Association between calprotectin and volatile fatty acids in patients with inflammatory bowel diseases

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Aim. To evaluate the content of calprotectin and volatile fatty acids (VFAs) in feces of patients with inflammatory bowel disease (IBD).

Materials and methods. 61 patients (33 men and 28 women) with IBD aged 20 to 66 years (the mean indicator was 41.80 ± 1.14 years) were examined. The patients were treated in the Department of Intestinal Diseases of SI “Institute of Gastroenterology of the National Academy of Medical Sciences of Ukraine”. All the patients were divided into two groups: Group I – 46 patients with ulcerative colitis (UC) and Group II – 15 patients with Crohn’s disease (CD). The control group consisted of 10 practically healthy people (donors).

Calprotectin detection in fecal samples was carried out using a kit “Immundiagnostik”, Germany. Fecal VFAs were analyzed using a hardware-software complex for medical research with a gas chromatograph Chromatek-Crystal 5000.

Results. A significant increase in the content of fecal calprotectin was found. Its amount depended on the disease nosology and was more expressed in patients with UC (3.5 times higher (P < 0.05) than that in patients with CD). The observed changes were accompanied by an increase in the content of propionic (C3) acid and a decrease in acetic (C2), butyric (C4) acids in coprofiltrates of the examined patients. The detected imbalance in the fecal content of VFAs in patients led to an increase in the amount of fatty acids, which was more pronounced in patients with IBD. An association between calprotectin levels and fecal VFA content was identified. Thus, correlation analysis allowed to establish a relationship between calprotectin levels and propionic acid content in patients with IBD (r = 0.370; P = 0.046).

Conclusions. In the case of active bowel inflammation, there is the increase in the fecal content of calprotectin and the decrease in VFAs (acetic and butyric acids) in accordance with the degree of disease activity, which allows the use of these indicators to assess the efficacy of therapies.

Keywords:
- calprotectin, volatile fatty acids, butyric acid, propionic acid, ulcerative colitis, Crohn’s disease.

Original research

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manifestations and polymorbid course [1]. The problem of diagnostics and treatment of UC and CD currently remains one of the most serious and unresolved issue in gastroenterological practice [4,5].

Genetic predisposition, intestinal microbiota, the immune system state and the external environment influence are the main factors in the pathogenesis of IBD [6–8]. It is known that the pathogenesis of IBD is based on impaired immune response, which leads to the development of nonspecific inflammation in the intestinal wall and mucous membranes [9,10]. The action of immune complexes and inflammatory mediators (cytokines, histamine, reactive oxygen species, NO) on intestinal wall cells contributes to their damage and tissue destruction. Excretion of fecal calprotectin (FC), a neutrophil protein that is a part of the inflammatory infiltrate in IBDs, reflects the transition of inflammatory cells into the intestinal lumen. FC performs a protective role. It was found that its concentrations were correlated with the neutrophilic infiltration intensity in the intestinal mucosa [11]. Due to this fact, FC was proposed as a noninvasive marker of intestinal inflammation [12,13].

Calprotectin is a calcium- and zinc-binding protein. Calprotectin of the S100 protein family was first discovered by I. Dale and co-authors in the cytoplasm of granulocytes as a protein with antimicrobial activity in 1983 [14]. It has antibacterial, antifungal, and antiviral activity. Calprotectin represents about 60 % of the total mass of soluble protein in human neutrophil cytoplasm and is also localized in monocytes, macrophages and epithelial cells [15]. Upon binding to calcium, it becomes resistant to high temperature and to degradation by leukocyte and microbial enzymes [16].

The colon is densely inhabited by a population of microorganisms, the so-called “gut microbiota”, capable of fermenting carbohydrates and proteins that elude absorption in the small intestine during digestion [17]. This microbiota produces a wide range of metabolites, including short chain fatty acids (SCFA). These compounds are absorbed in the large intestine and are defined as 1–6 carbon volatile fatty acids (VFAs) which can present straight or branched-chain composition [18]. The gut is the primary site where SCFA mediate their effect on either intestinal epithelial integrity or mucosal immune response. Disorders of gut microbiota leading to decreased SCFA are associated with colonic diseases, including IBD [19,20].

Aim
To evaluate the content of calprotectin and VFAs in feces of patients with IBD.

Materials and methods
A total of 61 patients (33 men and 28 women) with IBD aged 20 to 66 years (the mean indicator was 41.80 ± 1.14 years) were examined. The patients were treated in the Department of Intestinal Diseases of SI “Institute of Gastroenterology of the National Academy of Medical Sciences of Ukraine”. All the patients were divided into two groups: Group I – 46 patients with UC and Group II – 15 patients with CD. The control group consisted of 10 practically healthy people (donors).

The submitted materials for publication were consistent with the provisions of bioethics. All the patients signed an informed consent to participate in this study.

The diagnoses of CD and UC were established in accordance with generally accepted standards of diagnostics in gastroenterology. The disease severity was determined based on clinical data, laboratory, radiological, endoscopic and morphological examinations of intestinal mucosa samples. Calprotectin detection in fecal samples was carried out using a kit “Immudagnostik”, Germany. An absorption intensity was measured using a microplate photometer Stat Fax 303 Plus at a length of 450 nm. Calibration curves were used to determine calprotectin concentrations in the samples tested. The norm was a concentration of less than 50 µg/g of feces.

Fecal VFAs were analyzed using a hardware-software complex for medical research with a gas chromatograph Chromatek-Crystal 5000.

To optimize the mathematical processing, the results were entered into a spreadsheet database Microsoft Excel. Statistical analysis of the results was performed using the software package Statistica 6.1 (serial number AGAR090E-415822FA). To describe an extent of the central tendency in quantitative features, mean arithmetic (m) and standard error (SE), median (Me), 25 % and 75 % quartiles were used. Comparisons between mean values of variables were performed using a parametric method (Student’s t-test) with a normal distribution of these features expressed in an interval scale. Variables were found to be normally distributed as checked within each group by the Shapiro–Wilk’s test for normality. In other cases, a nonparametric method (U-Mann–Whitney test) was used. A difference between the mean values was considered significant at a level of P < 0.05. A strength of the relationships between the variables was assessed using significant Spearman correlation coefficients (r).

Results
FC levels were increased in 95.0 % (38/40) of patients with IBD and ranged from 43.8 µg/g to 1234.4 µg/g. A moderate increase in FC levels (from 50 µg/g to 120 µg/g) was observed in 20 % (6/40) of patients, and a significant increase – in 80 % (32/40).

A mean FC level was significantly higher than the norm and was 476.8 (150.8; 705.6) µg/g in the general group of patients with IBD. 521.3 (221.8; 839.3) µg/g – in patients with UC and 150.8 (93.2; 400.1) µg/g – in patients with CD. Its mean level was 3.5 times significantly higher (P < 0.05) in patients with UC than in patients with CD (Fig. 1).

There was a significant decrease in fecal acetic acid concentrations in patients with UC and CD compared with control values: by 13.3 times – 0.014 ± 0.003 µg/µl (P < 0.001) and 11.1 times – 0.018 ± 0.006 µg/µl (P < 0.001), respectively (Table 1).

A mean level of acetic acid (C2) exceeded the control values and was 0.014 (0.0040; 0.0018) µg/µl in patients with UC and 0.024 (0.009; 0.025) µg/µl – in patients with CD. The mean C2 level in patients with CD was 1.7 times higher (P < 0.05) than that in patients with UC (Fig. 2).

Analysis of the coprofiltrate VFA content in patients with IBD indicated a significant increase in propionic acid,
which was observed in both UC and CD patients by 1.7 times (0.0080 ± 0.0001 μg/μl, P < 0.001) and by 2.8 times (0.010 ± 0.001 μg/μl, P < 0.001), respectively (Table 1).

A mean level of propionic acid did not exceed the control values and was 0.008 (0.000; 0.001) μg/μl in patients with UC, but it was 2.8 times (P < 0.05) higher in CD patients compared with UC patients and above the control values – 0.022 (0.009; 0.025) μg/μl (Fig. 3).

The identified changes could indicate a violation of microcirculation in the intestinal mucosa and slowing down of metabolic processes, as propionic fatty acid is a regulator of lipid metabolic processes.

There was a decrease in butyric acid levels in UC patients by 2 times (0.039 ± 0.008 μg/μl, P < 0.001) and in CD patients by 1.7 times (0.046 ± 0.001 μg/μl, P < 0.001) as compared with the control (0.080 ± 0.001 μg/μl) (Table 1).

A median level of butyric acid (C4) was almost equal to the control values amounting to 0.041 (0.009; 0.017) μg/μl in patients with UC and was increased to 0.070 (0.005; 0.075) μg/μl in patients with CD (Fig. 4).

An observed imbalance in the fecal content of VFAs in patients with IBD led to an increase in the amount of fatty acids, which was 1.7 times more pronounced in CD patients as compared to UC patients.

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Table 1. VFA concentrations in coprofiltrates of patients with IBD, (M ± m)

<table>
<thead>
<tr>
<th>Indicator / Acid, μg/μl</th>
<th>IBD (n = 34)</th>
<th>UC (n = 25)</th>
<th>CD (n = 9)</th>
<th>Control (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetic</td>
<td>0.015 ± 0.002**</td>
<td>0.014 ± 0.003**</td>
<td>0.018 ± 0.008**</td>
<td>0.200 ± 0.003</td>
</tr>
<tr>
<td>Propionic</td>
<td>0.0090 ± 0.0001**</td>
<td>0.0080 ± 0.0001**</td>
<td>0.013 ± 0.001**</td>
<td>0.0045 ± 0.0002</td>
</tr>
<tr>
<td>Butyric</td>
<td>0.041 ± 0.007**</td>
<td>0.039 ± 0.008**</td>
<td>0.046 ± 0.001**</td>
<td>0.083 ± 0.001</td>
</tr>
<tr>
<td>Σ (C2-C4)</td>
<td>0.021 ± 0.003**</td>
<td>0.020 ± 0.003**</td>
<td>0.025 ± 0.002**</td>
<td>0.008 ± 0.001</td>
</tr>
<tr>
<td>Anaerobic index</td>
<td>4.81 ± 0.99**</td>
<td>5.23 ± 0.98*</td>
<td>3.61 ± 0.70*</td>
<td>0.735 ± 0.018</td>
</tr>
</tbody>
</table>

*: P < 0.01; **: P < 0.001 – significant difference compared with the control.
When determining an anaerobic index (AI), it was found to be equal to 0.735 ± 0.018 μg/μl in the control, while its a 6.5-fold increase was observed in the group of patients with IBD (4.81 ± 0.99 μg/μl, P < 0.001), a 7-fold increase – in the group of patients with UC (5.23 ± 0.98 μg/μl, P < 0.01), a 4.9 fold increase – in the group of patients with CD (3.61 ± 0.70 μg/μl, P < 0.01) (Fig. 5).

An association between fecal levels of calprotectin and VFAs was revealed. Thus, correlation analysis allowed to establish a relationship between the FC level and the propionic acid concentration in IBD patients (r = 0.370; P = 0.046).

Discussion

FC has been shown to be a product of neutrophilic granulocytes, which if detected at high levels in feces could indicate inflammatory processes in the intestine [1,6,7]. According to our study results, the increased levels of FC were detected in patients with IBD. Moreover, its level was significantly higher in UC patients than that in CD patients, which is consistent with the previous studies data [1,5,6].

Our study has shown the relationship between VFAs and the inflammatory parameter – FC which could reflect inflammation in the gastrointestinal tract and be useful as a biomarker of IBD and other inflammatory conditions [3,4,6].

VFAs are interesting metabolites because of their antiseptic activity, interacting with the immune system and improving the integrity of the intestinal barrier [13].

In our study, we have observed decreased VFA concentrations in patients with IBD, that was significant for acetic (C2) and butyric acids (C4). Acetic acid is considered to perform the most important physiological functions and is associated with body weight regulation, energy expenditure, lipid metabolism and insulin sensitivity. Since acetic acid has been demonstrated to affect the metabolism of muscle and fat tissues, it is possible that altered levels of acetic acid may affect metabolic disorders in these target tissues associated with IBD.

The detected changes are confirmed by the increased concentrations of propionic acid (C3) both in patients with UC and in patients with CD. Thus, the correlation analysis allowed to establish the relationship between the level of FC and the concentration of propionic acid in patients with IBD (r = 0.370; P = 0.046).

The examination of FC and VFAs in IBD can be used as a screening tool for the verification of intestinal diseases. In the case of active bowel inflammation, there were increased levels of FC and decreased fecal concentrations of VFAs (acetic and butyric acids) in accordance with the degree of disease activity, which allows the use of these indicators to assess the therapy efficacy. When using a FC test, a two-fold increase in normal indicator and a two-fold decrease in acetic (C2) and butyric (C4) acids can be considered diagnostically significant.

The use of non-invasive methods for the determination of calprotectin and VFAs in fecal samples in patients with IBD will reduce the frequency of invasive methods of examination.

Conclusions

1. Patients with IBD are characterized by increased levels of FC and propionic acid (C3), decreased concentrations of acetic acid (C2), and butyric (C4) acids, which could reflect inflammation in the gut. The positive correlation has been found between the level of FC and the content of propionic acid (r = 0.370, P = 0.046) in patients with IBD.

2. When using the FC test, the two-fold increase in the normal value and the two-fold decrease in acetic acid (C2) and butyric acid (C4) can be considered diagnostically significant.

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