

Serum Zonulin as a Biomarker of Disease activity in Pediatric Inflammatory Bowel Disease (IBD)

Engy A. Boshra², Ayman E. Eskander², Ahmed S. El-Gayed¹, Mariam Saad Shawky¹, Radwa M Abdel Halim³, Manar A. El Desouky¹

1 Pediatric Department, Faculty of Medicine, October 6th University, Egypt.

2 Pediatric Department, Faculty of Medicine, Cairo University, Egypt.

3 Clinical and Chemical Pathology Department, Faculty of Medicine, Cairo University, Egypt.

ABSTRACT

Current information shows that impaired intestinal permeability has an essential role in pathogenesis of inflammatory bowel disease (IBD). Zonulin is 47-KD protein, has a central role in regulating intestinal permeability via modulating intercellular tight junctions between enterocytes, so it may act as a novel biomarker of illness activity. This research aimed to assess serum Zonulin in pediatric with IBD, as a potential biomarker in disease activity and remission.

Methods: Eighty children: 50 Ulcerative colitis (UC) cases, 30 Crohn's disease (CD) cases were examined for serum Zonulin which tested by ELISA test. Serum Zonulin level was evaluated in relation to age, gender, anthropometric data, IBD type, onset of disease, disease activity, laboratory investigations, Esophago-gastro-duodenoscopy and ileo-colonoscopy and treatment modalities. Statistical tests have been applied for information analysis. **Results:** the median Zonulin in the studied group was 14.7ng/ml. Serum Zonulin level was statistically increased with higher disease severity compared to remission group: median for remission:10.8(10.2-14.6); mild exacerbation:16.9(11.7-29.7); moderate exacerbation:30.4(14.7-89.0) and severe exacerbation:64.3(45.8-225) ng/ml (P=0.002), strong correlation between serum Zonulin and Pediatric Ulcerative Colitis Activity Index (PUCAI), Pediatric Crohn's Disease Activity Index (PCDAI) Score ($p < 0.001$), there was a significant correlation among Serum Zonulin and fecal calprotectin ($r = 0.329, P = 0.003$), also results showed that serum Zonulin was statistically lower in children with apparently normal endoscopic examination ($p = 0.04$). No association was found between serum Zonulin and age, gender, anthropometric data, IBD type, onset of disease and treatment modalities. **Conclusion:** The study concluded that serum Zonulin concentrations are high among IBD patients and there is significant variance among cases in remission and those in activity, which may illustrate that serum Zonulin could act as a novel biomarker for IBD diagnosis and follow up in pediatric cases.

Keywords: Zonulin, inflammatory bowel disease, intestinal permeability

How to cite this article: Engy A. Boshra, Ayman E. Eskander, Ahmed S. El-Gayed, Mariam Saad Shawky, Radwa M Abdel Halim, Manar A. El Desouky, Serum Zonulin as a Biomarker of Disease activity in Pediatric Inflammatory Bowel Disease (IBD). 2026;16(19s): 77-83. DOI: 10.25258/ijddt.16.19s.9

Source of support: None

Conflict of interest: None

INTRODUCTION

Inflammatory bowel disease, comprising CD, ulcerative colitis and Indeterminate Colitis (IC), is distinguished by chronic inflammation of the gastrointestinal tract, and exhibit periods of remission and relapse¹. IBD is related to alterations in intestinal permeability and gut microbiota, which are thought to have a crucial role in illness pathogenesis². The intestinal barrier consists of a single layer of epithelial cells which tightly connected by protein complexes such as claudins, occludins, and zonula occludens. This structure allows selective permeability³. Zonulin is 47-KD protein, structurally related to pre-haptoglobin 2, and its gene is located on Chromosome 16⁴. Zonulin is a protein modulator that has a central role in regulating intestinal permeability by modulating intercellular tight junctions between enterocytes. Its discovery marked a significant advance in understanding

the dynamic nature of the intestinal barrier⁵. Zonulin regulation is tightly controlled under physiological conditions. However, in genetically susceptible individuals, dysregulated zonulin release results in persistent barrier dysfunction. This allows translocation of antigens, triggering immune response that can lead to chronic inflammation and autoimmunity⁶. This mechanism begins with zonulin binding to the epidermal growth factor receptor (EGFR) and protease-activated receptor 2 (PAR2), triggering a signaling cascade involving protein kinase C and the disassembly of tight junction complexes⁵. Impaired intestinal permeability represents an early event preceding the onset of inflammatory bowel disease. Since Zonulin has reflects of intestinal permeability, so it may act as a potential biomarker of disease activity in IBD². Research has shown increased concentration of serum Zonulin in individuals with autoimmune disorders, obesity, diabetes,

*Author for Correspondence: mariamsaad.med@obu.edu.eg

and inflammatory bowel diseases, supporting its role as a biomarker for impaired barrier function⁷.

MATERIAL AND METHODS:

This study is a cross sectional research has been done on 80 children between the age of 2 years and 16 years over the period of 6 months, registered in the pediatric Gastroenterology outpatient clinic, Abuelreish hospital, Cairo University, and pediatric outpatient clinic of October 6th University hospital, Egypt. The children were diagnosed with IBD based on clinical, laboratory, imaging, Endoscopic and histopathological evidence for diagnosis. The cases have been separated into two groups, Group A [IBD cases in activity] and group B [IBD patients in remission]. The study received approval from the ethical committee of the faculty of medicine, 6th of October University (Ethical Code: FWA 000017858). Following obtaining an informed written consent from parents, children were subjected to: Medical history assessment: data was collected by direct patient/parent interviewing including age, gender, age at diagnosis, consanguinity, similar condition in family, symptoms of disease. e.g., abdominal pain, diarrhea, number of stool, bloody stool, nocturnal stool, activity level, Peri-rectal disease, symptoms of extra intestinal manifestation e.g., fever, arthritis and uveitis, history of hospital admissions including cause, frequency and average duration of hospital stay, medication history and treatment modality. Clinical assessment: Anthropometry: to assess growth in children, we used several different Measurements comprising height for age, weight for age, and Body Mass Index (BMI). All of these measurements plotted Z-score and percentile growth curves for boys and girls. Assessment of the disease activity: The PUCAI has been developed to evaluate the activity of illness, Numerical scores are given to symptoms of rectal bleeding, stool consistency, abdominal pain, presence of nocturnal stools, stool frequency, and activity level, adding to a maximum score of 85. The PCDAI allows the calculation of a numerical score varying from 0 to 100 depending upon a child's well-being, bowel movements, degree of abdominal pain, linear growth, weight gain, hematocrit, physical examination, sedimentation rate, and serum albumin. Biochemical screening: the following laboratory data was ordered: Complete blood picture (CBC) with blood indices, hepatic function tests including alanine aminotransferase (ALT) and aspartate aminotransferase (AST), ESR, CRP, Stool Analysis and fecal calprotectin. Esophago-gastro-duodenoscopy and ileo-colonoscopy were done at time of diagnosis by OLYMPUS and FUJIFILM Endoscopy. Growth features according to Criteria of extent of disease by Pediatric Modification of the Montreal and Paris classification for UC and CD. Biopsy was taken from small intestine and colon for histopathological examination. Estimation of serum Zonulin level by ELISA test: Sample collection:- Two ml of venous blood in a sterile plain tube were collected from each participant. Serum samples are permitted to clot at room temperature then centrifuged at 3000xg for ten minutes for assay of serum Zonulin by ELISA. Assay procedure (Elabscience human zonulin ELISA kit Catalog

No: E-EL-H5560). The assay utilized the "sandwich" technique. The serum Zonulin result was given in ng/ml. Statistical analysis of the Information: The information that was gathered was revised, coded, and tabulated using the Statistical Package for Social Science (IBM Corp., 2017). IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corporation. Information was given, and appropriate analysis has been conducted based on the type of data acquired for each parameter. The Shapiro test has been conducted to assess the normality of information distribution. Descriptive statistics: Mean, Standard deviation (\pm SD) for parametric numerical information, whereas Median and range for non-parametric numerical information. Frequency and percentage of categorical information. Analytical statistics include the Student's T Test, Mann-Whitney Test (U test), Kruskal-Wallis Test, Chi-Square Test, Fisher's Exact Test, correlation analysis, and the ROC Curve (receiver operating characteristic). The area under the ROC curve (AUC) is classified as excellent for values between 0.9-1, good for 0.8-0.9, fair for 0.7-0.8, poor for 0.6-0.7, and failed for 0.5-0.6. All given p-values were two-tailed; P -value under 0.05 is considered significant (S)⁸.

RESULTS:

This research involved 49 males and 31 females; their mean age was 9.4 ± 3.2 years. Table 2 shows that their mean height was 128.8 ± 19.3 cm, $SD = -0.9$, their mean weight was 28.8 ± 12.1 kg, $SD = -0.7$, and their mean BMI was 16.9 ± 2.6 Kg/m². Table 3 shows that the median age at illness onset was five years, 62.5% had Ulcerative colitis and 37.5% had Crohn's disease, the median severity score was 12.5, 46.3% of cases had remission, 26.3% had mild exacerbation, 18.8% had moderate exacerbation, 8.8% had severe exacerbation. Study showed that Esophago-gastro-duodenoscopy and ileo-colonoscopy regarding gross features; 48.8% of cases was apparently normal, 43.8% had inflammation, 26.3% had ulceration, 3.8% had strictures, 10% had pseudo polyp, 2.5% had loss of haustration, 1.3% had nodular mucosa as seen in table 4. regarding extent of pathological findings, 48.8% had no lesion, 23.8% located in rectosegmoid, 7.5% on Right side of the colon, 17.6% had pancolitis and 13.7% on ilium as seen in table 5. Serum Zonulin level was done for all the patients. Figure 1 shows that the median Zonulin in the studied group was 14.7ng/ml. Table 6 shows that there was no statistical significant variance in Zonulin level as regarding to Gender. No statistically significant association was noted between serum zonulin, age and anthropometric measurements. Figure 2 shows that Zonulin level was statistically increased with higher disease severity. Serum Zonulin had significant positive correlations with PUCAI or PCDAI Score as shown in figure 3. ROC analysis was done to evaluate the correlation between serum Zonulin and fecal calprotectin predict severe exacerbation in children with IBD; at a cutoff point > 45.3 ng/ml of serum Zonulin, the sensitivity was 80.7% and specificity was 80.8%; at a cutoff point > 57 μ g/g of fecal calprotectin, the sensitivity was 71.4% and specificity was 79.5%. Table 8 shows that Zonulin level was

statistically lower in children with apparently normal endoscopic examination.

Table 1: Frequency distribution of age and gender among patients of the studied group.

		Study group	
		N=80	%
Gender	Male	49	61.3%
	Female	31	38.8%
Age (years)	Mean±SD	9.4±3.2	
	Range	2.5-16	

Table 2: Frequency distribution of anthropometric measurements among patients of the studied group.

		Study group	
		N=80	%
Height (cm)	Mean±SD	128.8±19.3	
	Range	89-171	
Height SD	Median	-0.9	
	IQR	-1.3 – 0.6	
Weight (kg)	Mean±SD	28.8±12.1	
	Range	10.5-78	
Weight SD	Median	-0.7	
	IQR	-0.8 - -0.2	
BMI (kg/m ²)	Mean±SD	16.9±2.6	
	Range	9.3-28.4	

Table 3: Frequency distribution of disease criteria of the patients of the studied group.

		Study group	
		N=80	%
Onset of disease (years)	Median	5.0	
	IQR	4-12	
Disease	Ulcerative colitis	50	62.5%
	Crohns Disease	30	37.5%
The pediatric UC/CD Activity index (PUCAI)(PCDAI) Score	Median	12.5	
	IQR	10-20	
The pediatric UC/CD Activity index	Remission	37	46.3%
	Mild exacerbation	21	26.3%
	Moderate	15	18.8%
	Severe	7	8.8%

Table 4: Result of the Endoscopy (Growth Features) of the patients of the studied group.

		Study group	
		N=80	%
Esophago-gastro-duodenoscopy and ileo colonoscopy (gross features)	Apparently normal	39	48.8%
	Inflammation	35	43.8%
	Ulceration	21	26.3%
	Stricture	3	3.8%
	Pseudo polyp	8	10.0%
	Loss of haustration	2	2.5%
	Nodular mucosa	1	1.3%

Table 5: Result of the Endoscopy (Extent of pathology) of the patients of the studied group.

		Study group	
		N=80	%
Esophago-gastro-duodenoscopy and ileo-colonoscopy (extent of pathology)	No lesion	39	48.8%
	Recto-sigmoid	19	23.8%
	Rt side of the colon	6	7.5%
	Pancolitis	14	17.6%
	Ilium	11	13.7%

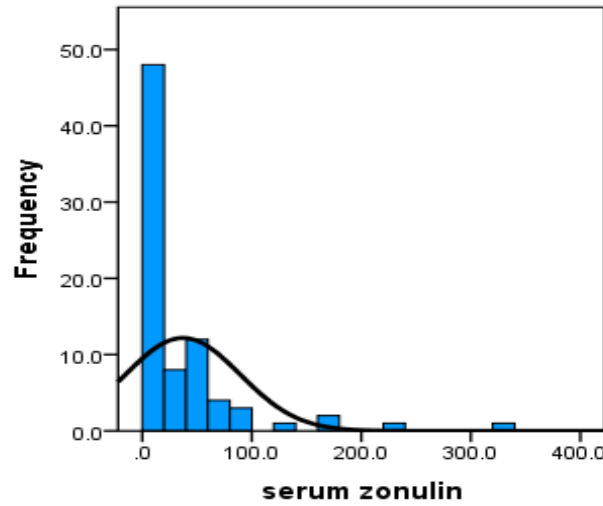


Figure 1: Serum Zonulin in the patients of the studied group.

Table 6: Correlation between serum Zonulin and gender of the patients of the studied group

		Serum Zonulin (ng/mL)		Test	P value
		Median	IQR		
Gender	Male	13.4	11.0-16.4	U=1.8	0.09
	Female	27.4	14.6-49.2		

Table7: Correlation between serum Zonulin in patients and age& anthropometric measurements of the patient of the studied group.

	serum zonulin	
	R	P value
Age (years)	-0.093	0.412
Height (cm)	0.020	0.860
Height SD	0.162	0.151
Weight (kg)	-0.045	0.693
Weight SD	-0.052	0.649
BMI	-0.115	0.313

*Author for Correspondence: mariamsaad.med@obu.edu.eg

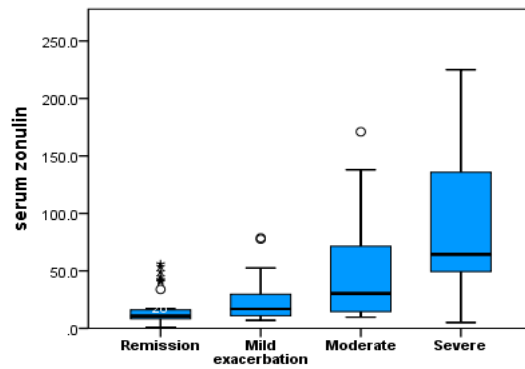


Figure 2: Serum Zonulin as regarding to disease activity index.

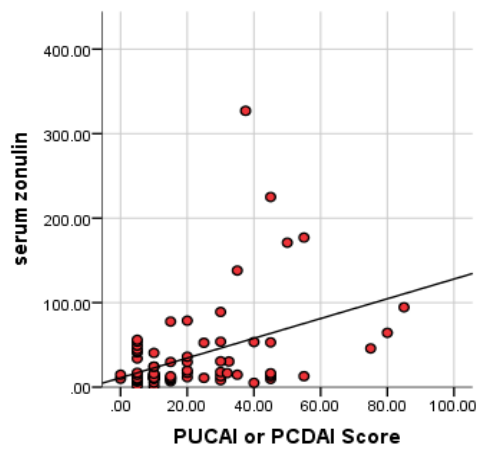


Figure 3: Correlation between serum Zonulin and PUCAI or PCDAI Score.

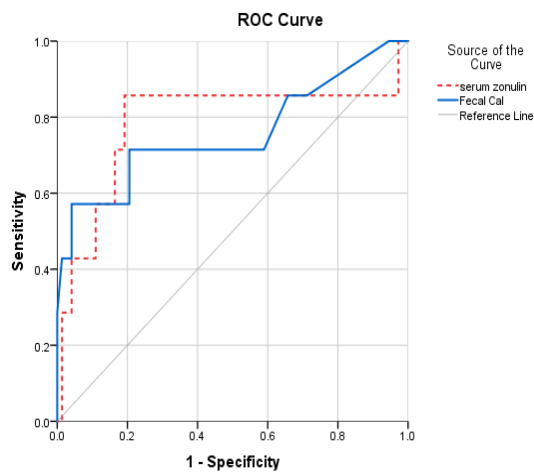


Figure 4: ROC curve of correlation of Serum Zonulin and fecal calprotectin predict severe exacerbation in children with IBD.

Table 8: Correlation between Serum Zonulin in the patients with the results of the endoscopic examination.

		Serum Zonulin (ng/mL)		Test	P value
		Median	IQR		
Esophago-gastro-duodenoscopy and ileo colonoscopy (gross features)	Apparently normal	11.9	7-16.6	K=26.1	0.043*
	Inflammation	16.9	11.7-53.4		
	Ulceration	22.0	8.9-64.3		
	Stricture	14.7	13.0-29.8		
	Pseudo polyp	44.8	12.9-49.2		
	loss of haustration	14.6	10.8-33.9		
Esophago-gastro-duodenoscopy and ileo colonoscopy (extent of disease)	Nodular mucosa	30.5	..	K=31.4	0.019*
	No lesion	15.3	11.5-29.8		
	Recto-sigmoid	17.7	11.0-49.7		
	Rt. Side of colon	37.6	10.8-64.3		
	Pancolitis	33.9	10.2-225.0		
	Ileum	30.4	5.1-53.8		

DISCUSSION:

The study showed that 80 children was 49 males and 31 females, their mean age was 9.4±3.2 years and their mean height was 128.8±19.3 cm, their mean weight was 28.8±12.1 kg, and their mean BMI was 16.9±2.6 Kg/m². This result is concordant with the retrospective cohort study of effect of IBD on growth at Jordan University Hospital by Almomani et al. (2025) conducted on 78 patients aged 5-18 years 40 male and 38 female, the anthropometric measurements illustrated a statistically significant rise in the 1st and 2nd years of monitoring than the diagnosis, this enhancement may be due to disease activity control⁹. Our study showed that the median age at illness onset was five years, 62.5% had ulcerative colitis and 37.5% had crohns disease with incidence of UC was higher than CD. This result is in concordant with Cenni et al. (2023) who measured fecal Calgranulin-C (S100A12) and fecal Zonulin in children showed that 60.7% with UC and 39.3% with CD¹⁰, but in contrast to cohort study by Szymanska et al. (2021) who measured fecal Zonulin in pediatric showed that CD was higher as 53.4% had CD and 46.6% had UC with mean age 12.8years ±3.9 years¹¹. Our study aimed to measure serum Zonulin to children and showed that the median serum Zonulin in the studied group was 14.7ng/ml. In our research a statistically insignificant correlation has been found between Zonulin level and age, gender, anthropometric measurements and regarding type of disease (UC or CD), concordant with the pilot study done at AOU Città della Salute e della Scienza di Torino – San Giovanni Antica Sede Hospital by Caviglia et al. (2019) who measured level of serum and fecal Zonulin in adult, found that no variance in serum Zonulin concentration has been found between cases with CD and those with UC², in contrast to the research by Malíčková et al. (2017) who measured serum and fecal Zonulin in adult IBD provided further evidence supporting Zonulin’s involvement in IBD by demonstrating increased zonulin levels in cases with Crohn’s disease than those with UC¹². In our study, illness activity in IBD in pediatric is commonly assessed by using

validated scoring systems: the Pediatric Ulcerative Colitis Activity Index and the Pediatric Crohn’s Disease Activity Index, these indices categorize disease severity into remission, mild, moderate, and severe based on clinical symptoms, physical examination, and laboratory findings. The principal finding of this study that serum Zonulin level was statistically increased with higher disease severity compared to remission group. This result is consistent with the observation reported In pilot study by Caviglia et al. (2019) who observed that serum Zonulin level were significantly elevated in adult with IBD, the results suggests that serum zonulin could have diagnostic value, particularly in identifying mucosal barrier defects, its levels in the bloodstream are thought to reflect dysregulation of tight junctions in the intestinal epithelium, making it a candidate for evaluating "leaky gut" syndromes². Szymanska et al., (2023) found that Fecal Zonulin higher in patients with IBD, but a statistically insignificant variance in concentration of serum Zonulin has been reported between children with IBD¹³. Our research illustrated that a significant positive correlation has been found between serum Zonulin and PUCAI or Pediatric Crohn’s Disease Activity Index Score. In a pilot research by Caviglia et al. (2019) who found that serum Zonulin levels were significantly elevated in adult with IBD, but no correlation has been found between serum Zonulin and disease activity index². In our research showed that Zonulin level was statistically lower in children with apparently normal Esophago-gastro-duodenoscopy and ileo-colonoscopy examination according to gross feature and extent of pathology. Our study showed that serum Zonulin had statistically significant positive correlations with fecal calprotectin. ROC analysis has been done to evaluate the correlation between Serum Zonulin and fecal calprotectin predict severe exacerbation in children with IBD; at a cutoff point > 45.3 ng/ml of serum Zonulin, the sensitivity was 80.7% and specificity was 80.8%; at a cutoff point > 57 µg/g of fecal calprotectin, the sensitivity was 71.4% and specificity was 79.5%. This contradicts to the study by

Caviglia et al. (2019) who reported that no correlation found between serum Zonulin in IBD patients with fecal calprotectin². Szymanska et al. (2023) mentioned that there was association between fecal Zonulin and fecal calprotectin, but no association between serum Zonulin and fecal calprotectin¹³.

CONCLUSION:

The study concluded that serum Zonulin concentrations are high among IBD cases and there is significant variance between cases in remission and those in activity, which may illustrate that Zonulin could act as a novel biomarker for diagnosis and follow up of IBD. Serum Zonulin concentrations are strongly correlate with fecal calprotectin this link positions zonulin not only as a local but also as a systemic indicator of disease severity. This thesis demonstrates that serum Zonulin presents as a significant biomarker for monitoring intestinal permeability and inflammation in pediatric IBD.

FUNDING

No funds.

DECLARATION OF CONFLICTS OF INTERESTS

Author declares that they have no conflict of interest.

USE OF ARTIFICIAL INTELLIGENCE

Not applicable

REFERENCE

1. Kobayashi, T., Siegmund, B., Le Berre, C., Wei, S. C., Ferrante, M., Shen, B., & Rogler, G. (2020). Ulcerative colitis. *Nature Reviews Disease Primers*, 6(1), 74.
2. Caviglia, G. P., Dughera, F., Ribaldone, D. G., Rosso, C., Abate, M. L., Pellicano, R., Bresso, F., Smedile, A., Saracco, G. M., & Astegiano, M. (2019). Serum zonulin in patients with inflammatory bowel disease: A pilot study. *Minerva Medica*, 110(2), 95–100.
3. Mowat AM, Millington OR, Chirido FG: Anatomical and cellular basis of immunity and tolerance in the intestine. *J Pediatr Gastroenterol Nutr*. 2004; 39 Suppl 3:S723–4.
4. Martinez, E. E., Mehta, N. M., & Fasano, A. (2022). The Zonulin pathway as a potential mediator of gastrointestinal dysfunction in critical illness. *Pediatric Critical Care Medicine*, 23(9), e424–e428.
5. Fasano, A. (2020). All disease begins in the (leaky) gut: Role of zonulin-mediated gut permeability in the pathogenesis of some chronic inflammatory diseases. *F1000Research*, 9, 69.
6. Laukoetter MG, Nava P, Lee WY, et al.: JAM-A regulates permeability and inflammation in the intestine in vivo. *J Exp Med*. 2007.
7. Fasano, A. (2021). Zonulin measurement conundrum: Add confusion to confusion does not lead to clarity. *Gut*, 70(10), 2007–2008.
8. Levesque, R. (2005). *SPSS programming and data management: a guide for SPSS and SAS users*. Spss.
9. Almomani H, Alsmadi A, Issi N, AlShurman M, Altamimi E. Inflammatory Bowel Disease in Jordanian Children: A Tertiary Center Experience. *Pediatr Gastroenterol Hepatol Nutr*. 2025 Nov;28(6):393-404. doi: 10.5223/pghn.2025.28.6.393. Epub 2025 Nov 6. PMID: 41282528; PMCID: PMC12636109.
10. Cenni, S., Casertano, M., Trani, M., Pacella, D., Martinelli, M., Staiano, A., ... & Strisciuglio, C. (2023). The use of calgranulin-C (S100A12) and zonulin as possible non-invasive markers in children with inflammatory bowel disease: A clinical study. *European Journal of Pediatrics*, 182(3), 1299-1308.
11. Szymanska E, Wierzbicka A, Dadalski M, Kierkus J. Fecal Zonulin as a Noninvasive Biomarker of Intestinal Permeability in Pediatric Patients with Inflammatory Bowel Diseases—Correlation with Disease Activity and Fecal Calprotectin. *J Clin Med*. 2021 Aug 30;10(17):3905. doi: 10.3390/jcm10173905. PMID: 34501351; PMCID: PMC8432014.
12. Malíčková, K., Francová, I., Lukáš, M., Kolar, M., Kralikova, E., Bortlik, M., Duricova, D., Stepankova, L., Zvolska, K., Pankova, A., et al. (2017). Zonulin is elevated in Crohn's disease and in cigarette smokers. *Practical Laboratory Medicine*, 9, 39–44.
13. Szymanska E, Bierla J, Dadalski M, Wierzbicka A, Konopka E, Cukrowska B, Kierkus J. New noninvasive biomarkers of intestinal inflammation and increased intestinal permeability in pediatric inflammatory bowel diseases and their correlation with fecal calprotectin: a pilot study. *Minerva Gastroenterol (Torino)*. 2023 Dec;69(4):504-510. doi: 10.23736/S2724-5985.22.03156-4. Epub 2022 Apr 19. PMID: 35436840.