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Inflammatory bowel disease and metabolomics- Are they connected?

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Abstract
Crohn's disease and ulcerative colitis are the two major diseases in the group of inflammatory bowel diseases of the GI tract. There is increasing incidence of both UC and Crohn's disease. Pathogenesis is not completely defined, the current theory being a complex interaction between genetics, the environment and the microbiota in a susceptible individual. Diagnosis requires clinical, laboratory, endoscopic, radiologic and histologic studies and in 15% of patients are it is not possible to differentiate between the two – so called "IBD-unclassified". Metabolomics measures metabolites in a biological sample (feces, serum, urine, tissue and air) thereby having the potential to clarify disease pathogenesis and find new biomarkers, which will then aid in diagnosis, guide therapy and give prognosis.

Keywords: IBD, Crohn's disease, Ulcerative colitis, microbiome, metabolome, metabolomics, biomarkers

Introduction

Crohn's disease and Ulcerative colitis are forms of chronic inflammatory diseases, characterized by transmural intestinal inflammation and stricture formation (Crohn's disease) or continuous inflammation involving the rectum (ulcerative colitis). There is increasing incidence of both forms of IBD in developed countries with one of the proposed factors being adopting the so called Western diet [1]. The pathogenesis of IBD is not fully understood, but microbiome and environmental factors play a role in genetically susceptible individuals [2, 3]. Diagnosis of IBD is complex and is based on clinical, biochemical, endoscopic, radiologic and histologic findings. It is important to discriminate between both IBD phenotypes in order to form a therapeutic strategy and predict prognosis.

Several biomarkers associated with IBD have been studied, such as perinuclear antineutrophil cytoplasmic (pANCA), anti-Saccharomyces cerevisiae antibodies (ASCAs), antibodies to exocrine pancreas (PABs), circulating noncoding RNAs such as miRNA and IncRNA, cathelicidin, CRP, and trefoil factor 3, but their use is not well adopted [4, 5] and new biomarkers are needed in order to support diagnosis and aid therapy in IBD patients.

Role of the microbiome

The human microbiome is defined as community of microorganisms living in different parts of the human body. More than 1000 species have been described (85). The biggest concentration of microorganisms is in the colon and distal ileum, 99% of them are of bacterial origin and 90% are of phylotypes Bacteroidetes and Firmicutes [6, 7]. One of the main changes in the gut microbiome in IBD patients is on one side increasing number of phylum Proteobacteria, most of which are pathogens, including Escherichia coli (a variant called adherent-invasive), Enterobacteriaceae, Klebsiela and Proteus spp, and reduced number of phylum Firmicutes on the other [8, 9, 10, 11]. Feacalibacterium prausnitzii and Roseburia homonis, which are butyrate-producing microorganisms are reported to be reduced in IBD patients. Butyrate is a short chain fatty acid, which is a main energy source for colonocytes and its lower concentrations in IBD patients aid inflammation and disrupts intestinal barrier integrity [12, 13, 14, 15, 16]. On the other hand, Ruminoccocus gnavus, also from phylum *Firmicutes* has mucin-degrading properties and is reported in increased concentrations in IBD patients, mainly in Crohn's disease, which aids in intestinal barrier integrity disruption [16, 17].

Paneth cell dysfunction is also reported in IBD individuals. These cells play a key role in maintaining intestinal homeostasis by producing and secreting antimicrobial peptides such as lysosym and alpha-dephensins [18, 19].

Change in gut microbiome is also associated in many trials with autoimmune conditions such as type 1 diabetes and rheumatoid arthritis [20, 21], type 2 diabetes and obesity [22, 23], colorectal cancer [24], heart disease [25] and IBD [26]. A key step in IBD pathogenesis is believed to be loss of immune tolerance to gut antigens of bacterial origin, which causes an abnormal immune response in a susceptible individual.

Oral dysbiosis is also reported in IBD represented by change in *Streptococcus* and *Prevotella* in saliva. Furthermore, a positive correlation is observed with increased fecal calprotectin and interleukins [16, 27, 28, 29]. In normal conditions gut microbiome has direct and indirect effect due to its metabolite diversity, thus aiding normal physiological processes, as well as other metabolic processes outside of the GI tract.

What is metabolomics?

Metabolites are products of many biological processes in the body. Their presence and quantitative measurement in different biological substrates in the human organism (urine, feces, serum, tissue) can provide detailed information about the specific state, which the body represents, reflecting a specific metabolic phenotype. [42]. Metabolomics is the study of these metabolites. It can be beneficial for diagnosis, monitoring therapy and determining the natural course of disease. The initiation of various metabolic reactions leads to the formation of the so-called metabolic markers that could subsequently be used to differentiate healthy from diseased [42].

There are two types of metabolomics – targeted analysis, where a particular metabolite is searched for and its concentration is measured, and untargeted, measuring the largest possible number of metabolites in a biological sample. Multiple methods are used to measure metabolites, but the main ones are proton nuclear magnetic resonance spectroscopy (H-NMR), liquid chromatography (LC), gas chromatography in combination with mass spectroscopy (MS), due to their high specificity and reproducibility of results.

Metabolites can be entirely of bacterial origin, but some of them after they are absorbed in the gastrointestinal tract, are processed (by hepatorenal conjugation, for example) and are then expelled from the body as co-metabolites.

Metabolomics in IBD – change in lipid metabolism

Altered metabolism has already been established in some diseases, such as type 1 and type 2 diabetes mellitus, liver diseases (bile acids), neurodegenerative diseases (tryptophan metabolites), cardiovascular diseases and colorectal cancer (long-chain fatty acids) [16,30,31].

In some of the patients with IBD (about 15%), despite having performed the necessary tests to establish the diagnosis, it is impossible to distinguish Crohn's disease from ulcerative colitis. In this context, metabolomics could distinguish healthy from diseased, as well as Crohn's disease from ulcerative colitis, by examining metabolites in various biological products such as urine [32, 33, 34], serum [35, 36, 37, 38, 39, 40], and feces [34, 40, 41, 42]. In a study by Elizabeth A. Scoville1 *et al*. from 2012 a total of 173 metabolites were found that differed in patients with IBD from healthy controls, 27 of them were increased and 146 were decreased, mainly affecting metabolites related to lipid metabolism - fatty acids, acylcarnitine, sphingolipids and bile acids. This difference was more pronounced in patients with Crohn's disease than in those with ulcerative colitis [50].

Fatty acids are important for maintaining intestinal homeostasis and have a role in inflammatory processes - some have a pro-inflammatory, others anti-inflammatory effect. Therefore, a change in their levels affects intestinal inflammation [43]. Short-chain fatty acids (SCFA) such as butyrate, acetate and propionate also have a trophic effect on the colonic mucosa and are a source of energy for colonocytes. Butyrate is also an important immunomodulator, inducing production of Tregs and mucus, thereby suppressing inflammation [44].

Short-chain fatty acids (SCFA) are one of the first identified abnormal metabolites in IBD. Decreased levels have been reported in a number of studies. [33, 41, 45, 40] One of the first by Marchesi *et al* from 2007 succeeded in differentiating healthy subjects from controls as well as Crohn's disease from ulcerative colitis. They reported low fecal levels of SCFA, including dimethylamine and trimethylamine. [41, 42].

Low levels of SCFA butyrate and propionate are associated with dysbiosis in Crohn's disease which is due to reduced levels of butyrate-producing organisms *Fecalibacterium prausnitsii* and *Roseburia homonis* [46]. In this regard, in is a study in which patients with Crohn's disease were treated with prebiotics (inulin and butyrate) a decrease in the levels of Ruminococcus gnavus was observed, high levels of which are associated with dysbiosis in IBD [46]. and an increase in the levels of *Bifidobacterium longum*, leading to a reduction in disease activity [47,16]. In a study by Pal *et al* from 2015 in children with IBD, treatment with butyrate resulted in an positive effect on intestinal disease activity [48]. The intake of food rich in fiber increases the level of SCFA, improves dysbiosis and quality of life in patients with UC [49, 7]. Another important energy source for colonocytes, responsible for about 30% of their energy needs, is glutamine, which also has altered levels, mostly reduced, which enabled Hisamatsu *et al* from 2012 in their study, calculating AminoIndex based on multivariate analysis of amino acid profiles from the serum of patients with IBD, to differentiate CD from UC. [50, 42]

Polyunsaturated fatty acids are also metabolites found at abnormal levels in patients with IBD. They are associated with intestinal inflammation through eicosanoids derived from arachidonic acid [50] and also correlate with inflammatory cytokines [50]. A 2019 study by Lai *et al* reported decreased levels of long-chain fatty acids such as docosahexaenoic, linolenic, and arachidonic acids and medium-chain fatty acids such as pelargonic and caprylic acids in the serum of patients with Crohn's disease [43, 5]. Increased levels of eicosatrienoic, omega-3-, docosapentaenoic, and omega-6 fatty acids, which are thought to have anti-inflammatory activity, have also been reported [45]. This increase could be due to malabsorption caused by the inflammation.

Another lipid-associated change is in LDL, HDL and VLDL. Patients with Crohn's disease have lower fecal cholesterol levels compared to healthy controls [45], as well as lower serum LDL and HDL levels, more pronounced in CD. The reason for this is that pro-inflammatory cytokines such as TNF-alpha, IL-1 and INF-gamma inhibit the expression of lipoprotein lipase. [50, 5].

Closely related to lipid metabolism are prostaglandins, which are produved from arachidonic acid. Low levels of prostaglandins have been found in CD, with the exception of PGE2, which activates Th17 lymphocytes, which in turn activate dendritic cells and increase IL-23 production [5, 50].

Bile acids –another altered metabolites, mainly secondary bile acids deoxycholic acid and lithocholic acid, are significantly reduced in UC patients. They suppress inflammation by inhibiting the synthesis of proinflammatory mediators and suppressing intestinal epithelial apoptosis [7, 0]. They also exert an antimicrobial effect and their absence contributes to dysbiosis [50, 16].

Alteration in the tricarboxylic acid (TCA) cycle and its metabolites

Tricarboxylic acid (TCA) cycle, also known as the Krebs cycle, is the final process in the oxidation of proteins, lipids, and carbohydrates. A significant reduction of intermediate metabolites such as citrate, aconitate, alpha-ketoglutarate, succinate, fumarate and malate was found in the serum of CD patients compared to controls and UC patients. Moreover, the metabolite reported in lowest levels in CD patients (11-fold compared to controls and 18-fold compared to CD patients) is beta-hydroxybutyrate, which is synthesized from acetyl-CoA [50]. In addition to serum, low levels of succinate and citrate are also observed in urine. [50, 37, 33]. Alonso *et al* even suggested citrate as a potential biomarker, mainly in CD patients, as its levels correlate with disease activity. [50, 5].

Alterations in other metabolites

Alterations in essential amino acid levels has also been reported. An example of this is the altered level of

tryptophan, which was elevated in the feces of patients with IBD compared to controls [50]. On the other hand, Nikolaus *et al* found low levels of this amino acid in the serum of patients with IBD [50]. In some studies, administration of tryptophan and its metabolites (indole-3-aldehyde, indole-3-propionic acid, and indole-3-acetic acid) suppresses colonic inflammation, protects epithelial integrity, and reverses colitis-associated microbial dysbiosis [7, 50]

Phenylalanine, another amino acid with anti-inflammatory effects (suppresses TNF production), was increased in serum and decreased in feces, reported by two different authors [43, 40]. High levels of taurine, glycine, lysine, alanine were also found in feces. This is most likely due to impaired epithelial absorption due to intestinal inflammation, but could also be due to dysbiosis, as some bacteria use amino acids for their metabolism [50, 16]. An increase in some polyamines - putrescine and cadaverine - was observed in the feces of patients with CD and UC, which suggests that these polyamines have a negative effect on disease [7]. An increase in fecal and serum taurine levels has also been reported [46, 40, 34, 7, 50].

Also of interest is the co-metabolite of mixed origin (mammalian and microbial) hippurate, or N-benzoylglycine. It is produced by bacterial fermentation of aromatic compounds introduced from the diet (aromatic amino acids, polyphenols and purines) to benzoic acid with subsequent conjugation with glycine in the liver. It was found to be significantly lower in the urine of patients with IBD compared to controls. Similar to it is formate, which is also in low amounts [35, 37, 33, 32, 16]. This suggests its potential use as a biomarker. A similar co-metabolite reported in low concentrations in urine is p-cresol sulfate, which is derived from bacterial metabolism of tyrosine, primarily by Clostridia spp [32, 44].

Histidine metabolism is also altered. It is converted by the microbiota into ergothioneine. This metabolite has antioxidant and neuroprotective properties. It was also found in low amounts reported by Lai *et al*. from 2019. It is assumed that this is due to the damage or lack of its transporter (OCTN1), which is expressed only in the small intestine, which suggests its use for the differentiation of CD from UC. [43, 5].

Conclusion

Based on all above, it can be seen that a number of metabolites in patients with IBD are altered to varying degrees. There is data for some of them that have the potential to be used as biological markers to differentiate Crohn's disease from ulcerative colitis, and some correlate with the activity of the inflammatory process. Commensal microbiota and their metabolites are candidates for the production of new probiotics containing F. prausnitzii, Akkermansia muciniphilia, Bacteoides fragilis due to their butyrate-producing properties. However, more data is needed in order for these finding to be standardized and implemented in routine clinical practice.

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