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SHARE OF REACTIVE OXYGEN SPECIES (ROS) IN INFLAMMATORY BOWEL DISEASE. THE DIAGNOSTIC USEFULNESS OF SELECTED MARKERS. PART 1

UDZIAŁ REAKTYWNYCH FORM TLENU (RFT) W NIESWOISTYCH ZAPALENIACH JELIT. UŻYTECZNOŚĆ DIAGNOSTYCZNA WYBRANYCH MARKERÓW. CZĘŚĆ 1

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Summary

Malfunctioning of environmental, immunologic or genetic mechanisms brings about a disorder of system homeostasis, which results in the development of diseases of arduous course. Inflammatory bowel diseases are a group of disorders which house a pathological inflammation of the wall of the gastrointestinal tract. It is postulated that one reason for the resulting changes may be free radical reactions. As a result of the ongoing inflammation under the course of the disease an influx of neutrophils into the lumen begins. Although endoscopic examination constitutes an irreplaceable method in the evaluation of the resulting changes, laboratory tests are an essential tool in the diagnostic process. In recent years it has been proven that the role of faecal calprotectin as a non-invasive test can be used to differentiate organic and functional gastrointestinal diseases, and evaluate remission or exacerbation of inflammatory bowel disease [6,28]. It has also been noted that there is a need to seek other new markers that would facilitate the diagnosis.

Streszczenie

W wyniku nieprawidłowego działania mechanizmów środowiskowych, immunologicznych czy genetycznych dochodzi do zaburzenia homeostazy ustrojowej, co skutkuje rozwojem chorób o uciążliwym przebiegu. Nieswoiste zapalenia jelit stanowią grupę schorzeń, w których dochodzi do patologicznego zapalenia ściany przewodu pokarmowego. Postuluje się, iż jedną z przyczyn powstałych zmian mogą być reakcje wolnorodnikowe. W wyniku toczącego się procesu zapalnego w przebiegu tych chorób rozpoczyna się napływ neutrofilów do światła jelita. Mimo, iż niezastąpioną metodą w ocenie powstałych zmian jest badanie endoskopowe, badania laboratoryjne stanowią niezbędne narzędzie w procesie diagnostycznym. W ostatnich latach udowodniona jest rola kalprotektyny kałowej, jako nieinwazyjnego badania służącego do różnicowania organicznych i czynnościowych chorób przewodu pokarmowego, oceny remisji bądź zaostrzenia nieswoistego zapalenia jelit [6,28]. Wskazuje się także na potrzebę poszukiwania innych nowych markerów, które przyczyniłyby się do ułatwienia diagnostyki.

Key words: oxidative stress, free radicals, inflammatory bowel disease
Słowa kluczowe: stres oksydacyjny, wolne rodniki, nieswoiste zapalenia jelit

Inflammatory bowel diseases (Inflammatory Bowel Diseases) are a group of chronic diseases of the gastrointestinal tract, which includes Crohn's disease and ulcerative colitis [25]. The above-mentioned diseases are characterized by the occurrence of periods of remission and exacerbation. The ethology of inflammatory bowel disease is not fully understood. Currently, the role of genetic factors (genes located on

chromosomes: 1, 5, 6, 12, 14, 16 and 19 are pinpointed as taking part), environmental, immunological disorders, viral or bacterial infections have been indicated [2, 43, 44].

Among the environmental factors, the role of trauma or abnormal response to stress is noted [10]. Due to the ongoing inflammation in the intestine, it is suggested that one of the causes of these diseases may be free radical reactions that lead to the development of the phenomenon referred to as oxidative stress [25]. Then, it leads to an oxidant-antioxidant imbalance, which results in the overproduction of reactive oxygen species (ROS) and the dominance of the oxidation reaction [16]. The oxidative stress leads to oxidation of cell membranes, changes in the structure and function of proteins, or DNA damage [23]. The oxidative stress also leads to lipid peroxidation, which is a process of oxidation of unsaturated fatty acids, leading to the formation of peroxides of such compounds. The main product of lipid peroxidation is malondialdehyde (MDA), the concentration of which, under the conditions of increased production of ROS, increases and causes a change in the permeability of the cell membrane [20].

The large intestine undergoes colonization by a very large number of bacteria that continuously stimulate the GALT system (Gut Associated Lymphoid Tissue) making a distinction between the symbiotic bacterial flora and the pathological one. When it comes to recognizing and destroying pathogenic organisms the so-called intestinal homeostasis is maintained. Then, the intestinal microflora and the GALT remain in balance. The tightness of the intercellular connections and the presence of a mucus layer ensure proper operation of GALT [25]. On the other hand, the intercellular damage is caused by hydrogen peroxide produced by colonocytes [32]. As a result of this process, the bacteria pass into the lamina propria, T cell stimulation and production of proinflammatory cytokines are initiated [33]. In the course of inflammatory bowel disease one can observe an increased number of T and B cells, mast cells, monocytes or macrophages. Among the latter, two types of M1 and M2 can be recognized.

It is the M1 type macrophages that play an important role in initiating and sustaining the inflammation.

They generate free radicals, an inducible nitric oxide synthase (iNOS), IL-12 or TNF-α [11].

An increase of leukocytes polymorphic nucleus (PMNs) in the lamina propria, which release large quantities of such proteases: elastase, cathepsin G or collagenase, is of great importance in the course of inflammatory bowel disease [14]. The effect of the above proteases leads to leakage junctions. Gouna-Berthold et al. observed increased concentrations of leukocyte elastase in the plasma of patients with ulcerative colitis. Furthermore, they proved that the concentration of the enzyme correlates with disease activity [13]. In turn, Kuno et al. demonstrated that elastase deriving from neutrophils affects the cell proliferation, and consequently interferes with repair processes in the intestinal mucous membrane layer [21]. And in the research of Gouna-Berthold et al. [13] it was demonstrated that plasma elastase is a useful and independent indicator of the activity of inflammatory bowel disease. The increased activity of proteases is also reinforced by a fall in concentrations of protease inhibitors. In the serum the main inhibitor of the serine protease is α1-antitrypsin, which protects the body from damage accompanying the inflammatory process [37].

α-1 antitrypsin is a glycoprotein which has a molecular weight of about 52 kDa. It is built of a single polypeptide chain comprising 394 amino acid residues. In its spatial structure we can discern nine domains of the α-helix structure and three β-bead [7.22]. At the carboxy terminal the domain is present, which comprises the following sequence of amino acids: proline, methionine, serine and isoleucine. It is the presence in the sequence of methionine at the 358 position of the polypeptide chain that is the cause of inactivation of the elastase-α by 1 antitrypsin [5].

α1-antitrypsin is synthesized primarily in the liver [39] and, in smaller quantities, also in monocytes, macrophages, intestinal epithelial cells and bowel. It strongly reacts with neutrophil elastase [19]. Determination of the concentration of the resulting complexes in serum or plasma can be used to assess the activity of inflammation and provide information on the stimulation of neutrophils in the inflammation focus. The α-1 antitrypsin is a recognized protein of an acute phase denoted in the blood, and has a relatively low susceptibility to damage caused by enzymes. Therefore, its amount determined in the feces reflects the actual concentration of the protein. Elevated levels of α1-antitrypsin indicate an increase in the permeability of the intestinal membrane. It also

constitutes an indicator of intestinal loss of protein [38].

In order to destroy the bacteria, the leukocytes of polymorphic nucleus produce reactive oxygen species such as the hydroxyl radical, superoxide anion or hypochlorite [3.31]. The resulting superoxide anion that occurs in neutrophils can also be generated by cells of the large intestine [30]. Superoxide anion and hydrogen peroxide can oxidize phospholipids of cell membranes. Additionally, the hydrogen peroxide may react with iron ions in the course of Fenton reaction, which leads to the formation of the hydroxyl radical. This, in turn, may lead to depolymerization of mucous cells in the colon; thus, to a damage to the natural intestinal barrier. This hydroxyl radical rapidly reacts with the cell membrane lipids, some of the side groups of amino acids and DNA, leading to the destruction of cell membrane and the formation of mutations [31]. Another compound with a high reactivity is the hypochlorite, produced by the myeloperoxidase (MPO) from activated neutrophils. It is under the influence of MPO that the hydrogen peroxide is transformed to hypochlorite [29], which can oxidize DNA, thiol groups, purine and pyrimidine nucleotides, polyunsaturated fatty acids or aromatic amino acids [37].

MPO is a protein indicator of a molecular weight of 140 kDa [12]. It is present in the granules of neutrophils and is released during the acute phase during the inflammatory condition. MPO, due to the catalysed reaction, is involved in an oxygen-dependent antimicrobial activity. Its determination in the stool can reflect inflammation in Crohn's disease or ulcerative colitis.

The formation of ROS, in addition to the MPO mentioned above, also involves such enzymes as: NADPH oxidase and inducible nitric oxide synthetase (iNOS) [34]. NADPH oxidase catalyses one-electron reduction of oxygen to superoxide anion. In the course of inflammatory bowel disease the concentration of nitric oxide (NO) increases as a result of increase of iNOS activity [25]. Keshavarzian et al. showed in their study a positive correlation between the level of NO and activity of the disease. Further they noted that, in the course of ulcerative colitis the nitric oxide concentration is higher than in Crohn's disease [15].

The migration of leukocytes and the phase of exacerbation of the disease is also caused by the production of Interleukin-8 (IL-8) [27] by neutrophils of proinflammatory cytokines. Neutrophils, in their granules, contain significant quantities of serine proteases including lysozyme, peroxidase, or free radicals, which once released, amplify their inflammatory response [11]. It is worth noting that increasing concentrations of ROS bring about lowering the level of antioxidants, which act as "scavengers" of reactive oxygen species [34]. Among the most important antioxidant systems, preventing free radical damage, are mentioned: superoxide dismutase (SOD), catalase (CAT), reductase (GR), glutathione peroxidase (GPx) and glutathione S-transferase (GST). These enzymes form a coherent system for protecting antioxidant. The role of superoxide dismutase is the dismutation of superoxide anion into oxygen and hydrogen peroxide which is then decomposed with the involvement of catalase and peroxidase to water and molecular oxygen.

In people with inflammatory bowel disease, a lower SOD activity has been shown as compared to healthy subjects. This enzyme activity was measured in plasma, biopsies and neutrophils, which originated from the cell culture [1,3,42].

The decrease in SOD activity leads to excessive accumulation of superoxide anion. On the other hand, Mulder et al. demonstrated a reduction in the SOD activity in the inflamed mucosa of patients, but the enzyme activity in the intact mucosa inflammation was comparable to the activity of SOD in healthy subjects. Researchers have also observed no significant difference between the activity of the enzyme in patients with Crohn's disease and ulcerative colitis [26]. However, in research by Oshita et al. [30] we did not observe any difference in the activity of SOD in the inflamed mucosa compared to healthy areas of mucosa in patients and compared to healthy subjects. The process of neutralization of hydrogen peroxide, which, as previously mentioned, is produced by colonocytes, the following are involved: catalase, and glutathione peroxidase. This, in turn, its operation requires reduced glutathione [34]. Ruan et al. observed no significant difference between the levels of glutathione in the mucosa in patients with active forms of Crohn's disease and ulcerative colitis. By contrast, during the Crohn's disease in its remission there were observed higher glutathione levels as compared to the active phase of the disease [35]. The concentration of glutathione is regulated with the participation of γ-glutamylcysteine synthetize and glutathione reductase, which increase its concentration. On the other hand, γ-glutamyl transpeptidase and glutathione oxidase lead to a

reduction in glutathione levels. Under conditions of oxidative stress due to the action of glutathione peroxidase, an oxidized form of glutathione, which is glutathione disulphide, is formed. If it does not undergo any re-reduction, it will go into the cytoplasm where it accumulates.

And the preferred glutathione escape from a cell is facilitated by the fact that the cell membrane is easily permeable to oxidized glutathione [35].

These data prove that the antioxidant enzymes, both directly and indirectly, are involved in the free radicals reactions, creating an integrated defence system of the body [36]. Determination of their activity in the serum or in biopsies, in the course of inflammatory bowel disease, can provide a lot of valuable information about the state of the body's antioxidant barrier. Furthermore, it can be used both for diagnosis, prognostic and preventive.

So far, it has not been proven that there is a sufficiently effective diagnostic laboratory test, which would allow differentiation between the Crohn's disease and the ulcerative colitis. Inflammatory bowel diseases, particularly ulcerative colitis, is characterized by the presence of infiltration of neutrophils and eosinophils. Therefore, attempts are made to assess the suitability of the diagnostic signs of substances secreted by neutrophils. Apart from the above mentioned elastase, calprotectin is also belongs to the acute phase proteins. Calprotectin is a heterodimer. In its construction, there are two subunits of S100A8 and two subunits of S100A9, of the size of 8 and 14 kDa respectively (MRP8 / MRP14 [18]. Calprotectin binds the Ca 2+ and Zn 2+ ions. This represents approximately 50-60% of soluble cytoplasmic protein neutrophils [9]. In small quantities, it is also present in monocytes and macrophages [4]. It plays a regulatory role in the inflammation process. It also has anti-microbial, anti-fungal or antiproliferative properties on some tumour lines [41]. There is also a chemotactic factor for neutrophils [10]. The bactericidal properties of calprotectin result from inhibition of bacterial zinc-dependent metalloproteinases. Calprotectin is released during the activation or cell death. By binding of calcium ions it is resistant to proteolytic degradation or by colonic bacteria and therefore, it proves stable in feces, even for few days [18].

As mentioned earlier in patients with inflammatory bowel disease there is a loss of containment of the intestinal barrier and the intensification of leukocyte migration through the intestinal wall, resulting in increased release of calprotectin to faeces [40]. Since one of the elements of the pathogenesis of inflammatory bowel disease is an inflammatory infiltration in the intestinal wall, which contains inter alia neutrophils, it can be concluded that the concentration of calprotectin in these patients will be higher. Thus, it may be a marker of intestinal inflammation. So far, studies have confirmed this hypothesis because the concentration of calprotectin in the faeces of patients with inflammatory bowel disease is higher than in patients with functional bowel disease and in healthy individuals [6,8,28].

Inflammatory bowel diseases are a group of diseases in the course of which there are diagnosed pathological inflammation of the wall of the digestive tract as a result of inflammation caused by the influx of neutrophils into the lumen. One of the reliable methods in the assessment of these changes is the endoscopic method. However, it belongs to invasive diagnostic methods. Therefore, it seems reasonable to seek for various markers that would facilitate diagnosis of the above diseases and constitute a useful tool in the differential diagnosis. A special role is attributed to the so-called stool markers in gastroenterological diagnostics [24].

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