the role of the mucosal barrier dysfunction in pathogenesis of the food hypersensitivity in children

Key words: *food hypersensitivity, children, gut permeability, mucosal dysfunction, calprotectin, hydrogen breath test.*

Recent studies have showed importance of the mucosal barrier in regulation of the oral tolerance formation and course of the clinical symptoms of the food hypersensitivity (FH). Dysfunction of the mucosal barrier nowadays is considered as new target for therapy. Recent studies demonstrated that this barrier has inner «physical» and outer «functional» immune layers [17]. Role of the microbiome in the pathogenesis of the FH is the subject for further studies, but effectivity of the probiotics in the treatment of the FH is still disputing due to the contradictory results of the trials [2,4]. Taking into consideration hygienic theory of the allergic diseases onset, it can be supposed that functional changes of the mucosal barrier can as accompany as precede formation of the immune and nonimmune FH [11]. **Objective** of the study was to assess role of the mucosal barrier dysfunction in children with FH with detection of the permeability level, mucosal inflammation and functional state of the microbiome.

Materials and methods

During 2011–2016 years primary medical source documents of 1780 children aged 1 month-18 years, who visited Children multiefield children hospital #5, Zaporizhya and University clinic, Zaporizhzhya, Ukraine due to the symptoms of the FH on the skin were studied. 760 patients of them were examined. Diagnosis of FH was made with EAACI criterions in case of FH skin symptoms reproducible occurrence on exposure to the suspected food. Patients with food anaphylaxis were not included. After assessment of the eligibility according to the inclusion/exclusion criterions 424 children were recruited for further diagnostics. An average age of the patients was 26,3 [12,1;54,2] months. Most part of them – 59,4% (n=252) were children of early age. There was no significant difference in quantity of both sexes (boys – 210 (49%) and girls – 214 (51%)).

Questioning was used for anamnesis assessment. Severity of the skin symtoms was assessed with SCORAD scale. Standard allergological diagnostics included skin-prick tests (SPT), serum specific IgE level detection and oral challenge test (OCT) with common food allergens. Mucosal permeability was studied with urine test with lactulose by Behrens et al. (1984) methodic, based on the enzyme hydrolysis of this molecule [13]. On the start of the «Silufol» plate ethanol extract of the biomaterial (2:1) and standard (10–5 M of lactulose in ethanol) were applied. Chromatogram was placed in the system of the propanom: ammonium hydroxide. When the solvent ran 10 cm of the plate it was dried and developed with 1% solution of the alloxane in DMF with further heating. After that lactulose was gathered to the tube with 5 ml of DMF, which was warmed in a water bath and centrifuged. Quantity of the lactulose was calculated with formula after spectrophotometry (540 nm, LibraS32 PC).

Blood samples were collected after fasting in cooling vacutaner and after that it was immediately centrifugated (4 °C for 3.000 × 30 min). Monocytes concentrate was prepared following the standard methodic in the mixture of the ficoll: verographin. Quantity of the eligible samples was 37, other were excluded due to the insufficient quality of the precipitate. Transcription factor STAT6 (Elabscience Biotechnology Co., Ltd., China), transcription regulator GATA3 (RayBiotech, Inc., USA) and – FOXP3 (RayBiotech, Inc., USA) were detected with ELISA method in the lysate of the monocytes concentrate. For serum levels of interleukin-2 (IL-2), IL-4 detection ELISA method was used (Human IL-2 Platinum ELISA

and Human IL-4 Platinum ELISA, produced by Affymetrix eBioscience, Austria).).

Hydrogen (H_2) breath test (Gastro+ Gastrolyzer® (Bedfont, Germany) with glucose was conducted during 90–120 min [9]. Predose measurement of the H_2 (PPM) was provided before glucose intake (1–2 g/kg), after that H_2 was measured every 15 min. Test was considered as positive when difference between pre and postdose was at least 6 PPM, optimal – 12 PPM and more [9]. 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.

All statistical analyses were performed in commercial software Statistica (Statsoft, USA, № AXXR712D833214FAN5). 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. Linear association between two variables was assessed with Spearman, gamma Rank order correlation. Differences from the three and more groups were analyzed using the non-parametric ANOVA (Kruskall–Wallis) test. On-to-one comparisons for unpaired data were done with non–parametric Mann–Whitney's test.

The study was executed in the frames of the scientific research of the faculty peadiatria department of the Zaporizhia state medical university, Ministry of Health of Urkaine and is part of the planned scientific theme «Elaboration of the methods of early diagnostics of the most spread allergic diseases in children from different age groups, prophylactics and treatment of the essential functional disturbances and comorbidities in this group of patients» (registration number 0112U005648).

Results and discussion

At the moment of the entrering into the study 34,4% (n=146) of patents can not report exact food which caused the symptoms. Only 14,6% (n=62) reported only one food. It was found that morphology of the clinical symptoms on the skin varied and, except atopic dermatitis (61%), it included urticarial (5%), oral allergic syndrome (7%)? generalized «measles like» form (14%), toxycodermia (4%), papulose «nodular erythema like» rush (4%). According to the standard diagnostic algorithm skin allergy tests were provided in 298 children due to the severity of the symptoms or age of the patients.

Increased serum common IgE level was detected in 46% (n=142). Oral challenge test was performed in 340 patients and was positive in 37% (n=128). In 72% of cases cow milk was detected as the causative product.

Results of the standard tests for allergy diagnostics revealed that only in ¼ of the recruited patients aged 1–3 years and less than in 1/5 of children aged 4 and over had IgE-dependent reactions, which was proved with elevated sIgE to food levels. This finding underlines need in further investigation of the mechanisms which are in the basis of the skin symptoms of the FH.

On the next step additional test were used. Urine lactulose test was positive in 66.7% of patients (96/144). Mean lactulose level was 2,58 [1,87;3,15] mmol/L. 4 samples were excluded due to the poor quality. The more was age of the patients the less lactulose level was detected (Fig. 1).

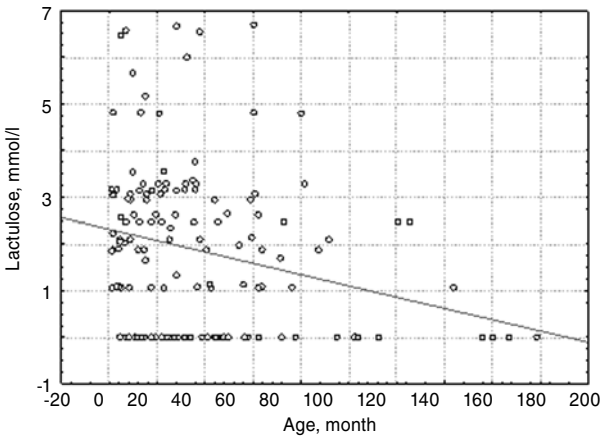

Figure 1. Mucosal permeability in children with skin FH symptoms

Table

Results of the mucosal barrier state study in children with clinical symptoms of the FH on the skin

Result age groups	Prevalence of the mucosal barrier dysfunction detected with method:		
	Urine lactulose test (n=144)	H_2 breath test with glucose (n=103)	Faecal Calprotectin (n=102)
up to 1 y. o.	31 (32%)	13 (30%)	13 (28%)
1–3 y. o.	43 (44%)*	16 (38%)*	27 (58%)*#
4–6 y. o.	17 (17%)	11 (26%)#	4 (9%)
7–11 y. o.	4 (4%)	1 (2%)	1 (2%)
12–18 y. o.	1 (1%)	1 (2%)	1 (2%)
total n (%)	96 (66,7%)#	42 (41%)	46 (45%)

Notation. * significance (p<0,05) of the difference between results of the same method in different age groups. # significance (p<0,05) of the difference between results of the different methods in one age group.

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Corresponding data were presented by other authors, who demonstrated that mucosal permeability is severest in the early childhood. Kalach N. et al. (2001) proved that children with cow milk allergy had negative correlation between mannitol/lactulose test results and age (r –0,33, p=0,01). [12]. Reason for such findings was presented on the animal model. It was found that early immune reaction to the formula based on the cow milk is accompanied with increased mucosal permeability [15].

In each third patient (32%, n=31) aged less than 1 year and almost ½ of the children (44%, n=43) aged from 1 to 3 y. o. had signs of the increased transmucosal transporting, which was proved with urine lactulose level (table).

As it can be seen from the table, it was found that 41% (n=42) and 45% (n=46) of children, correspondingly, had positive results of the H_2 breath test with glucose and Faecal Calprotectin test. Most part of such patients were children of the early age (68% (n=29) and 78% (n=40), correspondingly). Quantity of the positive tests varied according to the age. In age group up to 1 y. o. all tests had approximately the same results (32%, 30%, 28%). This fact indicated that in this period permeability, microbiome functioning abnormality and mucosal inflammation have the same prevalence. The more was duration of the disease the more H_2 level was detected with breath test (r = 0,63, p <0,05). This can be considered as the evidence of the gradual increase of the epitope disturbances in children with FH and that such changes have secondary place in the processes of the mucosal dysfunction onset which is obviously started with anatomical insufficiency of the tight junctions in the mucosa.

Faecal Calprotectin, which is considered as the most reliable marker of the mucosal inflammation as it is resistant to the digesting [6], was significantly more frequently detected in children aged 12 months and over, which indicates that previously diagnosed increased permeability and microbiome dysfunction lead to the inflammatory reaction in the mucosa. Other authors demonstrated that disaccharide insufficiency – risk factor of the FH symptoms, as it can change functioning of the Treg and cause mucositis, can increase permeability due to the butyrate accumulation [1].

It should be noticed that in age group from 4 y.o. and over fecal calprotectin was detected in much less quantity of patients – only in 9%, and H_2 almost in 2 times less (17% in compare with 44% and 32%). But in the same time H_2 breath test positive results were detected with the same frequency as in the younger children (26% in compare with 38% and 30%). This fact reflects that symptoms of FH on the skin in children aged 4–6 years in 26% of cases can have mechanisms of FH manifestation which involve dysbiosis as the cause of the maldigestion, pathological stimulation of the mucosal immune system by microbal antigens, hypersecretion of the biological amines. It was found that increased permeability which was detected with urine lactulose test correlated with positive H_2 breath test results (r=0,45, p<0,05). This fact evidence persistence of the pathological permeability in patients with abnormal epitope functioning. On the other hand, poor correlation between faecal calprotectin and lactulose levels (r=0,35, p<0,05) reflects that other factors are important for asymptomatic mucosal inflammation.

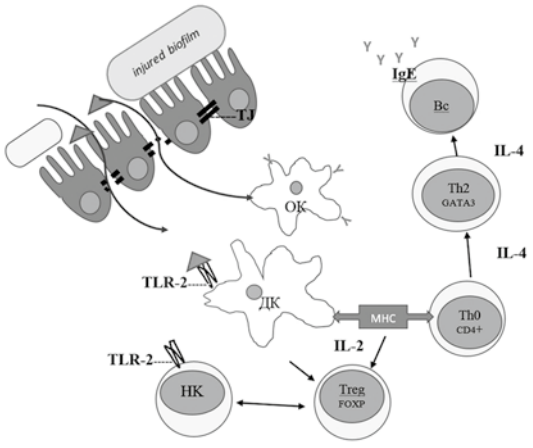
All three tests were positive in less than 5% of patients aged 7 years and over. This indicates that in these age groups skin symptoms of FH are not associated with mucosal barrier functioning and this can be taken into consideration during differential diagnostics and treatment. Other authors demonstrated that patients with systemic immune reactions have primary or secondary increased intestinal permeability [5]. Studies showed that this process can independently influence on serum IgE levels [17]. No correlation was detected either between IgE levels and results of the tests of the intestinal wall condition which were used in our research.

Kalach N. et al (2013) demonstrated that oral challenge test showed higher diagnostic value for detection of the pathological transmucosal transport. The same time they found that patch test was valuable only in 68%, IgE levels – 60%, prick-test – 55% [11]. Results of our study revealed that positive H_2 breath test correlated with serum IL-4 level (r=0,35, p<0,05) and positive result of the urine lactulose test negatively correlated with FOXP3 (r= – 0,52p<0,05). Such findings correspond to the hypothesis of the Th-2 associated mucosal barrier damage and decrease of it permeability after oral tolerance induction.

Taking into consideration results of our research and basing on the literature review data regarding structure and features of the functioning of the mucosal barrier new paradigm of the pathogenetic changes of the mucosal barrier in children with FH was proposed. The main diagnostics markers and possible therapeutic targets were noted schematically (modified from Chinthrajah R.S. et al., 2016) [7] (Fig.2).

Conclusions

Retained data demonstrated that more than 1/2 of children with FH symptoms on the skin have intestinal



Uncontrolled permeability of the mucosa leads to the specific and nonspecific activation of the cells

Notation: Bc – B-lymphocyte, Th – T-lymphocyte helper, MC- mast cell, NK – natural killer, DC – dendritic cell, TJ –tight junction, Treg – regulatory T-lymphocyte.

Figure 2. Scheme of the molecular-genetic mechanisms of the formation of the «phenotype of the increased permeability» of the intestinal mucosa in children with FH symptoms on the skin

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mucosa dysfunction which is proved in 66,7% of patients with urine lactulose test, in 40,7% – H_2 breath test with glucose and in 45% – faecal calprotectin test. Age features of the state of the mucosal barrier which were detected can be used for target therapy. Correlative analyzes proved that increased intestinal permeability during first 12 months precedes other changes. With the course of the disease biofilm functioning abnormality ($r=0,45$, $p<0,05$) and granulocyte infiltration of the intestinal wall appear ($r=0,35$, $p<0,05$), that was detected in 58% of children with FH symptoms from 1 to 3 y. o.

Gradual changes of the mucosal barrier condition lead to the reactivity of the immune system, which was proved by correlation between H^2 breath test positive results and polarization of the immune response through IL-4 ($r=0,35$, $p<0,05$) and link between urine lactulose test and intolerance to food allergens (negative correlation with FOXP3 ($r= – 0,52$, $p<0,05$)). Obtained data regarding mucosal barrier functioning in children with FH symptoms on the skin can lead to the as immune as nonimmune reactions. They should be taken into consideration during differential diagnosis and personalized therapy of FH in children.

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ОЦІНКА РОЛІ ДИСФУНКЦІЇ МУКОЗАЛЬНОГО БАР'ЄРУ У ФОРМУВАННІ ХАРЧОВОЇ ГІПЕРЧУТЛИВОСТІ У ДІТЕЙ

О.П. Пахольчук

Резюме

Метою роботи стало дослідити роль дисфункції мукозального бар'єра у дітей із симптомами харчової гіперчутливості (ХГ) на шкірі шляхом визначення рівня проникності та наявності реакції запалення стінки, функціонального стану біоплівки.

Матеріали та методи дослідження. З 2011 по 2016 р. було включено 424 пацієнта віком 26,3 [12,1;54,2] міс.

Результати. Тест сечі з лактулозою був позитивний у 66,7% пацієнтів (96/144). У 41% (n = 42) та 45% (n = 46) – позитивний результат H_2-дихального тесту з глюкозою та аналізу копрофільтрату на кальпротектин. Виявлено етапність змін стану мукозального бар'єра в дітей залежно від віку, часу маніфестації й тривалості хвороби.

Висновки. Діти із симптомами ХГ на шкірі мають дисфункцію мукозального бар'єра, що чинить системний вплив і потребує окремої корекції.

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Ключові слова: харчова гіперчуливість, діти, підвищена проникність, мукозальна дисфункція, кальпротектин, водневий дихальний тест.

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ОЦЕНКА РОЛИ ДИСФУНКЦИИ МУКОЗАЛЬНОГО БАРЬЕРА В ФОРМИРОВАНИИ ПИЩЕВОЙ ГИПЕРЧУВСТВИТЕЛЬНОСТИ У ДЕТЕЙ

О.П. Пахольчук

Резюме

Целью работы стало исследовать роль дисфункции мукозального барьера у детей с симптомами пищевой гиперчувствительности (ПГ) на коже путем определения уровня проницаемости и наличия реакции воспаления в стенке, функционального состояния биопленки.

Материалы и методы. С 2011 по 2016 г. было включено 424 пациента в возрасте 26,3 (12,1; 54,2) мес.

Результаты. Тест мочи с лактулозой был позитивным у 66,7% пациентов (96/144). У 41 и 45% – позитивный результат H_2-дыхательного теста с глюкозой и анализ копрофильтрата на кальпротектин. Вявлена этапность изменений состояния мукозального барьера у детей в зависимости от возраста, дебюта и длительности болезни.

Выводы. Дети с симптомами ПГ на коже имеют дисфункцию мукозального барьера, что имеет системные эффекты и требует отдельной коррекции.

Ключевые слова: пищевая гиперчувствительность, дети, повышенная проницаемость, мукозальная дисфункция, кальпротектин, водородный дыхательный тест.

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