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EVALUATION OF EFFECT *CAT -262C/T, SOD + 35A/C, GPX1 PRO197LEU* POLYMORPHISMS IN PATIENTS WITH IBD IN THE POLISH POPULATION*

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Inflammatory bowel disease (IBD) are a heterogeneous group of disorders in the course dominated by chronic, recurrent gastrointestinal inflammation. It is believed that the activation of IBD occurs in patients with a genetic predisposition to their development. Chronic inflammation develops as a result of an excessive reaction of the immune system principally under the influence of environmental risk factors. Among them, it has been shown that the mechanism of oxidative stress is associated with the pathophysiology of inflammatory bowel disease, responsible for the commencement and progress of these diseases.

The aim of the study was the relationship between single nucleotide polymorphisms (SNPs) of individual antioxidant enzymes, and the prevalence of inflammatory bowel disease that may be associated with increased levels of oxidative stress.

Material and methods. A total of 111 IBD patients, including 65 patients with ulcerative colitis (UC) and 46 with Crohn's disease (CD) and 125 healthy controls recruited from the Polish population, were genotyped for *CAT -262C / T* (rs1001179), *SOD + 35A / C* (rs2234694), *GPx Pro 197 Leu* polymorphisms. Genotyping of *CAT, SOD, GPx* gene polymorphism was performed by a RFLP-PCR.

Results. The performed analysis of genetic polymorphisms of antioxidant enzymes showed that polymorphic variant of the CAT -262 C / T may have protective effects in patients with ulcerative colitis in the range of genotype C / T; OR = 0.49 (0.25-0.99), p = 0.044. Trend protective, but statistically unrelated, it was also observed for genotype T / T and T allele of the same polymorphism and genotypes and alleles + *35A / C SOD1* in UC as well as polymorphic variants *CAT -262 C / T*, *Pro197Leu of GPx1*, + *35A / C SOD1* in CD. The results were compared with a control group of potentially healthy individuals without such diseases.

Conclusions. It has been shown that the polymorphism of antioxidant enzymes *CAT* gene *-262 C / T* may have protective effects in patients who are carriers of a genotype *C / T* at the UC. The potential protective effect without statistical relationships were also observed for other genotypes and alleles studied polymorphic variants of antioxidant enzymes in CD and *CAT- 262C / T* and + *35 A / C SOD1* in UC. Conducted our audit should be extended to more group of patients in order to assess whether or not to confirm the observed during analysis, the protective effect of CAT-262 C / T in ulcerative colitis and other trends observed for other polymorphic variants tested genes.

Key words: genetic polymorphism, antioxidant enzyme, inflammatory bowel disease

Inflammatory bowel disease is a group of very troublesome gastrointestinal disorders. Among a number of unspecified forms most frequently encountered two forms of Crohn's disease (CD) and ulcerative colitis (UC). CD in their entire length can cover all sections of the gastrointestinal tract from the esophagus to the anus while UC is localized predomi-

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nantly in the colon. These diseases run with periods of spontaneous exacerbations, during which the inflammation gets out of control, and can be difficult to control the course of. In severe cases often require surgical intervention, which in extreme cases it can be completed a total colectomy.

Often in these diseases the exacerbation itself periods of remission where symptoms completely or partially disappear. Remission of the disease can take several years, and after this period, followed by re-tightening. After about 10 years disease to a long-lasting, chronic inflammation may occur foci of dysplasia, and the subsequent course of these diseases often leads to the development of colon cancer. The etiology of these diseases has not been fully explained It is believed that the activation and development of these conditions occur in individuals with a genetic predisposition due to the interaction of various environmental factors and excessive immune response. occur in individuals with a genetic predisposition due to the interaction of various environmental factors and immune disorders. As a result of a complex combination of these factors occur erode intestinal homeostasis and development of chronic inflammation.

Inflammation occurring in the course of IBD is closely related to the relationship with the formation of reactive intermediates, including reactive oxygen and nitrogen species (ROS / RNS), and oxidative stress has been proposed as a mechanism underlying the pathophysiology of IBD (1). Due to these exceptional hardship diseases, as well as the fact that chronic inflammation contributes to the formation of tumors, tests should be performed the early diagnosis of these diseases. Oxidative stress is thought to be one of the causes of the development of IBD, and antioxidant enzymes protects cells from the effects of Catalase (CAT) are involved in the production and inactivation of the hydrogen peroxide (H_2O_2), and a functional *CAT* gene polymorphism *-262C / T* is involved in the regulation of the concentration of H_2O_2 (2). Polymorphism in the promoter region *-262 C / T CAT* is associated with altered activity of catalase. The intensity of its actions may depend also upon the age, physical activity, seasonal fluctuations and other chemicals (2).

Glutathione peroxidase 1 (GPx1), and catalase (CAT) encode the two enzymes antioxidants that reduce the toxic effect of H_2O_2. They protect cells against reactive oxygen species ROS controlling the H_2O_2 concentration, by conversion into water and oxygen thereby protect cells from oxidative damage (3). Superoxide dismutase is also a strong antioxidant enzyme which plays an important role in the defense mechanisms against oxidative stress (4).

As the antioxidant enzymes are the first line of defense against ROS assessed the impact of polymorphic variants of antioxidant enzymes *Pro197Leu* of *GPx1, + 35A/C SOD1, -262C/T CAT* in patients with inflammatory bowel diseases. The relationship between the polymorphic variants of genes encoding enzymes such as SOD1, CAT and GPx1 and the risk of these diseases has not yet been clearly defined. Conducting research we want to test whether occurring polymorphisms will affect the evolution of the antioxidant enzymes. This may contribute to developing IBD, through greater damage caused by oxidative stress.

MATERIAL AND METHODS

We investigated a total of 111 patients with IBD, including 65 patients with ulcerative colitis (UC) and 46 with Crohn's disease (CD) and 125 healthy controls. All subjects were from the Polish population. Analyzed polymorphic variants of antioxidant enzymes *CAT -262C / T, SOD + 35A / C, GPx Pro197Leu.* Genotypes *CAT, SOD, GPx* was performed by PCR-RFLP. The control group consisted of 125 healthy individuals without symptoms of upper gastrointestinal tract and intestinal mucosa. The study was qualified persons aged 18 to 60 years hospitalized in the Department of Gastroenterology and Internal Medicine at the Medical Universityin Lodz and at the Department of General and Colorectal Surgery, Medical University in Łódź. UC and CD were diagnosed on the basis of radiological, pathological and clinical criteria.

On the test it is expressed consent Bioethics Committee of the Medical University in Łódź number RNN / 835/09 / KB. Patients who agreed to participate in the study.

Three milliliters of peripheral blood taken from the patient into EDTA tubes. Lymphocytes were obtained from these samples were used for DNA isolation. The isolation was done

using the Genomic Blood Mini Ax in accordance with the manufacturer's instructions.

Analysis of polymorphic variants of antioxidant enzymes *CAT -262C/T, SOD + 35A/C, GPx Pro197Leu* was performed using PCR-RFLP.The PCR reaction used oligonucleotide pairs restricting the polymorphic site of the studied gene sequence to the following:

CAT F; 5'TAAGAGCTGAGAAAGCATAGCT-3 „

CAT R; 5'AGAGCCTCGCCCCGCCGGACCG-3 ,;

GPx1; 5'TGTGCCCCTACGCAGGT-3 ,;

GPx1; 5CCAAATGACAATGACACAGG-3,;

SOD1; 5'CTATCCAGAAAACACGGTGGGCC-3 ,;

SOD1; 5'TCTATATTCAATAAATGCTACAAAACC-3 ,.

The PCR reaction was performed in a Multi-Gene thermocycler (Labnet International Inc) in a total volume of 15 µl, containing 100 ng of genomic DNA, primer, polymerase mix and water in a total volume of 15 µl.The reactions for *CAT* were conducted under the following temperature conditions: predenaturation step at 94°C for 5 min, and then in 16 cycles: denaturation at 95°C for 30 s, oligunucleotide annealing: 68°C for 45 s, amplification of PCR productsat 72°C for 60 sec and then 25 cycles: denaturation at 95°C for 30 sec, annealing oligunucleotide: 60°C for 45 seconds, amplification of PCR products at 72°C for 60 s and final incubation at 72°Cfor 10 minutes.

The reactions for *GPx1*were conducted under the temperature conditions following: predenaturation step at 94°C for 5 min, and then in 35 cycles: denaturation at 95°C for 30 sec, annealing oligunucleotide 58°C for 30 s, and amplification of PCR productsat 72°C for 90 s and final incubation at 72°C for 10 minutes.

The reactions for *SOD1*where conducted under the temperature conditions following: predenaturation step at 94°C for 5 min, and then in 32 cycles: denaturation at 94°C for 40 s, annealing oligunucleotide 55°C for 40 s, amplification of PCR products at 72°C for 40 s and final incubation at 72°C for 2 minutes.

Products of the PCR reaction were subjected to digestion by the enzyme restrictive

Smal (1.5 U) 1.5 µl at 37°C for *CAT*, ApaI 0.2 µl at 37°C for *GPx1*, HhaI 0.25 µl at 37°C for *SOD1*. After 16 hours of digestion to a restrictive enzyme, the samples were separated by electrophoresis on a 2% agarose gel and fragments obtained restrictive were visualize by staining with ethidium bromide.

The distribution of electrophoretic gel homozygous C/C polymorphic variant of catalase is shown as a single band at a height of 155 base pairs, heterozygous C/T is exemplified by the two levels of 185 and 155 bp, homozygous T/T shows the band of 185 bp.

Homozygous C/C polymorphic variant of glutathione peroxidase gel is shown as two bands at levels of 257 and 81 bp, the heterozygote C / T illustrate three bands at the height of 338, 257 and 81 bp homozygous T/T shows the band of 338 bp.

The distribution of electrophoretic gel homozygous A/A polymorphic variant of superoxide dismutase is shown as a single band on a 277 bp, heterozygote A/C is exemplified by the two levels of 206 and 277 bp, homozygous C/C shows the band of 206 bp.

Chi^2 test was used to test significance of differences in the genotype and allele frequency distribution between the two study groups. The control group was able to Hardy-Weinberg equilibrium. Odds ratio (OR) and corresponding 95% confidence intervals (CI) were used to assess correlations betweengenotypes and alleles and IBD.

RESULTS

Table 1 and 2 show schedules genotype and allele frequencies in controls and patients with UC tab. 1 and tab. 2 for the CD polymorphic variant enzyme *SOD A35C*. The control group was consistent with the distribution of Hardy-Weinberg equilibrium. Genotype A/C OR = 0.56 (0.19-1.59) p = 0.271 and C allele OR = 0.46 (0.17-1.26) p = 0.121 for patients with UC and genotype A/C OR = 0.67 (0.21-2.12) p = 0.493 and C allele OR = 0.55 (0.18-1.65), p = 0.277. Within the activities of the analyzed polymorphism was not found statistical relationship with IBD, but the observed tendency protective.

Tables 3 and 4 show schedules genotype and allele frequencies in controls and patients with UC in table 3 and table 4 for the CD polymorphic variant enzyme *CAT C-262T*. Genotypes C/T OR = 0.49 (0.25-0.99), p = 0.044, T/T OR = 0.69 (0.22-2.11), p = 0.512 and T allele OR = 0.64 (0.38-1.07), p = 0.086 in the UC and

Table 1. The genotype and allele frequency and odds ratios (OR) of the *SOD* A35C polymorphism in healthy subjects and patients with CU

Aenotype/ allele	Patients (%) (n=65)	Control group (%) (n=125)	OR (95% CI)
A/A	60 (0,92)	107 (0,86)	Ref.
A/C	5 (0,08)	16 (0,13)	0,56 (0,19-1,59) p=0,271
C/C	0 (0,00)	2 (0,02)	-
A	125 (0,96)	230 (0,92)	Ref.
C	5 (0,04)	20 (0,08)	0,46 (0,17-1,26) p=0,121

Table 2. The genotype and allele frequency and odds ratios (OR) of the *SOD* A35C polymorphism in healthy subjects and patients with CD

Aenotype/ allele	Patients (%) (n=44)	Control group (%) (n=125)	OR (95% CI)
A/A	40 (0,91)	107 (0,86)	Ref.
A/C	4 (0,09)	16 (0,13)	0,67 (0,21-2,12) p=0,493
C/C	0 (0,00)	2 (0,02)	-
A	84 (0,95)	230 (0,92)	Ref.
C	4 (0,05)	20 (0,08)	0,55 (0,18-1,65) p=0,277

Table 3. The genotype and allele frequency and odds ratios (OR) of the *CAT* C-262T polymorphism in healthy subjects and patients with CU

Aenotype/ allele	Patients (%) (n=65)	Control group (%) (n=125)	OR (95% CI)
C/C	45 (0,69)	68 (0,54)	Ref.
C/T	15 (0,23)	46 (0,37)	0,49 (0,25-0,99) p=0,044
T/T	5 (0,08)	11 (0,09)	0,69 (0,22-2,11) p=0,512
C	105 (0,81)	182 (0,73)	Ref.
T	25 (0,19)	68 (0,27)	0,64 (0,38-1,07) p=0,086

Table 4. The genotype and allele frequency and odds ratios (OR) of the *CAT* C-262T polymorphism in healthy subjects and patients with CD

Aenotype/ allele	Patients (%) (n=46)	Control group (%) (n=125)	OR (95% CI)
C/C	30 (0,65)	68 (0,54)	Ref.
C/T	13 (0,28)	46 (0,37)	0,64 (0,30-1,36) p=0,244
T/T	3 (0,07)	11 (0,09)	0,62 (0,16-2,38) p=0,359*
C	73 (0,79)	182 (0,73)	Ref.
T	19 (0,21)	68 (0,27)	0.70 (0,39-1,24) p=0,218

* Fisher test

genotypes C/T OR = 0.64 (0.30-1.36), p = 0.244, T/T OR = 0.62 (0.16-2.38), p = 0.359 and T allele OR = 0.70 (0.39-1.24) p = 0.218 for patients withCD.The polymorphic variant of the enzyme *CAT C-262T* showed a protective effect forgenotypes C/T. Other genotypes and alleles also demonstrate a propensity towards the protective action.

Table 5 and 6 shows the schedules genotype and allele frequencies in controls and patients with UC and CD polymorphic variant enzyme *GPx1 Pro197Leu*. The OR value for genotype C/T1.05 (0.55-1.99) p=0.888, TT1.00(0.36-2.79) p=0.609*and T allele1.01 (0.65-1.58) p=0.518*in the UC and genotype C/T OR = 0.77 (0.36-1.66), p = 0.507, TT OR = 0.99 (0.31-3.15), p = 0.620 and T allele OR = 0.92 (0.54-1.57), p = 0.764 in CD. The analyzed indicate a polymorphic variant of a protective trend only CD

Table 5. The genotype and allele frequency and odds ratios (OR) of the *GPx1* Pro197Leu polymorphism in healthy subjects and patients with CU

Aenotype/ allele	Patients (%) (n=64)	Control group (%) (n=125)	OR (95% CI)
C/C	25 (0,39)	50 (0,40)	Ref.
C/T	32 (0,50)	61 (0,49)	1,05 (0,55-1,99) p=0,888
T/T	7 (0,11)	14 (0,11)	1,00 (0,36-2,79) p=0,609*
C	82 (0,64)	161 (0,64)	Ref.
T	46 (0,36)	89 (0,36)	1,01 (0,65-1,58) p=0,518*

* Fisher test

Table 6. The genotype and allele frequency and odds ratios (OR) of the *GPx1* Pro197Leu polymorphism in healthy subjects and patients with CD

Aenotype/ allele	Patients (%) (n=40)	Control group (%) (n=125)	OR (95% CI)
C/C	18 (0,45)	50 (0,40)	Ref.
C/T	17 (0,43)	61 (0,49)	0,77 (0,36-1,66) p=0,507
T/T	5 (0,13)	14 (0,11)	0,99 (0,31-3,15) p=0,620*
C	53 (0,66)	161 (0,64)	Ref.
T	27 (0,34)	89 (0,36)	0,92 (0,54-1,57) p=0,764

* Fisher test

DISCUSSION

Inflammatory bowel disease is characterized by chronic inflammation of the gastrointestinal wall of unknown etiology and pathogenesis is not fully understandable. Crohn's disease and ulcerative colitis are associated with the genetic disorder as a consequence of which there is an excessive T cell responses of commensal intestinal bacteria. Under the influence of environmental factors is interrupted intestinal barrier, a disorder of the immune response and also the balance between commensal and pathogenic bacteria. These changes can be generally described as a cause defects in the mucosal barrier function and immunoregulation (5). Among a range of symptoms of these extremely arduous conditions depending on the site location of the lesion is dominated by abdominal pain, diarrhea alternating with constipation, bloating, nausea, vomiting and bleeding from the gastrointestinal tract, factors immunological and genetic are involved in the inflammatory process and play an important role in the development of these diseases.

Oxidative stress is also considered as a factor related to the pathophysiology of IBD (1). Antioxidant enzymes are the first line of defense against ROS. Operation of reactive oxygen speciessuch as a hydroxyl radical, superoxide anion, hydrogen peroxide and nitric oxide in the absence of an effective antioxidant defenses contribute to the development of various pathological conditions associated with pregnancy complications as well (3). Homeostasis caused by the action of reactive oxygen species associated with the onset and progression of chronic diseases such as cardiovascular diseases, neurodegenerative disorders and cancer. Gene polymorphisms *GPx1 Pro198Leu* and *Pro197-Leu* significantly increased risk of cardiovascular disease in the population of East Asia (6). In addition *Pro197Leu GPx1* were significant risk factors in the development of familial Mediterranean fever (FMF) (7). Catalase is one of the most important endogenous antioxidants present in high levels in liver, kidney and erythrocytes (3). In humans, it plays an important role in various physiological and pathological states, protects cells from the toxic effects of hydrogen peroxide, and regulates the expression of the *CAT* gene using a variety of mechanisms, plays an important role in its levels.

Changes in the levels of CAT enzyme observed in various diseases, and the *CAT* gene polymorphisms associated with a number of pathophysiological conditions such as hypertension, diabetes, insulin resistance, dyslipidemia, asthma, bone metabolism or vitiligo (8). Increased oxidative damage and *CAT* gene polymorphism -262 of the promoter region, C/T associated with the development of asthma in children (9). Single nucleotide polymorphism (SNP) catalase -262 C/T also been associated with the occurrence of breast and prostate

cancer (10, 11). It is believed that the balance between oxidants and antioxidants affects many genetic variants, as well as endogenous and exogenous factors (12, 13).

High levels of ROS is harmful, since it can cause lipid peroxidation in cell membranes to oxidative damage to proteins, DNA mutations (14). Then, if the mechanisms are properly antioxidant under high levels of ROS are formed oxidative damage, which is responsible for initiating and modulating disease progression (9). Disturbances in the system of antioxidant defense can play an important role in the development and progression of distal symmetric polyneuropathy associated with type 2 diabetes (DSPN) (15).The presence of a single nucleotide polymorphism (SNP) in the genes encoding the antioxidant enzymes can lead to impaired and slowing ROS disposal, thus contributing to the expansion of oxidative damage (16).

Our study involved the analysis of genetic polymorphisms of antioxidant enzymes *Pro197Leu*of *GPx1, + 35A/C SOD1* and *-262C/T CAT* in patients with IBD. Such polymorphisms may can change the properties of antioxidant enzymes, which may lead to increased oxidative stress-induced damage, and thus contribute to the development of the disease. So far, no conclusive reports on the role of polymorphic variants described in the above diseases. Since ROS are involved in inflammation, because oxidative stress may play a role in the pathophysiology of IBD (1). The effect obtained as a result of our analysis suggests that the polymorphic variant of the antioxidant enzyme CAT gene *-262C / T* may be protective in ulcerative colitis. This effect was confirmed for genotype C / T 0.49 (0,25-0,99), p = 0.044. Genetic analysis showed no statistical relationships between the variant *GPx1 Pro197Leu* and UC, in other variants of polymorphic enzymes observed a trend towards protectiveactions. The results obtained were likened to the control group of potentially healthy without of these disease.

Kodayari et al. obtained in contrast to our results suggest that polymorphism *-262 C/T CAT* may be associated with UC, a genotype C/T may be a risk factor for developing this disease. In holders of genotype C/T risk of UC was 4.88 times higher compared with genotype C/C (95% CI 1.73-13.75, p = 0.002). There was no evidence, however, a significant increase in the frequency of the T allele in patients compared to healthy controls (p = 0.077). Studies have shown the lack of acknowledgment between the polymorphism of *CAT C-262T* and clinical function of UC (17).

The main limitation of our study was the small sample size. It should be increased in order to confirm the observed relationship. This also suggests KodayariSet al. who have received conflicting results, but these relate only to the *CAT C-262T* polymorphism. The results may also depend on the population studied.

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