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Evaluation of intestinal permeability on the basis of zonulin levels, in children with inflammatory bowel disease

Ocena przepuszczalności jelitowej na podstawie stężenia zonuliny u dzieci z nieswoistymi zapaleniami jelit

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S u m m a r y

Introduction. Non-specific inflammatory bowel disease in children is often associated with other autoimmune disorders, additionally abdominal pain, weight loss and growth disturbance occur in celiac disease and food allergies. In the pathogenesis of these diseases the impact of increased intestinal permeability is considered. The aim of the study is an assessment of an incidence of celiac disease and food allergy in children with inflammatory bowel disease and determination of their correlation with serum zonulin levels.

Materials and methods. The analysis included 71 children in age from 4 to 18 years with the active form of Crohn's disease and ulcerative colitis. The control group consisted of 32 children in the same age range without gastrointestinal symptoms. In the group of examined children immunological tests were performed to diagnose celiac disease and food allergy. In all children, quantitative determination of the zonulin in the serum was made.

Results. In the examined group of patients with inflammatory bowel disease celiac disease has not occurred, while in the case of atopic increased zonulin levels and significantly positive correlation between serum zonulin levels and total IgE was found.

Conclusions. Increased zonulin levels among children with atopic in the course of inflammatory bowel disease confirm its use as a serum biomarker of intestinal permeability. In the future, measurement of its levels among children with inflammatory bowel disease may also be one of non-invasive tests used in practice in the evaluation of the intestinal mucosa.

Key words: inflammatory bowel disease, celiac disease, allergies, intestinal permeability, children

S t r e s z c z e n i e

Wstęp. Nieswoistym zapaleniom jelit u dzieci nierzadko towarzyszą inne zaburzenia o charakterze autoimmunologicznym, a bóle brzucha, niedobór masy ciała i zaburzenia wzrastania występują również w celiakii oraz alergii na pokarmy. W patogenezie tych chorób rozważa się udział zwiększonej przepuszczalności jelitowej.

Cel. Celem pracy jest ocena częstości występowania celiakii i alergii na pokarmy u dzieci z nieswoistymi zapaleniami jelit oraz określenie ich korelacji z poziomem zonuliny w surowicy krwi.

Materiały i metody. Analizą objęto 71 dzieci w wieku od 4 do 18 lat w aktywnej fazie choroby Leśniowskiego-Crohna i wrzodziejącego zapalenia jelita grubego. Grupę kontrolną stanowiło 32 dzieci w tym samym wieku bez objawów ze strony przewodu pokarmowego. W grupie dzieci chorych wykonano: IgA, IgG, stężenie IgA tTG lub IgA EmA (przy niskich wartościach IgA całkowitego zostały pobrane IgG tTG bądź IgG EmA), IgE całkowite oraz IgE specyficzne na wybrane alergeny pokarmowe. U wszystkich dzieci oznaczono ilościowo zonulinę w surowicy krwi.

Wyniki. W badanej grupie chorych z nieswoistymi zapaleniami jelit nie stwierdzono celiakii, natomiast w przypadku atopii stwierdzono podwyższony poziom zonuliny oraz istotnie dodatnią korelację pomiędzy stężeniem zonuliny a IgE całkowitym.

Wnioski. Podwyższone stężenie zonuliny u dzieci z atopią w przebiegu nieswoistych zapaleń jelit potwierdza jej rolę jako biomarkera przepuszczalności jelitowej. Pomiar jej stężenia u dzieci z nieswoistymi zapaleniami jelit w przyszłości może stanowić również jeden z nieinwazyjnych testów wykorzystywanych w praktyce w ocenie stanu błony śluzowej jelita.

Słowa kluczowe: nieswoiste zapalenia jelit, celiakia, alergia, przepuszczalność jelitowa, dzieci

INTRODUCTION

Inflammatory bowel disease in children is often associated with other autoimmune disorders. Additionally, an abdominal pain, weight loss and growth disturbance occur in celiac disease, food allergies and also in IBD (1, 2). In the pathogenesis of these diseases the

impact of increased intestinal permeability is considered (3). In recent years the diagnosis of celiac disease has been extended by assessment of serum zonulin levels. Zonulin is a mediator which mediates in physiological movement of molecules from the intestinal lumen by relaxation of tight junctions (4-6). These cell junctions belong to occluding junctions – zonula occludens (ZO). These structures reduce passage of harmful particles into the mucosa. Under conditions of excessive secretion of zonulin increased movement of macromolecules through the intestinal mucosa may cause immunization leading to intestinal and extraintestinal autoimmune diseases, what is genetically determined (7-9).

Until now, three ways of transepithelial transport were identified. These are: the transcellular way – through the surface of the enterocyte; the paracellular way – through intercellular connections, in which zonulin is involved; and the way through dendritic cells. In recent years, the hypothesis that the increased intestinal permeability is the basis in inflammatory process of IBD are put forward (10, 11).

It has been shown that due to a direct effect of gluten on intestinal mucosa in acute celiac disease the increase in zonulin activity occurs. In this case the zonulin release depends on MyD88 factor (mylloid differentiation factor). The increase in zonulin activity causes a direct flow of gliadin and other antigens into the intestinal mucosa (12, 13). Also, in patients with acute celiac disease the results of immunofluorescence studies on intestinal tissue showed increased zonulin levels in the submucosal layer (14).

There is a growing trend of allergies in recent years (15, 16). In the literature, the impact of food allergy on the etiopathogenesis of inflammatory bowel disease is considered. This is explained by the increase in the permeability of the intestinal mucosa, and easier penetration of different antigens through the intestinal barrier and excitation of immune response (17, 18).

Histological changes in the course of allergy in the gastrointestinal tract are seen as the increase in the amount of lymphocytes in the epithelium of the intestine, the result of this is a deepening of crypts and a progressive villous atrophy. Morphological abnormalities of intestinal villi have an impact on their absorption functions, what gives symptoms of malabsorption syndrome (19).

AIM

The aim of the study was an evaluation of prevalence of food allergy and celiac disease in children with inflammatory bowel disease and determination of their correlation with serum zonulin levels.

MATERIALS AND METHODS

The analysis included 71 children with active IBD, average age – 12.9 years: 36 children (17 girls and 19 boys; average age – 13,36 years) with Crohn's disease (CD) and 35 children (17 girls and 18 boys; average age – 12.44 years) with ulcerative colitis (UC).

The diagnosis was made using Porto criteria (20).

The control group consisted of 32 children without gastrointestinal symptoms (14 girls and 18 boys, average age – 12.09 years) classified for planned laryngological procedures. After legal guardians got familiar with the information concerning the nature of procedure and agreed in writing to its performance the study was conducted. Planned work received the positive opinion from the Bioethics Commission of the Medical University of Silesia in Katowice. All patients were subjected to subjective and physical examination.

To diagnose celiac disease and food allergy in examined group of children with CD and UC following immunological studies were performed:

1. Evaluation of IgA, IgG by immunoturbidimetric metod. In the interpretation of the results standards for age were applied.
2. tTG IgA levels by ELISA (standards: < 20 EU/ml negative result; 20-25 EU/ml questionable result, > 25 EU/ml positive result) or IgA EMA by indirect immunofluorescence (at low values for age the total IgA, tTG IgG or IgG EmA were used).
3. Total IgE levels determined by chemiluminescence. In the interpretation of the results standards for age were applied.
4. Specific IgE for selected food allergens (milk, wheat flour, egg white and egg yolk) determined by chemiluminescence. Reagents produced by DPC Krakow. Standard < 0.35 kU/l.

In all patients and in the control group: quantification of serum zonulin levels was performed using a set for research purposes – the Zonulin ELISA kit from Immundiagnostik. Determination of zonulin in serum was performed using 1cm^3 blood sample collected for other examination. The serum was diluted 1:2 with extraction buffer (EXBUF), and the results of zonulin levels (expressed in units of ng/ml) were read from the curve defined by the calibration.

The results of zonulin levels were compared to control group.

Laboratory researches were conducted at the Department of Laboratory Diagnostics in the Upper Silesian Center of Child's Health in Katowice. Statistical analysis was performed using STATISTICA program. The average values and standard deviation of examined parameters were calculated. The following tests were used: the Kolmogorov-Smirnov test, Student's t test, Mann-Whitney test, chi-square test or chi-square with Yates' correction, [R] Spearman Rank Correlation test. Level of $p < 0.05$ considered to be statistically significant.

RESULTS

In the examined group of children and the control group differences in gender has not been demonstrated. In the group of children with CD average age was about one year higher than in children with UC, however, this difference was not statistically significant. In examined group, in patients with CD zonulin levels

ranged from 2.87 to 46.2 ng/ml and in patients with UC form 1.93 to 61.43 ng/ml. In the control group zonulin levels ranged from 0.51 to 15.46 ng/ml. Zonulin levels in children with CD and UC were similar and significantly higher compared to the control group (tab. 1).

Table 1. Average values of zonulin levels in the examined groups of patients with Crohn Disease (CD) and Ulcerative Colitis (UC), and in the control group.

	CD	UC	Control group
Average values of zonulin levels (ng/ml)	18.96	18.40	7.84
+/- SD	+/- 14.2	+/- 16.05	+/- 5.78

CD – Crohn Disease, UC – Ulcerative Colitis

IgE values in children with IBD

In both groups, regard to the age standard in more than a half of children, elevated total IgE values occurred (tab. 2).

Table 2. IgE values in the examined groups of children.

Group	N	Total IgE IU/ml		
		Mean values	Elevated	Proper
CD	36	79.0 ± 78.5	21 (58.3%)	15 (41.7%)
UC	35	73.2 ± 71.8	22 (62.9%)	13 (37.1%)
Comparison		NS	NS	

CD – Crohn Disease, UC – Ulcerative Colitis

The presence of positive specific IgE for selected foods (milk, chicken eggs, chicken, wheat flour) was observed in 3 children (8.33%) with CD and in 4 children (11.42%) with UC. In patients with CD, with elevated total IgE levels, significantly higher zonulin levels occurred ($p = 0.01$). Also in patients with ulcerative colitis, with elevated total IgE, significantly higher levels of this protein occurred ($p = 0.03$). In both groups, statistically significant correlation between total IgE and zonulin levels has been shown (tab. 3).

Intestinal biopsy in children with IBD

In patients with CD and UC, there were no positive tissue transglutaminase antibodies, or antibodies against the endomysium of the smooth muscle. In the examined group, in 7 patients with IBD (9.85%) villous atrophy was observed. Among patients with CD following Marsh stages of villous atrophy were found: in 2 patients stage IIIA (zonulin levels in these patients were as follows: 42.73 ng/ml, 10.99 ng/ml), in 2 patients stage IIIB and in 3 patients with UC stage IIIA (zonulin levels were as follows: 39.56 ng/ml, 17.97 ng/ml, 17.37 ng/ml).

DISCUSSION

The discovery of zonulin has resulted in a number of studies on the nature and significance of intestinal permeability in the pathogenesis of many diseases of the digestive system diseases (such as celiac disease), nervous system, and some cancers and allergies (8).

Most recently there has been a growing interest in the subject of intestinal permeability disorders and its impact on the pathogenesis of many diseases, and also the coexistence of some of them (9). The issue of this work is to evaluate the coexistence of IBD, food allergies and celiac disease. Currently, many studies relate to assessment of the intestinal permeability in terms of an initiating disease factor – is it the primary mechanism or is it occur secondary to lasting inflammation? According to Kalach et al., maturity and therefore sealing of the intestinal barrier occurs in children who have completed one year of age. Thus, in infancy an increased tendency to be allergic to food occurs (21-23). Also, the long-term oral antibiotic treatment, the usage of NSAIDs and intestinal infections do affect the balance of intestinal flora – damage the function of the intestinal epithelium. Increased susceptibility to the harmful factors as well as genetic predisposition to the occurrence of increased intestinal permeability are indicated in the research by Hilsedena et al. This research showed increased intestinal permeability in 30% of healthy relatives of patients with IBD. Easier penetration of antigens through the intestinal barrier undoubtedly predisposes to the development of allergies (24, 25).

Bartunkowa et al. found the presence of IgE-mediated food allergy in 14.3% of children with CD and in 9% of patients with ulcerative colitis. Van den Bogaerde observed hypersensitivity to foods in the half of patients with CD. Arienzo showed the coexistence of food allergies in 22% of patients with ulcerative colitis (26-28). In our previous studies, IgE-mediated selected food allergy was found in 21% of patients with CD and in 32% of patients with UC. Features of atopic in the form of high total IgE were shown twice as often in CD (29). Results of this study show the IgE-mediated allergy to selected foods in 3 (8.33%) children with CD and in 4 (11.42%) children with UC, and features of atopic in the form of increased total IgE were found much more often because in more than half of the patients. In both disease groups, also the positive correlation between zonulin levels and total IgE in patients was demonstrated. However, until now, it has not been proven if the increase in total IgE is in the primary or secondary mecha-

Table 3. Correlations between zonulin levels and total IgE in the examined groups of children with Inflammatory Bowel Disease (IBD).

Correlated parameter	CD + UC	P	CD	P	UC	P
	Spearman rank		Spearman rank		Spearman rank	
IgcEc	0.4669	< 0.001	0.3509	0.0359	0.5574	< 0.001

CD – Crohn Disease, UC – Ulcerative Colitis

nism to disorders of intestinal permeability. But it has been proven, that in the course of IBD the atopic is most probably related to immune disorders, at the root of which lies increased intestinal permeability.

In 2009, Fasano et al. published the research in which they showed the gliadin effect on the intestinal mucosa, what leads to the relaxation of the actin microfilaments and increased intestinal permeability, while the amount of secreted zonulin depends on levels of gliadin (30). Celiac disease coexists with some disease conditions in the course of which an increased intestinal permeability was found (31, 32). In none of the patients with UC and CD positive tissue transglutaminase antibodies or antibodies against the endomysium of the smooth muscle were detected. Among examined group with IBD in 7 patients (9.85%) intestinal villous atrophy was found. In this group of patients zonulin levels were high, what confirms abnormalities in intestinal permeability.

Perhaps if the study included a larger group of children with IBD, it would show the coexistence of these two diseases. However, analyzing epidemiological data in literature, which determined this rate at the level of 0.83% what is equal to the population ratio, the much higher results should not be expect (33).

CONCLUSIONS

Increased zonulin levels in children with atopic in the course of inflammatory bowel disease confirm its use as a serum biomarker of intestinal permeability. In the future, measurement of its levels among children with inflammatory bowel disease may also be one of non-invasive tests used in practice in the evaluation of the intestinal mucosa.

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