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Mini Review

OXIDATIVE STRESS AND INFLAMMATORY BOWEL DISEASE IN PEDIATRICS

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ABSTRACT

Inflammatory bowel diseases (IBD) are chronic, idiopathic and complex diseases of the gastrointestinal tract. Their two most common forms are ulcerative colitis (UC) and Crohn's disease, (CD).

The incidence and prevalence of IBD increase, the age profile of IBD patients is also changing, in a direction that includes childhood. C-reactive protein (CRP) and calprotectin have been traditional inflammatory markers in IBD. The primary diagnostic tool in the hands of gastroenterologists remains endoscopy, which is an invasive, expensive, and patient-unwanted procedure. Furthermore, a wide range of diseases, from malignant and infectious to functional, can present with similar symptoms as IBD. This makes the diagnostic process even more complicated and requires a wide range of laboratory and instrumental studies, takes a lot of time and money.

The oxidative imbalance presence in patients with IBD has been demonstrated. In addition, some markers of oxidative stress in IBD have the potential to become markers for the differential non-invasive diagnosis of inflammatory bowel diseases (IBD and CMA) and would shorten the time to diagnosis. Further, would become a suitable method for monitoring inflammation and the effect of treatment in these diseases.

Key words: oxidative stress, IBD, UC, CD, pediatric.

INTRODUCTION

Inflammatory bowel diseases (IBD) are chronic, idiopathic and complex diseases of the gastrointestinal tract. Their two most common forms are ulcerative colitis (UC) and Crohn's disease, (CD) (1, 2). The incidence and prevalence of IBD (Figure 1) have increased in recent years, and the epidemiology of the disease is best established in developed countries: in North America > 1 million and in Europe, > 2 million people are IBD affected (3).

In the last decade, the incidence and prevalence of IBD increased, and the age profile of IBD patients is decreasing, in a direction that includes childhood (4, 5). Although, IBD is the focus of attention in the field of translational medicine, the main diagnostic tool in the hands of gastro-enterologists remains endoscopy, which is an invasive, expensive and patient-unwanted procedure (1). In addition, the wide spectrum of malignant, infectious to functional diseases, manifesting with similar symptoms as IBD, complicates the diagnosis and requires additional laboratory and instrumental studies, consuming time and money (3).

The IBD pathogenesis is not fully understood. There is convincing evidence for the role of free-radical processes and oxidative stress (OS) in the pathogenesis and progression of these diseases (5-9).

The oxidative stress, defined as a prooxidants and antioxidants imbalance, is closely related to inflammatory responses and has been implicated in the spread and IBD exacerbation. The search for new non-invasive diagnostic methods is of great importance, especially for children and adolescents with IBD (10-12).

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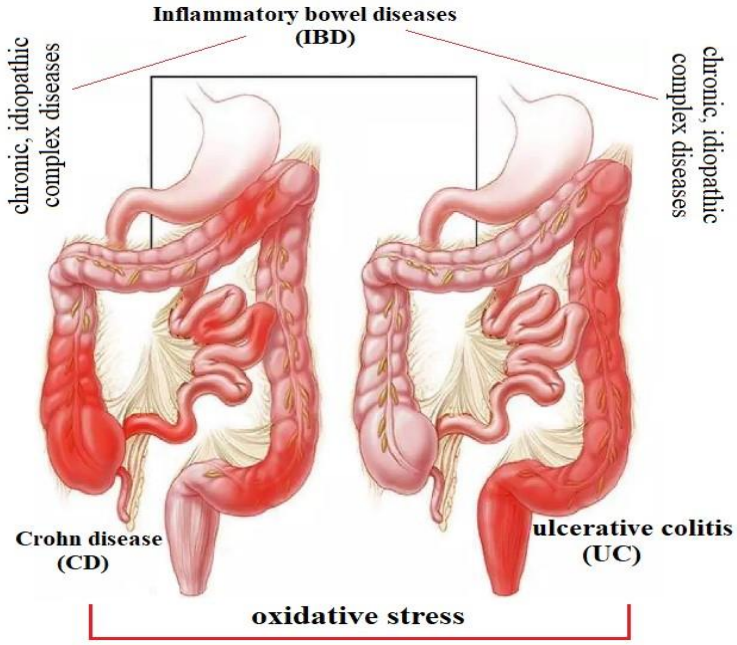

Figure 1. Inflammatory bowel diseases (IBD) are chronic, idiopathic and complex diseases of the gastrointestinal tract; and oxidative stress (OS) as factor in the pathogenesis and progression of these diseases.

Considering the limited number of biomarkers in pediatrics, some authors such as Savage and Everett, 2010 (13) argue that children are "biomarker orphans". Menzel et al., 2021(14), point out, that further studies conducted in larger cohorts and including detailed characterization of OS markers in IBD are needed to have the potential to become indicators of clinical relevance. It is considered, that the IBD pathogenesis (CD and UC) involves genetic, immune, and environmental factors that together lead to disruption of the delicate homeostasis between host immunity and the gastrointestinal microbiome (5, 15, 16).

All parts of the gastrointestinal tract can be affected in Crohn disease – from the mouth to the rectum. Lesions alternate with healthy fragments. Inflammation is most often located in the terminal ileum and the initial colon and it is characterized by transmural inflammation. UC typically involves superficial inflammation of the rectum with extension into adjacent mucosa in a continuous fashion. Inflammation forms in the mucosa and submucosa, leading to ulceration. Patients with UC usually present with diarhoea, which may be associated with blood. Bowel movements are frequent and small in volume as a result of a rectal inflammation. Associated symptoms include colicky pain, tenesmus, anemia and weight loss (4, 6, 17).

Recently, attention has been paid to multi-pronged research on the IBD mechanisms. Mucosal tissue infiltration with activated phagocytic immune cells generating reactive oxygen and nitrogen species (ROS/ RNS) induces a switch to prooxidants (18-21).

Therefore, attention is turning to OS as a potential IBD trigger. Increased OS (Figure 2) and a disturbed antioxidants-prooxidants balance, induced ROS (such as: superoxide ($O_2 \cdot^-$), hydroxyl ($HO\cdot$), peroxyl ($RO_2\cdot$), alcoxyl ($RO\cdot$), and hydroperoxyl ($HO_2\cdot$) radicals; lipid hydroperoxides; and reactive nonradical compounds including singlet oxygen (O_2), hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl), chloramines (RNHCl)) and RNS (such as: nitric oxide ($\cdot NO$), nitrogen dioxide ($\cdot NO_2$), nonradical compounds, peroxynitrite ($ONOO^-$)), are the most common natural by-products of metabolism (22, 23).

In addition, ROS and RNS are predominant mediators responsible for the intracellular damages of macromolecules (proteins, lipids, nucleic acids) and as highly reactive radicals, upregulate the genes expression and reduce immune responses adaptation (22, 23). ROS and RNS mainly produced by the intracellular organelles: endoplasmic reticulum, mitochondria and peroxisomes, as well as by some enzymes such as peroxidase, NADPH oxidase, xantin-oxidase, lipo-oxigenase, gluco-oxidase and epoxidase (4).

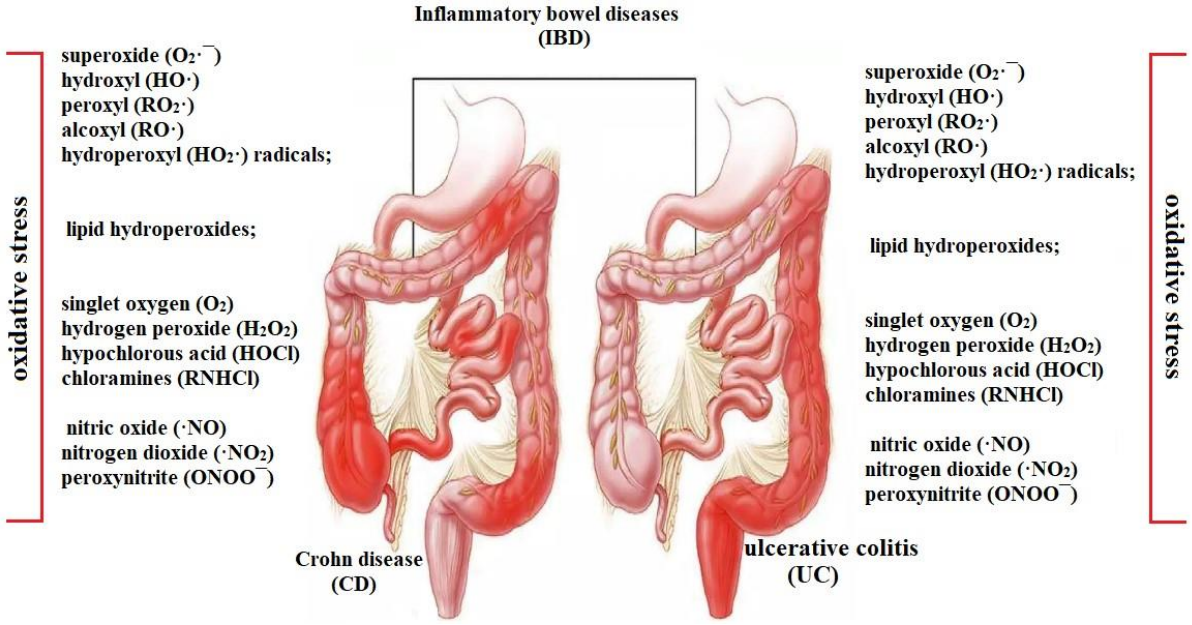

Figure 2. Increased OS and a disturbed antioxidants-prooxidants balance, induced ROS, RNS, nonradical compounds, IBD responsible.

Endogenous and exogenous antioxidant systems, on the other hand, consist of enzymatic and non-enzymatic defenses. Enzyme defenses including catalase (CAT), superoxide dismutase (SOD) and glutathione peroxide (CPx) exist in all cells. Intracellular non-enzymatic antioxidants include glutathione, and extracellular antioxidants including vitamin E, C, A and metal elements (zinc, copper, manganese and iron) minerals, ceruloplasmin, and uric acid (20, 22, 23). ROS determine a number of homeostatic functions, such as cell signal transmission, growth differentiation, apoptosis and inflammation. In parallel, deposited ROS in OS damage some macromolecules such as membrane lipids, DNA and proteins (10, 20-23). Different investigations, compared OS markers between IBD and healthy subjects. Alzoghaibi, 2013 and Balmus, 2016, comment, that antioxidant enzymes (PON1, SOD, CAT, GP-x) and enzymatic antioxidants (vitamins A, C, E; β-carotene) are higher in healthy subjects compared to IBD patients (12, 20). Frequently studied total antioxidant capacity (TAC), directly reflects the overall antioxidant capacity of an individual. Studies have shown that TAC in the serum of IBD patients generally decreases. Conversely, pro-oxidant agents such as MPO, NO, spermine oxidase, COX2 and NOS2 are increased in the intestinal mucosa and serum of affected individuals. Moreover, the lipid peroxidation products (4-hydroxynonenal; MDA), DNA oxidative products (8-OHdG) and oxidative protein (hydroxylated or carbonated) products with proinflammatory activity correlate with the IBD severity (12, 20).

Other studies have demonstrated that OS is closely related to inflammation in IBD. A correlation was found between OS markers and reactive protein levels (CRP) (18). OS is thought to lead to the overexpression of inflammatory factors, which favors the entry of opportunistic bacteria into the intestinal mucosa and perpetuates inflammation in a vicious cycle (18). OS has been implicated in the IBD pathogenesis for another reason: several genetic risk loci associated with OS have been identified (24). The OS undoubtedly is a major trigger for neoplastic transformation in IBD patients, as well (6). The MDA and GSH are promising OS markers and diagnostic tools in IBD and CD in its active phase (9), and 8-OHdG as a progression marker (8). The stable ROS and RNS structures possess excellent sensitivity and selectivity and can be used as routine analysis in the OS assessment and its induced disorders and/or determination of macromolecules changes, in numerous diseases, including IBD and other gastrointestinal diseases. Compared with conventional redox-imbalance research methods applied in standard biochemical screening, nitroxide-enhanced EPR spectroscopic analysis, in combination with stable nitroxides TEMPOL, Mito-TEMPO, 3-Maleimido-PROXYL,(1-hydroxy-3-methoxycarbonyl-2,2,5,5-tetramethylpyrrolidine) CMH, etc., has outstanding advantages. Taking into account

the easy sample preparation and the limited use of additional reagents and consumables, the performed analyzes are not aggravated. They are also characterized by the stability of the spin-probes at physiological/non-physiological pH, and a short running time (14, 25). Regarding the pro-oxidant enzymes investigated as markers, intracellular SOD was increased in UC patients in contrast to CD patients (7). Researcher reports of SOD enzyme activity in erythrocytes in pediatric CD being either decreased or unchanged, are conflicting. The discrepancy in SOD levels is probably due to different facts. Koláček et al. (5) studied exclusively children in the inactive phase of the disease, while Pácal et al. (24) probably also includes children with active disease. The role of glutathione (GSH) in IBD is positive. The antioxidant acts as a protector that directly neutralizes ROS and RNS; or indirectly- aids in the ROS and RNS reduction as a cofactor for enzymes, GPx and others. However, the reported results are not consistent regarding its level in IBD patients compared to healthy individuals, with reduced serum GSH being demonstrated in UC (26). Ozhegov E et al. (12) demonstrated that CD patients have increased mucosal indices after ROS and RNS induction and increased peroxide radical generation (H_2O_2) and susceptibility to peroxidation than patients with irritable bowel syndrome (a functional bowel disorder). These patients also show, possibly as a compensatory mechanism, an increased TAC.

Electron paramagnetic resonance analyzes (EPR) in IBD

Real time in vivo study and assessment of residual lipid peroxidation (ROS production); nitrogen oxide (NO•), hydroxide (•OH) and superoxide ($•O_2^-$) radicals; structural damage to albumin, etc., assessed using spin-conjugates. New markers are also being introduced, including the determination of nitroxide radicals as OS detectors and spin-markers to prove hypoalbuminemia/ anemia (22, 25). Redox-based theranostics includes redox monitoring and therapeutic agents that normalize the redox imbalance and is a promising approach to significantly improve the quality of life of IBD and UC patients through rationalized treatment (26). The authors address the development of functional nitroxyl radicals, to readily localize inflammation in a target organ, tissue, or cell that has specific reactivity for the redox-related biomolecule (26).

Immunoenzymatic assays (ELISA methods) for the study of pro-inflammatory mediators

The protein, fatty acid and DNA metabolism is a highly regulated process in which dysregulation can lead to an imbalance of pro- and anti-inflammatory mediators that determine both the type and extent of inflammatory responses in the gut during IBD. Notably, plasma or serum levels of proinflammatory cytokines (interleukins: IL-1β, IL-6, IL-10, and tumor necrosis factor (TNF)), as well as colonic mRNA expression, were significantly increased in untreated patients (IBD) and decreased in groups treated with additional anti-inflammatory drugs. preparations, demonstrating the critical role of macromolecule metabolism in the regulation of gastrointestinal inflammation (22, 27).

CONCLUSION

In recent years, IBD has been the focus of attention in the field of translational medicine, but accurate diagnostic biomarkers are still lacking. Despite the exponential progress in the study of IBD pathogenesis, the time from the appearance of the first symptoms to the final IBD diagnosis has not been shortened, and the main diagnostic tool in the hands of gastroenterologists remains endoscopy, however invasive, expensive and unwanted a procedure is by patients. Furthermore, the wide spectrum of diseases, from malignant and infectious to functional, that can present with similar symptoms as IBD, makes the diagnostic process even more complex. The studies conducted so far with the most reliable markers of oxidative stress are at an early stage but offer information about markers that are elevated in patients with active disease compared to healthy individuals. Therefore, OS markers in IBD have the potential to become indicators for differential non-invasive diagnosis of some inflammatory bowel diseases (IBD and CMA) and would shorten the time to diagnosis and become a suitable method for monitoring inflammation and the effect of treatment in these diseases. This would provide a better prognosis for pediatric IBD patients.

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