UDC 616.34-002-036.1-093-07-053.2:577.112
DOI: 10.15587/2519-4798.2022.265388

DIAGNOSTIC APPROACH TO CHRONIC BOWEL DISEASES IN CHILDREN

Tetiana Yaroshevska, Kateryna Skriabina

Diagnosing inflammatory bowel diseases in children and differentiating them from functional disorders is complicated based on clinical assessment, laboratory data, and radiological, endoscopic, and histological signs. Therefore, in paediatrics, substantial attention has been given to standard invasive evaluation methods. Recently, research efforts have been directed towards using faecal calprotectin as a sensitive non-invasive stool test.

The aim *of the research was to study the diagnostic value of faecal calprotectin in pediatric chronic inflammatory bowel diseases. The paper discusses faecal calprotectin's diagnostic and monitoring role in 35 children aged 3 to 17 years with ulcerative colitis, non-specific non-ulcerative colitis, and irritable bowel syndrome. The concentration of the faecal calprotectin level was examined by ELISA using monoclonal antibodies.*

Results. *The study shows that in patients with non-specific ulcerative and non-ulcerative colitis, the faecal calprotectin level exceeds the normal ranges 2-5 times, corresponding to clinical and morphological manifestations of the diseases and the level of inflammatory markers. However, this biological marker does not exceed the average values in children with irritable bowel syndrome.*

Conclusions. *The faecal calprotectin test allows us to differentiate organic and functional intestinal diseases, monitor chronic inflammatory bowel disease activity dynamics, and estimate treatment effectiveness. Applying the faecal calprotectin test within the framework of primary medical care can decrease the number of referrals for invasive endoscopic, laboratory, and radiologic examinations in pediatric patients with inflammatory bowel diseases*

Keywords: *calprotectin, ulcerative colitis, non-ulcerative colitis, irritable bowel syndrome, diagnostics, children*

How to cite:
Yaroshevska, T., Skriabina, K. (2022). Diagnostic approach to chronic bowel diseases in children. ScienceRise: Medical Science, 5 (50), 00–00. doi: http://doi.org/10.15587/2519-4798.2022.265388

1. Introduction

Diagnosing and treating chronic inflammatory bowel diseases (IBD) in children are complicated and invasive. Pediatric-onset IBD is associated with higher disease activity and is more complicated than adults. IBD and irritable bowel syndrome develop in approximately 10 % of pediatric patients [1]. Epidemiologic studies suggest an increasing incidence and prevalence of IBD in developed and developing countries [2]. For example, in Ukraine, bowel diseases accounted for 3.7 % of the structure of chronic digestive diseases. In addition, researchers have recorded an increasing incidence of IBD in Ukraine [3]. The general global trend toward increasing the prevalence of these diseases among children and adults highlights the importance of methods for early non-invasive diagnostics. Recently, research efforts have been directed towards using faecal calprotectin (FC) as a non-invasive sensitive stool test.

Ulcerative colitis (UC) is a chronic inflammatory disease of unknown aetiology, characterized by continuous mucous membrane inflammation (less often penetrating the submucosa) and dissemination of the process proximal from the rectum. UC is characterized by chronic relapse and slow progression of the disease. Typical symptoms are diarrhoea, hemorrhagic colitis, abdominal pain, toxic syndrome, and late physical development. Based on the literature, the inflammatory process most often affects the large intestine (59.6 % of cases), followed by left-sided colitis (28.5 %), and lastly, proctosigmoiditis (11.9 %) [4].

Chronic non-specific non-ulcerative colitis (NUC) is a chronic poly-etiological disease characterized by inflammatory and atrophic changes in the mucous membrane of the large intestine, morphological signs of epithelial dystrophy, decrease in crypt depth, and development of lymphocytic infiltration. Instability of stool with a predominance of constipation, false urge to defecate, and feeling of incomplete bowel movement dominate clinically. In addition, the patients feel dull pain relieved upon the evacuation of gases. An exacerbation of chronic colitis is characterized by the appearance or intensification of symptoms of chronic intoxication: increased fatigue, loss of appetite, headache, and sub-febrile temperature [5].

Another common disease is irritable bowel syndrome (IBS). The prevalence of IBS in children of different ages is 19.4–21.2 %. This functional disorder is characterized by a combination of abdominal pain and abnormal presentation of stool. In conjunction with mobility disorders and visceral sense of the digestive

organs, secretory function, and immune response, the disease is based on changes in the composition of intestinal microflora. The main manifestations of this syndrome are digestive disorders (steatorrhea, amenorrhea, steatorrhea); an asthenic vegetative syndrome is also possible [6].

Diagnosing inflammatory bowel diseases and their differential diagnosis with functional disorders in children is a difficult task, and it is based on clinical, laboratory, radiological, endoscopic, and histological signs [1, 5]. The endoscopic method with a morphological examination of the biopsy material is the most informative; however, it is invasive. It requires the careful preparation of the patient for an examination. The degree of specialism and experience of the endoscopist and morphologist are also essential considerations [4]. Therefore, non-invasive methods for diagnosing IBD as faecal markers of inflammation, including faecal calprotectin, are increasingly used [7, 8].

FC is a calcium-binding protein SS100, discovered in 1980 by Fagerhol et al. [9]. Calprotectin is a heterodimeric protein capable of binding to calcium and zinc. This protein exists in neutrophil cytosol and monocyte membranes. Following the activation of neutrophils or binding of monocytes to endothelial, calprotectin is released, and its level in the serum or body fluids is an important indicator of inflammation. Calprotectin has both a bacteriostatic effect and a cytokine-like effect on the local environment. Calprotectin is released from damaged cells and enters the faeces. The content of FC is proportional to leukocyte migration; therefore, in IBD, the marker increases, and in functional bowel diseases, it remains within the normal range. [10]. The calprotectin concentration in faeces has been shown to correlate with IBD's histological and endoscopic activity [7]. Usually, the concentration of FC is less than 50 μg/g of faeces, an increase in it above 100 μg / g of faeces is observed in inflammatory bowel diseases [11].

The aim of the study was to evaluate the diagnostic role of faecal calprotectin in chronic IBD in children.

2. Materials and methods

The study was conducted at the Dnipro State Medical University, Department of Propaedeutics of Children Diseases (based on the CNP “City Children Clinical Hospital No 6” of Dnipro City Council) for 2019–2020. ll studies with patients were conducted following the provisions of the Declaration of Helsinki of the World Medical Association "Ethical Principles of Medical Research Involving Human Subjects" (Helsinki, Finland, 1964), which was revised by the 59th General Assembly of the WMA (Seoul, 2008). The Local Ethics Commission of the CNP "City Children Clinical Hospital, No 6" of DCC approved the research. (Protocol No. 1 dated 12.01.2019 of the Local Ethics Commission). In addition, the parents of all patients have given and signed consent for the research participation.

Thirty-five children from a city area aged 3 to 17 years (average age is 8.7±5.7 years), 22 boys and 13 girls, were enrolled in the study.

The patients were divided into three groups depending on their pathology. UC was diagnosed in 10 patients (first group), 13 patients had NUC (second group), and 12 patients (third group) presented with IBS. The average duration of the disease was 1.8±0.9 years. On average, patients in the first group were 8.2±5.4 years old, 7 boys and 3 girls. The second group consisted of 9 boys and 4 girls, 9.3±6.2 years old; the third group included 6 girls and 6 boys, 8.7±5.7 years old on average.

All children were admitted to the department during the period of exacerbation of the disease, the activity of the UC was moderate in 7 children and minimal in 3 patients. A complex of clinical-anamnestic, laboratory, and instrumental methods: endoscopic and morphological examination, clinical and biochemical blood tests, scatological examination, and finally, faecal occult blood test were performed to verify diagnosis according to the protocols of medical care approved in Ukraine.

The diagnosis was based on several parameters: history, detailed patients' clinical examination, the results of the ileocolonoscopic evaluation with multiple biopsies, and histological tests. When necessary, faecal culture to exclude infectious diarrhoea and allergy tests were used to rule out allergic colitis. The activity of IBD was determined while considering a child's general condition, the intensity of the pain syndrome, fever, stool frequency, manifestations of hemorrhagic colitis, and changes in the blood test like leukocytosis, and high erythrocyte sedimentation rate (ESR), increased C - reactive protein, and dysproteinemia. Endoscopic activity assessment was performed to assess the extent of the lesion, presence of ulcers, erosions, granulations, contact bleeding, changes in the vascular pattern, mucus deposition, fibrin, and pus on the intestinal mucosa. The level of calprotectin in stool samples was studied in all patients.

The concentration of the FC level was examined by ELISA using monoclonal antibodies. A calprotectin concentration of more than 50 μg/g was a positive result [10]. The diagnostic value of the method was assessed based on comparing calprotectin in different groups of patients.

The license program "Statistica 6.1" (serial number - AGAR909E415822FA) was used for the statistical processing of the obtained results. Quantitative and qualitative indicators were evaluated. The mean value (M) and its standard error (m) were determined with the normal distribution of parameter values. Next, the significance of differences in quantitative traits was determined using a Student's t-test for dependent populations. The results were considered statistically significant if the p-value was less than 0.05 ($p<0.05$) [12].

3. Results

UC symptoms were manifested with abdominal pain syndrome, diarrhoea, symptoms of hemorrhagic colitis in all patients (100 %), sub-febrile fever (3 cases), inflammatory changes in the hemogram (5 patients), an increase in C-reactive protein (3 cases), an increase in α2- globulin (3 patients). The clinical characteristics of patients are presented in Table 1.

Table 1

Clinical manifestations in examined patients

Symptoms	Group 1 (%) n=10	Group 2 (%)n=13	Group 3 (%)n=12
Constipation	–	–	83.3
Diarrhoea	100	23.1	16.7
Hematochezia	100	–	–
Abdominal pain	80	100	100
Fever	30	7.7	–
Weight loss	40	15.3	8.3

Note: group 1 – ulcerative colitis; group 2 – nun-ulcerative colitis; group 3 – irritable bowel syndrome; n – number of patients

Our study's most typical symptoms of UC were: abdominal pain (before, during, and after defecation, localized in the left iliac region or lower abdomen); frequent stool with blood and mucus in the faeces; symptoms of intoxication; and poor appetite.

Common laboratory markers of inflammation, such as leukocytosis, neutrophilia with left shift, high ESR, increased C-reactive protein, and dysproteinemia, were notable. At the endoscopic examinations, total colitis occurred in only one patient out of 10; left-sided colitis was observed in 50 % of cases, and proctosigmoiditis in 4 patients. Typical signs of inflammation, like oedema, hyperemia, changes in the vascular pattern of the mucous membrane, contact bleeding, erosions, and ulcerative defects of the intestinal mucosa were revealed at the morphological examination - decreased number of goblet cells, crypt deformation, lymphocytic infiltration.

Clinical symptoms at NUC were characterized by abdominal pain syndrome and predominance of constipation; weight loss was not characteristic (Table 1). High body temperature was noted in 1 patient; there were no symptoms of hematochezia, and laboratory parameters in most cases (69.2 %) remained within normal limits. Hyperemia of the mucous membrane and changes in the vascular pattern without detecting defects were revealed endoscopically. Lymphocytic infiltration, dystrophic changes in the mucosa, and crypt flattening were usually noted morphologically.

Intestinal symptoms of IBS were manifested by impaired defecation, paroxysmal dull pressing or bursting pain in the low abdomen, and flatulence. Pain and flatulence frequently appeared during stressful situations, with intensified pain after food intake and before a bowel movement. Symptomatic relief occurred after defecation. As a rule, our patients had a variant of IBS with constipation: they had defecations 2–3 times a week with tension, feeling of incomplete emptying of the intestines, change in the shape and nature of faeces - hard, dry, "sheep" faeces. All children presented with complaints: headache, fatigue, tingling or slight pain in the heart region, or a lump in the throat. Children's total blood count and chemistry were within the average age range; clinical urine tests were standard. X-ray examination of the large intestine with contrast revealed signs of decreased motility in 3 patients and colon ptosis in 2 children. X-ray examination of the intestine in 2 patients showed the presence of spasmodic areas. The relief of the mucous membrane was unchanged in all cases.

Calprotectin levels in stool samples were examined in all 35 patients. Average concentrations of faecal calprotectin in patients are presented in Table 2.

Table 2

The concentration of faecal calprotectin in examined patients

Groups of examined patients	M±m, μg/g	n
Group 1	269.7±20.03	10
Group 2	111.56±14.09	13
Group 3	16.18±4.35	12

Note: group 1 – ulcerative colitis; group 2 – nun-ulcerative colitis; group 3 – irritable bowel syndrome; n – number of patients

Table 2 shows that the highest average calprotectin values (269.7±20.03 μg/g) were in children with UC. Since the patients were examined during the period of exacerbation of the disease, no one had average values of FC in the group of children with UC. At NUC, as a rule, calprotectin levels were increased, and the average values exceeded the normal level by about two times (111.56±14.09 μg/g). At the same time, in all patients with IBS, FC was within the normal range, less than 50 μg/g, on average 16,18±4,35 μg/g.

4. Discussion

According to the study results, the maximal activity of the pathological process was prominent in patients from the first group. Expressed clinic and laboratory signs of inflammation provided additional proof. At the endoscopic examination, oedema, hyperemia, vascularization of the mucous membrane, contact bleeding, erosions, ulcerative defects, decreased number of goblet cells, crypt deformation, and lymphocytic infiltration were found.

In our study, patients with UC demonstrated the highest average FC level, backed up by literature with similar results. [7, 9].

NUC is a disease with fewer signs of intestinal inflammation. Milder forms of clinical and laboratory manifestations were visible in this group. At endoscopy, hyperemia of the gut's mucous membrane, lymphocytic infiltration, dystrophic changes in the mucosa, and crypt flattening were usually noted. The level of FC had an intermediate value. In 2 out of 13 cases in children with NUC, the faecal calprotectin test was negative. This data emphasizes the importance of differential diagnostics with functional bowel disease, mainly since clinical symptoms such as diffuse abdominal pain associated with defecation, stool abnormalities with a predominance of constipation, and symptoms of asthenia can also be found in IBS. In these cases, NUC was diagnosed by laboratory data showing mild non-specific signs of inflammation. This finding was also

confirmed by using instrumental examination (X-ray and endoscopy).

IBS is known as a functional disease. Although patients demonstrated numerous subjective symptoms, objective manifestations were minimal. Colon ptosis presents in spasmodic areas, and colon decreased mobility was only noted during X-ray examination of the large intestine with contrast. Per other research, all children of this group had an average level of FC [6].

Thus, the elevation of FC level correlates with clinical, laboratory, and endoscopic signs of inflammation in examined patients. Indeed, performing colonoscopy and histopathologic evaluation of bowel biopsy specimens are considered gold standards for diagnosing and managing IBD. However, these techniques are invasive, expensive, and undesirable for pediatric patients. In recent years, FC as a biomarker has received much attention for diagnosing and non-invasive management of IBD. However, using this parameter has limitations, as mentioned in existing literature [8, 11]. FC is not a specific marker of IBD. This protein is found in the cytosol of neutrophils and the membranes of monocytes. Under inflammatory processes in the intestine, calprotectin is released from damaged cells and enters the faeces. Thus, against the background of infectious and allergic diseases, the level of FC also increases. As a result, during the study, a detailed collection of patients' medical histories was performed. Children with intestinal infection or food allergy signs were excluded from the study based on medical history and laboratory data (faecal culture or allergy diagnosis according to the indications).

Our study showed the efficacy of faecal calprotectin in the IBD differentiation from IBS, their management, and the correspondence of endoscopic and histologic activities of IBD with the level of FC. We aimed to use FC tests to allow differentiation of organic and functional bowel diseases, monitor the dynamics of chronic IBD activity, and estimate treatment effectiveness. The application of the FC test can decrease the number of invasive endoscopic, laboratory, and radiologic examinations in pediatric patients with IBD.

Study limitations. This study had a relatively small sample size for the determination of faecal calprotectin.

Prospects for further research. Prospects for further research are the development of a differentiated approach to the management of chronic inflammatory bowel diseases in children.

5. Conclusions

1. According to the study results, in the period of exacerbation of the disease, the level of faecal calprotectin was maximal in children with ulcerative colitis, did not exceed average values in patients with irritable bowel syndrome, and was moderately increased in chronic non-ulcerative colitis.

2. The level of calprotectin in faeces correlated with clinical, endoscopic and laboratory signs of an inflammatory process in the intestine; that is why in the active period of inflammatory bowel disease, faecal calprotectin can be used to monitor the disease course and the effectiveness of therapy.

3. To summate, the faecal calprotectin test for inflammatory bowel diseases has a high diagnostic value for screening to exclude the organic nature of the intestinal disease and determine the indications for invasive instrumental diagnostic methods.

Conflict of interests

The authors declare that they have no conflict of interest concerning this study, including financial, personal, authorship, or any other, that could affect the study and its results presented in this article.

Financing

The study was performed with no financial support.

Acknowledgement

The authors are grateful to the patients for their participation in the study and wish to thank the staff of the Pediatric Department of Dnipro CNP “City Children Clinical Hospital No 6” DCC, Svitlana Ivanus and Natalia Sapa for providing support in this study.

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Received date 12.07.2022
Accepted date 20.09.2022
Published date 30.09.2022

Tetiana Yaroshevska*, PhD, Associate Professor, Department of Propaedeutic of Children Diseases, Dnipro State Medical University, Volodymyr Vernadskyi str., 9, Dnipro, Ukraine, 49044

Kateryna Skriabina, Assistant, Department of Propaedeutic of Children Diseases, Dnipro State Medical University, Volodymyr Vernadskyi str., 9, Dnipro, Ukraine, 49044

***Corresponding author:** Tetiana Yaroshevska, e-mail: tatyana.yaroshevskaya@gmail.com*