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THE VALUE OF POLYMORPHISM RRO12ALA GENE PPARG IN VIOLATION OF LIPID PEROXIDATION AND ANTIOXIDANT PROTECTION IN PATIENTS WITH TYPE 2 DIABETES MELLITUS

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Resume. The incidence of type 2 diabetes mellitus is increasing in Ukraine and worldwide. The severity of this disease is determined by the number of complications, which are based on lipid peroxidation (LPO). Today, the influence of gene polymorphisms Pro12Ala PPARG on oxidative and antioxidant processes is not in doubt. We studied the association between gene polymorphism Pro12Ala rs1801282 PPARG and intensification of lipid peroxidation and antioxidant systems (AOS) in 88 patients with type 2 diabetes, using analysis of variance. In the 12Pro allele carriers male found probable increased intensification of lipid peroxidation than in women, with increasing levels of DC (p=0,034) and MDA (p=0,001). Reducing the enzyme catalase level of AOC in patients with type 2 diabetes was observed in the case of genotype Pro12Pro gene PPARG on 21.7% compared with heterozygotes (F=8,17; p=0,005) and the presence of the allele 12Pro (F=6,28 , p=0,013). Found significantly higher activity of AOC in the form of increasing the level of α-TF (p=0,016) and catalase activity (p=0,034) among male patients with gene polymorphism Pro12Ala PPARG, than homozygotes for allele 12Pro.

Key words: **lipid peroxidation; antioxidant system; type 2 diabetes mellitus; polymorphism Pro12Ala rs1801282 of gene PPARG**

Introduction. In 2014, the worldwide incidence of diabetes mellitus (DM) was 9% in the adult population, and type 2 diabetes was found in 90% of patients with diabetes [1]. A similar trend is also typical for Ukraine, which currently has over 1.3 million. Patients with type 2 diabetes, and the true prevalence of the disease at least three times higher, confirming control epidemiological studies [2,3].

One of the most important links in the pathogenesis of type 2 diabetes and its complications is considered to oxidative stress and its direct consequence - lipid peroxidation (LPO) [4-6].

Proved, that type 2 diabetes - a free radical pathology [7-14]. The urgency and necessity of forecasting disturbances in the antioxidant protection system due to the fact that a cascade of free radical reactions started before clinical manifestation of type 2 diabetes and the observed failure mechanisms of antioxidant defense [15,16]. A sharp intensification of free radical processes leads to increased formation of reactive oxygen species, resulting in the development of oxidative stress [17-19]. Increased secondary product of lipid peroxidation (MDA) in type 2 diabetes proved by many scientific studies even been proposed to use indicators MDA as a marker for early diagnosis of this disease [17,19,20]. Moreover, the results of scientific works Lamichhane A. exhibit significantly ($P < 0.001$) increase MDA levels in patients with type 2 diabetes with acute form and the presence of complications [21,22].

The gene product PPARG - the main factor regulating differentiation of adipocytes [23], as it also promotes the expression of the protein, that transports fatty acids, increases the expression and activity of acetyl-CoA synthase, fosfatidilinozitol-3-kinase and increases the expression of adiponectin gene, glucose transporter (GLUT- 4), inhibits leptin gene expression, participates in the regulation of protein oxidative breathing, inhibits the expression in adipose tissue TNF-alpha, accompanied by a decrease in insulin resistance and an increase in β-cells insulin secretion [24]. PPARG gene polymorphism rs1801282 association of type 2 diabetes was confirmed in studies in Finnish, Swedish, British, French [25-27] and Russian populations [28-30].

Localization polymorphism rs1801282 gene PPARG - Chr.3: 12393125 on NCBI Build 37. Sikvens areas analyzed AACTCTGGGAGATTCTCCTATTGAC [C / G] CAGAAAGCGATTCCTTCACTGATAC, polymorphic codon CCA / GCA. This polymorphism is an easy-nucleotide substitution C to G, which leads to the replacement of the amino acid proline to alanine at position 12 in the protein gamma receptor that activates peroxisome proliferation (PPARG). Ancestral allele is C and allele G - minor. According to MAF Source: 1000 Genomes (http://www.1000genomes.org/node/506) last frequency is T = 0,0703 / 352.

Studies conducted in Chinese populations demonstrate that gene polymorphism Pro12Pro PPARG promotes oxidative stress, and patients with genotype Ala12Pro less prone to complications

of type 2 diabetes [31]. Also on cardiomyocyte studies demonstrated that cells overexpressing PPARG, more resistant to oxidative stress [32,33]. Today, the influence of polymorphisms rs1801282Pro12Ala PPARG gene on oxidative and antioxidant processes is not in doubt, as recent research Chia-Ter Chao demonstrate the relationship of genotype Pro12Ala and SOD activity in renal disease ($p < 0.028$) [34]. According to our previous studies, found an association 12Pro allele polymorphism rs1801282 Pro12Ala gene PPARG with the disease in type 2 diabetes [35]

Therefore, the main **purpose** of this study was to examine the influence of gene polymorphisms rs1801282 Pro12Ala PPARG and the intensification of LPO and antioxidant systems (AOS) in patients with type 2 diabetes.

Materials and Methods

In this study involved 88 patients with type 2 diabetes. LPO evaluation activity measured in terms of indicators of diene conjugates (DC) and malonic dealdehid (MDA). DC content of unsaturated fatty acids in plasma were determined by Z. Placer modifications in V.B. Gavrilov et al. (1983) [36]. MDA concentration was determined by its reaction with thiobarbytur acid and subsequent quantitative determination of colored product on a spectrophotometer «Specord» (of Germany in), the MDA level expressed in mmol/g protein [37]. The AOC status was evaluated in terms of the activity of superoxide dismutase (SOD), catalase and content of α-tocopherol (α-TF). Determination of α-TF was carried out by J. Biery modification R.SH. Kysylevych al. (1973), and SOD - by AP Makarevich et al. (1983). To determine the activity of catalase was used method for spectrophotometric measurement, MA Koroliuk al. (1998), catalase activity per unit of blood taken, mkkat / L.

With a view to providing genomic DNA were used reagents PureLink® Genomic DNA Kits For purification of genomic DNA, manufacturer INVITROGEN (USA). For the analysis of polymorphic DNA loci using a standardized test system TaqMan Mutation Detection Assays Life-Technology (USA).

Data analysis was performed by using the statistical package MedCalc v.15.11.0 (MedCalc Software bvba, 1993-2015 years).

Results and Discussion

As shown in Table 1, statistically significant influence of gene polymorphisms Pro12Ala PPARG on DC and MDA levels in patients with type 2 diabetes were not found ($p = 0.865$ and $p = 0.783$ respectively).

Table 1. Levels of DC and MDA depending on the genotype Pro12Ala gene PPARG in patients with type 2 diabetes (M ± m)

PPARG (Pro12Ala)	(Together) Cases	Men	Women	P**
DC U/ml				
Pro12Pro	3,39±0,102	3,58±1,170	3,28±0,127	0,478
Pro12Ala	3,35±0,397	3,82±0,639	3,08±0,510	0,300
P*	0,865	0,625	0,586	-
MDA mmol/g protein				
Pro12Pro	9,59±0,375	10,93±0,648	8,78±0,419	0,103
Pro12Ala	9,95±1,425	11,74±3,126	8,95±1,439	0,813
P*	0,783	0,685	0,878	-

Notes: * - comparison between groups at different polymorphisms;

** - comparison between women and men in the respective groups

Multiplicative model study found no significant effect 12Pro allele or 12Ala at the level of DC in patients with type 2 diabetes (tab.2), than increase this indicator among men with 12Pro allele, compared to women (p = 0,034). The level of MDA in men with 12Pro allele, was at 20,4% higher than in women (p = 0,001), while in patients with this allele 12Ala differences between gender were not found (p = 0,219). Analysis of MDA between carriers allele 12Pro and 12Ala found no significant difference (p=0,808).

Table 2. Distribution of MDA and DC levels depending on the availability allele 12Pro or 12Ala in patients with type 2 diabetes (M ± m)

PPARG (Pro12Ala)	(Together) Cases	Men	Women	P**
DC, U/ml				
12Pro	3,39±0,074	3,62±0,12	3,26±0,092	0,034
12Ala	3,29±0,37	3,61±0,56	3,08±0,51	0,321
P*	0,698	0,973	0,599	-
MDA, mmol/g protein				
12Pro	9,63±0,27	11,04±0,48	8,79±0,29	0,001
12Ala	9,87±1,33	11,24±2,6	8,95±1,44	0,219
P*	0,808	0,908	0,882	-

Notes: * - comparison between groups for the presence of allele gene PPARG;

** - Comparison between women and men in the respective groups

No statistically significant effect of polymorphism Pro12Ala on SOD activity in patients with type 2 diabetes were found (p = 0,734) (Table. 3). In patients with genotype Pro12Pro, catalase activity was lower by 21.7% than in the case Pro12Ala (F = 8,17; p = 0,005), and this difference was observed among men (F = 6,48; p = 0,016). The study revealed a significant reduction in α-TF in men with Pro12Pro polymorphism, compared to Pro12Ala, at 29,5 % (F = 4,93; p = 0,034) and among homozygotes, the figure was significantly lower at 37,5% in women, by using Student's criterion (p = 0,032).

Table 3. Average performance SOD, catalase and α-TF depending on the genotype Pro12Ala gene PPARG in patients with type 2 diabetes (M ± m)

PPARG (Pro12Ala)	(Together) Cases	Men	Women	P**
SOD, U/ml				
Pro12Pro	0,43±0,0211	0,44±0,031	0,42±0,028	0,063
Pro12Ala	0,41±0,062	0,46±0,130	0,38±0,068	0,737
P*	0,734	0,852	0,592	-
Catalase, mkkat/l				
Pro12Pro	22,12±0,746	22,94±1,218	21,61±0,948	0,393
Pro12Ala	28,24±2,988	31,91±5,083	26,18±3,731	0,117
P*	0,005	0,016	0,094	-
α-TF, mkmol/l				
Pro12Pro	8,28±0,367	8,67±0,613	8,06±0,459	0,326
Pro12Ala	9,33±1,068	12,29±1,803	7,68±1,016	0,032
P*	0,283	0,034	0,742	-

Notes: * - comparison between groups at different polymorphisms;

** - Comparison between women and men in the respective groups

No statistically significant effect of alleles in SOD activity and the level of α-TF were detected (Table. 4). Availability 12Pro allele resulted in a significant decrease in catalase activity in patients with type 2 diabetes (p = 0,013).

Published research results by Molodan V.I., showed no significant associative link between gene polymorphism Pro12Ala PPARG and intensification of lipid peroxidation in patients with hypertension (p>0,05)[38]. Our data indicate the presence of influence polymorphic marker Pro12Ala gene PPARG the processes of lipid peroxidation, which is increased intensification of lipid peroxidation in men suffering from type 2 diabetes, with a significant increase DC (p = 0,034) and MDA (p = 0,001) levels.

Proved significant decrease in activity of the enzyme catalase AOC level in patients with type 2 diabetes which have Pro12Pro polymorphism of PPARG gene by 21.7% compared with heterozygotes (F = 8,17; p = 0,005), and the analysis of multiplicative models, 12Pro allele carriers, also had significantly lower activity of catalase (p=0,013). Found significantly higher activity of AOC in the form of increasing the level of α-TF and catalase activity among men with a gene

polymorphism Pro12Ala, than carriers Pro12Pro genotype (p=0,016 and p=0,034 for α-TF and catalase respectively).

Table 4. Average performance SOD, catalase and α-TF depending on the availability allele 12Pro or 12Ala in patients with type 2 diabetes (M ± m)

PPARG (Pro12Ala)	(Together) Cases	Men	Women	P**
SOD, U/ml				
12Pro	0,43±0,015	0,45±0,022	0,42±0,02	0,209
12Ala	0,39±0,059	0,42±0,12	0,38±0,069	0,791
P*	0,514	0,673	0,605	-
Catalase, mkkat/l				
12Pro	22,66±0,56	23,73±0,94	22,02±0,69	0,224
12Ala	27,74±2,83	30,07±4,55	26,18±3,73	0,563
P*	0,013	0,057	0,108	-
α-TF mkmol/l				
12Pro	8,37±0,25	8,96±0,44	8,02±0,31	0,178
12Ala	9,32±0,99	11,79±1,56	7,68±1,015	0,132
P*	0,285	0,060	0,751	-

Notes: * - comparison between groups for the presence of allele gene PPARG;

** - Comparison between women and men in the respective groups

The mechanism of action of the gene product PPARG not entirely clear, but one can assume that the ligand-dependent activation of gene products of oxidation of unsaturated fatty acids, more pronounced in the case of polymorphism Pro12Ala, accompanied by the activation and proliferation of adipocytes peroxisomes. Sufficient peroxisomes provide adequate synthesis of catalase in response to the excessive formation of reactive oxygen species.

Conclusions

1. Availability 12Pro allele in the polymorphism Pro12Ala gene PPARG causes increasing intensification of lipid peroxidation in patients with type 2 diabetes male.

2. Proved significantly lower activity of catalase enzyme AOC level in patients with type 2 diabetes, in the case of Pro12Pro polymorphism gene PPARG, and in case of presence 12Pro allele in this genotype.

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