Changes of Oxidative Stress and Haematological Indices in Case of Glutenic Enteropathy Associated with Rheumatoid Arthritis

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The aim of the work was to evaluate changes in the peroxidative–antioxidative status of the organism in case of co-occurrence of glutenic enteropathy and rheumatoid arthritis, to determine changes of haematological indices and their incidence among patients with glutenic enteropathy associated with rheumatoid arthritis and only rheumatoid arthritis and to compare the data on patients and the control group. The study cohort comprised 75 patients and 21 patients with inactive gastroduodenal ulcer disease and chronic inactive gastritis (control group). The study demonstrated that in case of rheumatoid arthritis changes in lipid peroxidation indices take place: MDA increases 1.5 times, DC – 1.7 times, catalase activity decreases 1.3 times. In cases of glutenic enteropathy associated with rheumatoid arthritis, changes of lipid peroxidation indices are as follows: MDA increases 1.4 times, DC – 1.6 times, catalase activity decreases 1.3 times.

In patients with glutenic enteropathy associated with rheumatoid arthritis, the blood serum concentration of iron, calcium, total cholesterol and albumins was significantly lower in comparison with patients affected by rheumatoid polyarthritis alone.

Key words: glutenic enteropathy, rheumatoid arthritis, oxidative stress, lipid peroxidation

INTRODUCTION

Many diseases, including digestive tract pathology (1) and rheumatoid arthritis (RA) (2), are related to oxidative stress. Oxidative stress is produced by the excess of free radicals in cells. Indices characterising the peroxidative and oxidative status of the organism undergo changes. Free radicals are chemical substances able to exist with one or more unpaired electrons. One of the targets for free radicals are lipids of cell membrane, specifically one of their components – fat acids (3).

Free radical peroxidation of lipids is a normal metabolic process of all organs and tissues. In norm, peroxidation of lipids is strictly reglamented by endogenic antioxidants, and enzymes such as superoxide dismutase, catalase, glutationperoxidase, glutationreductase play a crucial role in this process (4). The antioxidative system is one of the adaptation systems of the organism. It regulates lipid peroxidation processes. It is established that disruption of the regulation of lipid peroxidation and exhaustion of the antioxidative system are closely related with numerous diseases (5).

Physiologically, lipid peroxidation takes place at a certain limited rate and is one of the chains of cell metabolism. Particles of reactive oxygen are included into the processes of cell growth, division and death. Low concentrations of reactive oxygen particles are useful to the organism, as they take part in intracellular signalisation processes and protect the cells from microorganisms. High concentrations of free radicals play an important role in the pathogenesis of some diseases. Toxic oxygen formulations, peroxides, free radicals change the permeability of cell membranes, thus causing inflammation and impairment of the functions of organs.

Glutenic enteropathy (GE), a chronic disease classified as one of the intestinal enzymopathies, is related to diseases of impaired immunity and autoimmune diseases (6), as well as to changes in the indices of oxidative stress (7). GE is considered

to be induced by prolamines, an integral part of the protein gliadine of wheat, rye and barley. Morphological changes of intestinal mucosa vary from lymphocyte infiltration into the epithelium and lamina propria to total atrophy of mucosal villi and hyperplastic changes of crypts. Clinically, dyspeptic and malabsorption syndromes are characteristic of glutenic enteropathy (8). Malabsorption can predispose to changes in the antioxidative system (9).

GE is closely related with some immune characteristics – HLA class II haplotype DR3 (DQ2) and presence of antigliadine, antiendomysium, antireticuline antibodies in blood serum. Antiendomysium antibodies which are related to transglutaminase of tissues have the highest specificity (10). Recently tissue transglutaminase (tTG) as an autoantigen of the endomysium has been identified. The expression and frequency of glutenic enteropathy recently has changed significantly; the number of latent and low symptomatic cases has increased, and their diagnosis requires much more sensitive diagnostic procedures (8, 11, 12).

Over the last years, attention has been drawn to the association between rheumatoid arthritis and glutenic enteropathy (13, 14). The immune complexes produced in the intestinal mucosa of patients with coeliac disease are thought to accumulate in other organs and trigger appearance and progression of autoimmune diseases (15). According to literature data, coeliac disease is associated with rheumatic diseases in 0.4 to 2 percent of cases (16, 17). P. Collin, after analysis of 5,600 individuals, has diagnosed glutenic enteropathy in 1 from 243 rheumatic patients. It was established that arthralgy and RA can be the first and single symptom of this enzymopathy (14). RA is most often considered as an extraintestinal clinical symptom of glutenic enteropathy, but exact data on its occurrence are still lacking (13). High frequencies of antigliadine IgA and IgG antibodies were established among patients with rheumatoid arthritis (37%). Presence of antigliadine antibodies in early stages proves the importance of intestinal immune system for the development of rheumatoid arthritis (17).

AIMS OF THE STUDY

To evaluate changes in the peroxidative-antioxidative status of the organism in cases of co-occurrence of glutenic enteropathy and rheumatoid arthritis. To determine changes of haematological indices and their incidence among patients with glutenic enteropathy associated with rheumatoid arthritis and with rheumatoid arthritis alone and to compare the data on patients and control group.

STUDY SAMPLE AND METHODS

In total, 75 patients were studied, of them 19 were ill with glutenic enteropathy associated with rheumatoid arthritis (mean age 59.41 ± 2.7 years), and 56 had rheumatoid arthritis alone (mean age 52.39 ± ± 1.3 years). The control group consisted of 21 patients with inactive gastroduodenal ulcer disease and chronic inactive gastritis. The mean age of the control group was 48 ± 1.5 years.

The diagnosis of glutenic enteropathy was established after evaluation of anamnesis, data of physical, clinical and biochemical blood examination, determination of the concentration of IgA class antigliadine antibodies, intestinal biopsy with a Crosby capsule or duodenoscope.

The peroxidative status of the individuals studied was evaluated according to blood serum concentrations of diyenic conjugates (DC) and malondialdehide (MDA). For quantitative lipid peroxides determination, their transformation into a coloured complex with thiobarbituric acid was applied [18]. Malondialdehide (MDA), the final product of peroxidation of fatty acids, reacts with thiobarbituric acid to produce a coloured complex characterised by the maximum absorption at a wavelength of 532 nm. Data are expressed in nmol/ml. For DC blood concentration, lipid extract absorption in ultraviolet area at a wavelength 232–234 nm was measured. Data are expressed in nmol/ml [19].

The status of antioxidative system was analysed by measuring the activity of catalase. The principle of catalase determination is that a decrease of hydrogen peroxide quantity in time is measured; hydrogen peroxide makes a stable coloured complex with ammonium molibdate at a wavelength of 410 nm. Enzyme activity is expressed in nmol/l/min [20].

Blood indices were studied by standard clinical and biochemical methods. Concentration of IgA class antigliadine antibodies in blood serum was determined by immunoezyme (ELISA) method. The reaction was positive when the concentration of antibodies was higher than 11 U/ml, and negative when the concentration of antibodies was below 9 U/ml. Biopsy material from intestinal mucosa was studied using standard histologic techniques.

Statistical data were processed using an EpiInfo2000 computer statistic programme.

RESULTS AND DISCUSSION

Comparison of changes in the oxidative stress indices of the blood serum of rheumatoid arthritis patients and control group revealed an increase in lipid peroxidation. Activation of cell inflammation and hyperproduction of free radicals takes place, both

having a great impact on the circulation of lipid peroxidation products. The data are presented in Table 1. This phenomenon is confirmed also by other authors (21, 22). Increase of serum MDA and DC as well as decrease of catalase activity prove the importance of free radicals in the development of inflammatory processes. In case of rheumatoid arthritis, joints damaged by inflammation suffer from oxidative effects, as there are numerous active phagocytes producing radicals of superoxide, excess of which damages tissues of the joints affected by the disease.

Our study demonstrated that both in cases of rheumatoid arthritis and glutenic enteropathy associated with rheumatoid arthritis catalase activity increased 1.4 times, the concentration of MDA increased 1.7 times in cases of rheumatoid arthritis associated with glutenic enteropathy (RA + GE) and 1.5 times – in cases of rheumatoid arthritis (RA) alone. The results correlate well with the data of other authors [23]. However, some investigators point out that in rheumatoid arthritis patients the concentration of MDA and the activity of superoxide dismutase increase, but catalase activity remains unchanged (2).

Recent studies of intracellular antioxidant level show the importance of cellular immunity protection (24). Data on antioxidative system in rheumatoid arthritis are controversial – there are indications that, although MDA intensifies, catalase activity is constant (25, 26). Our data prove that the indices of oxidative stress in patients with glutenic enteropathy associated with rheumatoid arthritis do not differ significantly from those in patients affected by rheumatoid arthritis alone.

Literature data suggest that in cases of glutenic enteropathy the concentration of MDA, a lipid peroxidation marker, significantly increases in comparison with controls, and the level of the antioxidant alpha-tocoferol decreases (9, 27). We failed to find in literature data on the indices concerning glutenic enteropathy associated with rheumatoid arthritis included into our study.

Analysis of some haematological indices of patients with glutenic enteropathy associated with rheumatoid arthritis revealed that concentrations of haemoglobin ($p < 0.001$), albumins ($p < 0.001$), iron ($p < 0.01$), calcium and total cholesterol ($p < 0.001$), erythrocyte number ($p < 0.001$), average erythrocyte volume ($p < 0.05$) were significantly lower than in control group. The average haemoglobin concentration in erythrocytes was similar in both groups.

A comparison showed that in patients with rheumatoid arthritis alone only the concentrations of haemoglobin and total cholesterol ($p < 0.001$), as well as erythrocyte number ($p < 0.001$) were statistically significantly lower than in control group. All data are presented in Table 2.

In patients with glutenic enteropathy associated with rheumatoid arthritis, the concentration of iron ($p < 0.05$), calcium ($p < 0.001$), total cholesterol ($p < 0.05$) and albumins ($p < 0.001$) was signifi-

Table 1. **Changes of oxidative stress in the study sample**

Group of patients	Number of patients	DC conc. in blood serum	MDA conc. in blood serum	Catalase activity
RA + GE	19	9.91 ± 0.65 ***	4.79 ± 0.22 ***	30.26 *
RA	56	9.23 ± 0.75 ***	5.82 ± 0.33 ***	29.61 ± 2.2 *
Control	21	5.7 ± 1.8	3.44 ± 0.1	43.36 ± 2.1

*$p < 0.05$, *** $p < 0.001$.

Table 2. **Haematological indices of patients and control group**

Patient group	Number	Haemoglobin	MCV	MCH	Erythrocytes	Iron	Calcium	Total cholesterol	Albumins
RA + GE	19	122.4 ± 26 ***	86.91 ± 6.06 *	28.55 ± 0.6	4.28 ± 0.09 ***	7.6 ± 0.8 **	2.11 ± 0.05 ***	4.84 ± 0.3 ***	35.25 ± 0.6 ***
RA	56	125 ± 1.9 ***	87.14 ± 1.7	28.81 ± 0.3	4.31 ± 0.05 ***	11.1 ± 0.5	2.47 ± 0.02	5.71 ± 0.2 ***	41.41 ± 0.7
Control	21	146 ± 2.1	92 ± 0.89	29.9 ± 0.7	5.16 ± 0.1	11.73 ± 07	2.35 ± 0.02	8.01 ± 0.1	41 ± 0.26

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

cantly lower than in patients with rheumatoid arthritis alone.

Our data revealed high frequencies of changes in the blood indices of patients ill with glutenic enteropathy associated with rheumatoid arthritis: decreased concentrations of haemoglobin (35.29%), iron (29.41%) ($p < 0.005$), calcium (17.64%), total cholesterol (23.52%), albumins (23.52%) ($p < 0.005$), average erythrocyte volume (23.52%), average erythrocyte haemoglobin concentration (23.52%) as compared to patients ill with rheumatoid arthritis alone.

Over the last years clinical signs of adult glutenic enteropathy have changed significantly. The subclinical and latent types of the disease prevail. Typical malabsorption syndrome and marked anaemia became less frequent, and for the majority of patients only minor changes of blood indices are observed. Folic acid, iron and calcium deficiency is still widely spread, but thus deficiency is not necessarily manifested clinically. Erythrocyte macrocytosis or microcytosis without anaemia can be a single trait of the disease (29, 28). According to data of S. Bode and co-authors, the following changes of haematological indices for glutenic enteropathy patients were established: decreased concentrations of haemoglobin (22%), iron (32%), calcium (43%), albumins (26%), decreased prothrombin activity (21%), average erythrocyte volume (10%). The data are similar to the results of our study.

Numerous literature sources describe association of non-specific mono- or polyarthritis with glutenic enteropathy. This enteropathy is also related to a number of connective tissue diseases, as well as with rheumatoid arthritis (28, 30). According to K. Chakravarty and D. Scott, arthritis is one of prevailing traits of subclinical celiac disease. However, blood analysis reveals only minor normochromic and normocytic anaemia, a slightly decreased haemoglobin concentration and average erythrocyte volume. Thus polyarthritis should be differentiated from glutenic enteropathy, especially if it is followed by weight loss and anaemia, as treatment with aglutenic diets decreases the symptoms (31).

Our data indicate that for patients ill with GE associated with RA the majority of haematological indices are substantially lower. It is possible that these haematological changes are related with a persisting lesion of intestinal mucosa and impaired absorption. When patients with joint diseases have persisting slight anaemia or signs of malabsorption, glutenic enteropathy should be considered.

CONCLUSIONS

1. In cases of rheumatoid arthritis, changes in lipid peroxidation indices take place: MDA increases 1.5 times, DC 1.7 times, catalase activity decreases 1.3 times.

2. In cases of glutenic enteropathy associated with rheumatoid arthritis, changes in lipid peroxidation indices are as follows: MDA increases 1.4 times, DC 1.6 times, catalase activity decreases 1.3 times.

3. Comparison of lipid peroxidation indices between patients with rheumatoid arthritis and glutenic enteropathy associated with rheumatoid arthritis revealed no differences.

4. Blood serum concentrations of iron, calcium, total cholesterol and albumins were found significantly lower in patients with glutenic enteropathy associated with rheumatoid arthritis than in patients affected by rheumatoid arthritis alone.

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OKSIDACINIO STRESO IR HEMATOLOGINIŲ RODIKLIŲ POKYČIAI SERGANT GLIUTENINE ENTEROPATIJA, ASOCIJUOTA SU REUMATOIDINIU ARTRITU

S a n t r a u k a

Įvertinti organizmo peroksidacinės-antioksidacinės būklės rodiklių pakitimai sergant gliutenine enteropatija ir reumatoidiniu artritu. Nustatyti hematologinių rodiklių pakitimai bei jų dažnis tarp sergančiųjų gliutenine enteropatija, asocijuota su reumatoidiniu artritu, ir tarp sergančių tik reumatoidiniu artritu; palyginti tirtų ligonių ir kontrolinės grupės asmenų duomenys. Iš viso ištirti 75 ligoniai, iš kurių 19 sirgo gliutenine enteropatija, asocijuota su reumatoidiniu artritu, 56 – tik reumatoidiniu artritu. Kontrolinę grupę sudarė 21 asmuo. Kontrolinės grupės ligonių amžiaus vidurkis – 48 ± 1,5 metų. Sergant reumatoidiniu artritu lipidų peroksidacijos rodikliai: MDA padidėja 1,5 karto, DK – 1,7 karto, katalazės aktyvumas sumažėja 1,3 karto. Sergant gliutenine enteropatija, asocijuota su reumatoidiniu artritu, lipidų peroksidacijos rodikliai: MDA padidėja 1,4 karto, DK – 1,6, katalazės aktyvumas sumažėjo1,3 karto. Palyginus sergančių reumatoidiniu artritu ir gliutenine enteropatija, asocijuota su reumatoidiniu artritu, LP rodiklius, skirtumas nenustatytas. Sergančiųjų gliutenine enteropatija, asocijuota su reumatoidiniu artritu, kraujo serume statistiškai reikšmingai mažiau nustatyta geležies, kalcio, bendrojo cholesterolio ir albuminų, palyginus su sergančiais tik reumatoidiniu artritu.

Rakatažodžiai: lipidų peroksidacija, oksidacinis stresas, gliuteninė enteropatija, reumatoidinis artritas