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Current Biomarkers in Irritable Bowel Syndrome

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Abstract.

The irritable bowel syndrome is one of the most common gastrointestinal disorders with chronic progression whose diagnosis is often difficult to establish, requiring multiple investigations, long-term treatment, and in the absence of any structural damage, the symptoms are not accompanied by radiological or endoscopic changes and laboratory tests are deficient. The aim of this review is to synthesize the information regarding serum biomarkers, biomarkers from faeces or volatile organic compounds from exhaled air, as studied in patients with irritable bowel syndrome that might be introduced in testing procedures, in order to help diagnose this condition.

Keywords: irritable bowel syndrome, biomarkers, serum, faeces.

Introduction

Irritable bowel syndrome (IBS) is one of the most common gastrointestinal disorders with a global incidence of 1-2% [1-5], with chronic progression whose diagnosis is often difficult to establish requiring multiple investigations, long-term treatment and an increased discomfort of the patient. This condition is characterized by discomfort or even abdominal pain, changes in stool frequency

and consistency, abdominal meteorism, incomplete defecation accompanied by pain or even burning sensation, changes in intestinal transit (constipation / diarrhoea) in the absence of any structural damage, symptoms that unfortunately for the diagnosis procedure of the disease are not accompanied by radiological or endoscopic changes and laboratory tests are deficient [6-8].

General Aspects

To support the diagnosis of irritable bowel syndrome, the symptoms of the disease must meet Rome IV criteria (so called after the place where they were established), namely: discomfort or abdominal pain at least 3 days per month in the last 3 months, disappearance of pain after defecation and installation of pain when the number and consistency of the stools change. These criteria must be met within the last 3 months with the onset of symptoms, at least 6 months before the diagnosis and must be accompanied by the exclusion of metabolic or organic diseases, malignant or benign, including diabetes mellitus, thyroid disorders, lactose intolerance, psychiatric disorders, abdominal surgery, and possible adhesions [9-13].

In terms of gender distribution, it was found that women are more affected by this disease than men, and the ratio in the western countries is 2: 1 [14-15], just as female patients who see a doctor more often than men which is indicated by a ratio of 3: 1 to the detriment of the latter [16-17].

IBS can be categorized into four subtypes: IBS with constipation (IBS-C), IBS with diarrhoea (IBS-D), mixed type and unclassified [18].

The Bristol Stool Form Scale (BSFS), published for the first time in 1997, is a system that calculates a score with visual illustrations and verbal descriptions that make it easy for patients to describe the stool consistency. It classifies the form of human faeces in seven categories as a result of the conclusion that the shape of the stool is a surrogate measure of transit time in the colon [19].

Given the complexity of this condition, it is assumed that there are several responsible factors involved, which are described below [20].

1. Inflammation

Lately it is believed that actually there are significant increases in lamina propria immune cells in patients with irritable bowel syndrome [21]. T and B lymphocytes actively participate in the body's immune response to pathogens [22]. Increases in T-lymphocytes, particularly in the rectum [23-25], rectosigmoid colon [26], colon [27-29], cecum [30], jejun [31] and duodenum [32] have been observed in patients suffering from irritable bowel syndrome. On the other hand, low levels of secretory B cells were identified in the colon [33].

In IBS cases followed by Campylobacter jejuni infection, there is a decrease in CD68 + macrophages due to the cytotoxic nature of the infectious agent inside the host cell [34-36]. Similar situations have been encountered with Shigella spp. [37-38], Salmonella [39-40], although it appears to be less cytotoxic to macrophages [41] and induces gamma interferon synthesis, a situation that will cause elevated levels of activated T-lymphocytes in the infected intestine [42-46].

2. Post-infection

It is considered that this cause is implicated in about 20-30% of cases and is associated with infection with Shigella, Salmonella, E. coli, Campylobacter or viral infections caused by Rotavirus, with a predisposition to smokers, vegetarians, youngsters, whose duration of infection is greater than 3 weeks [47-49].

3. Bacterial Intestinal Growth

In addition, bacterial intestinal growth is observed in less than 10% of the cases and associated with aging or other comorbidities such as diabetes mellitus, collagenosis, colon diverticulosis [50].

4. Imbalance in the Production of 5-hydroxytryptamine

There are also reports suggesting an increase in the production of 5 HT in IBS C and decrease it in IBS-D [51-52].

5. Psychosocial Determinants

The biopsychosocial approach allows the understanding of the symptoms both from the psychological and social perspective [53]. The link between psychosocial and gastrointestinal factors through the gut-brain axis allows the

connection between neurological and gastrointestinal spheres using neuroendocrine and neuroimmune pathways [54].

Certain events in the patient's life can trigger this condition. For example, a study of 333 IBS patients less than 15 years old revealed that 31% are orphans or come from broken families, 19% have an alcoholic parent, 61% have poor parenting relationships, or even parents are in tense relationships [55], so these aspects can influence the aetiology of the disease. Also, stressors such as life events, daily stress may have a negative influence on the good functioning of the digestive system, the symptom perception, and, inevitable, on the quality of life [56].

Although many people are confronted with these situations in the living environment, only certain elements characteristic to each of them such as personality or psychiatric disorders can determine the illness. The most common psychiatric disorders associated with IBS are depression [57-58], anxiety and post traumatic stress [59], neurasthenia, similar to chronic fatigue [60].

6. Genetic Determination

Recent studies have indicated that the genetic factor can influence and trigger clinical manifestations of irritable bowel syndrome. Cytokines are involved in regulating the immune response. A genetic predisposition to produce higher or lower amounts of cytokines can direct susceptibility to a particular disease or even alter its manifestations. A low prevalence of IL-10 genotype was also observed in patients with IBS indicating an impairment of the inflammatory response [61-62], also, an increase in the TNF-ά genotype was evident in IBS patients versus controls [63]. It is believed that the discovery of genes involved in somatization may facilitate the understanding of the aetiology of this condition [64].

Serum Biomarkers

The complexity of IBS diagnosis makes it necessary to identify biomarkers whose quantification is to help clinicians. An ideal biomarker should meet criteria of high specificity and sensitivity, reproducibility, repeatability, and low cost [65].

Studies have suggested that of a total of 140 possible biomarkers could be described here, with a set of 10 have a 50% IBS prevalence in the validation cohort. These are represented by: interleukin-1β (IL-1β), growth- related

oncogene-a (GRO-a), brain- derived neurotrophic factor (BDNF), anti-saccharomyces cerevisiae antibody (ASCA IgA), antibody against CBirl (Anti-CBirl), antihuman tisue transglutaminase (tTG), tumor necrosis factor(TNF)-like weak inducer of apotosis (TWEAK), antineutrophil cytoplasmic antibody (ANCA), tissue inhibitor of metalloproteinase-1 (TIMP-1), neutrophil gelatinase-associated lipocain (NGAL) [66].

Another study published in 2014 highlighted a combination of other 10 biomarkers that could differentiate patients with irritable bowel syndrome from healthy patients with a sensitivity of 83% and a specificity of 86%, these being: histamine, prostaglandin E2, tryptase, serotonin, P substance, IL-2, IL-6, IL-8, IL-10 and TNF-ά [67]. On the other hand, increased levels of IL-6, TNF-α and IL-10 have been identified in patients with IBS-D versus controls [68] and low levels of leptin, which is a hormone involved in regulating body weight, in patients with IBS compared with controls [69].

From the need to differentiate irritable bowel syndrome (IBD) from IBS-D, two serum biomarkers were put in the foreground, namely antibodies (Abs) to cytolethal distending toxin B (CdtB), which is a bacterial toxin produced either by Campylobacter jejuni, or Escherichia coli, Salmonella and Shigella, and antivinculin antibodies.

Thus, in a study conducted on 2681participants with ages between 18-65 years, the elevation of anti CdtB Abs in patients with IBS-D compared to the reference group was revealed, as did the increased levels of antivinculin Abs [70].

On the other hand, elevated levels of ileal peptides YY (responsible for water and electrolyte absorption) and low levels of duodenal cholecystokinin synthesized by I cells of the small intestine mucosal epithelium and secreted in the duodenum were distinguished in patients with IBS-C [71].

Considering the acute phase reactant, C-reactive protein (CRP) is used in laboratory screening to identify certain inflammatory processes, post-operative infections and graft rejection [72]. Although nonspecific, it can be used in the diagnosis of IBS as a general biomarker along with erythrocyte sedimentation rate (ESR), cortisol, chromogranin or other proinflammatory cytokines.

Furthermore, Hod et al. Has clearly demonstrated the role of high sensitivity CRP (hs-CRP) as a biomarker of microinflammation in IBS. Although within

normal limits as laboratory values, there were notable differences between hs-CRP values in patients with IBS versus healthy patients, which reinforced the idea of microinflammation in IBS [73].

ESR, also a non-specific marker of microinflammation, is considered to be helpful because of ease of testing, and more than that, clinicians have the advantage of being able to delineate patients with mucosal inflammation by those with biopsychosocial disorders [74].

In addition, cortisol is the most important glucocorticosteroid and is essential for maintaining many functions of the body. Like other glucocorticosteroids, cortisol is synthesized in the zona fasciculata of the adrenal cortex from a common precursor with cholesterol. Synthesis and secretion of cortisol are controlled by negative feedback by the hypothalamic-pituitary-adrenocortical axis. Cortisol itself acts through a negative feedback mechanism on pituitary and hypothalamus. In addition, stress causes increased cortisol secretion [75-78].

Chronic stress and certain traumas may be trigger factors for IBS, hence the idea of quantifying cortisol in patients susceptible to this disease, as well as assessing the HPA axis in the context of the need to get to the origins of the disease [79-80].

Starting from this idea, Kennedy et al., using the Trier Social Stress Test, which is a method of testing patients in laboratory undergoing artificial stress [81], have identified elevated levels of salivary cortisol in patients with IBS tested and concluded that this is due to the poor functioning of HPA that did not close after the removal of the stressor. However, the results could be less relevant if they do not take into account several aspects such as age, gender, the presence of certain psychiatric comorbidities, menstrual cycle in female patients, type of IBS, type of stressor, genetic and environmental factors [82].

Controversial studies linking chromogranin A to irritable bowel syndrome require further testing to qualify it as a biomarker. Chromogranin A (CgA) is part of the family of chromogranins that are known to modulate intestinal inflammation and can be a ligand of communication between neuroendocrine and immune systems. Thus, until further discoveries, we can rely on this possible biomarker to assess the possibility of neuroendocrine tumors [83].

The controversy arises from the fact that some authors have shown elevated levels of CgA in patients with IBS-D [84], although tests have been performed on a relatively small number of patients, while other authors have not identified variations in serum CgA in patients with IBS or controls, instead they reported low cell densities containing CgA in the duodenum and colon of patients with IBS-D and IBS-C, which suggested that the density of CgA intestinal cells could be considered as a histopathological marker [85].

Faecal Biomarkers

Given the link between irritable bowel syndrome and faeces, the idea of testing biomarkers in the stool that can help diagnose, treat, and prevent this disease has become of great help, especially if we take into account the non-invasive nature of the method.

Calprotectin is an important component of the cytoplasm of polymorphonuclear granulocytes (PMN), so that up to 60% of the proteins dissolved in the cytosolic granulocytes are represented by this non-glycosylated protein. It was first isolated from granulocytes by Fagerhol and called the L1 protein; was later renamed calprotectin due to its intracellular calcium binding properties, likely to protect the cell from its own catalytic enzymes as well as bacterial [86]. The fact that it is eliminated during inflammation of the colon and especially that it is resistant to degradation makes it easy to identify it in the faeces [87].

Significantly elevated levels of faecal calprotectin (FC) have been identified in patients with IBD [88], as compared with those with IBS, with a sensitivity of 93% and a specificity of 94%, which helps to differentiate the two diseases [89].

Another faecal possible biomarker could be represented by human β-defensin-2 (HBD-2), whose expression is induced by probiotic microorganisms and proinflammatory cytokines [90-93]. Recent results imply that HBD-2 is expressed in active intestinal inflammation. Faecal specimens were collected from a total of 100 participants, faecal inflammation markers lactoferrin (Lf) and calprotectin (Cal) were measured by enzyme-linked immunosorbent assay (ELISA) and the faecal HBD-2 was measured by ELISA and immunoblots. The highest values

were highlighted at HBD-2 compared with Lf and Cal in IBS patients, making human β-defensin-2 a possible reliable biomarker [94].

A study published in 2014 also highlights significant increases in a set of serum and faeces biomarkers in IBS patients compared to controls: MCP-1, MIP-1β, TNFα, IFN gamma, IL-1β, IL- 10, IL-4, IL-3, and CXCL16, being the first study to identify the two MCP-1 and MIP chemokines [95].

The microbiome metabolises non-digestible food constituents into short-chain fatty acids (SCFA) that have extensive immunological and regulatory functions and appear to be the link in the host-microbe interactions. SCFA include acetic acid, propionic acid, butyric acid, iso-butyric acid, valeric acid and iso-valeric acid [96].

Also, another study on 50 participants, half suffering from IBS, the rest being controls, revealed differences in levels of propionic acid and butyric acid, but due to the fact that the diets of the studied participants were not taken into account, the results were considered not consistent [97].

Respiratory Biomarkers

Another noninvasive approach to identifying possible biomarkers of irritable bowel syndrome is the respiratory analysis.

A study on 170 participants with IBS and 153 controls evaluated a set of 16 volatile organic compounds (VOCs) and it was observed that besides other volatile organic compounds found, n-hexanes, 1,4-cyclohexadienes, n-heptanes and aziridines have elevated levels in patients with the aforementioned condition, whereas increases in butane, tetradecanol, 6-methyloctadecane, nonadecatetraene, methylcyclohexane, 2-undecene, benzyl-oleate 6,10-emethyl-5, 9-undecadin-2-one and 1-ethyl-2-methyl-cyclohexane were found in control group [98]. These findings need to be studied in depth, just as the tests should go further.

Other recent studies have highlighted the association of IBS-C with methane [99-100]. From 1277 participants, 319 methane producers and 958 non-producer methanes, methane was observed to be associated with IBS-C cases [101]. Lactulose breath test (LBT) measures methane and hydrogen from breathing expired at 15-20 minutes after ingestion of 10 frames of lactulose up to 2 hours, using gas chromatography [102]. A higher than 3 ppm of methane in breath define

methane producers [103-104], and this method has become a fairly accurate one in the IBS-C prediction with a sensitivity of 91% and a specificity of 81.3% [105]. Moreover, the amount of methane of LBT is directly proportional to the severity of constipation, and although this cannot reap the distinction between healthy and IBS patients, there is a greater possibility for methane producer patients to have constipation episodes compared to non-methane producers [106].

Concluding Remarks

Given the complexity of the disease and its multifactorial nature, the diagnosis made by the clinician can often be aggravated. Unfortunately, gaps in laboratory testing come to reinforce this argument. In an attempt to improve this situation, recent years of research have brought to the forefront a number of possible biomarkers (e.g. both seric and obtained by non-invasive methods such as from faeces or volatile organic compounds obtained from the exhaled air). Some biomarkers have been shown to be linked to irritable bowel syndrome or other gastrointestinal disorders, others still need to be studied, but whatever the situation, it is imperative to continue research to make the most of the results already achieved and to reach out biomarkers required to facilitate the medical act in order to diagnose, possibly treat or even prevent irritable bowel syndrome.

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