UDC 616.379-008.64:577.352.38:575.11

Polymorphism Pro_{12}→Ala of the peroxisome proliferator-activated receptors gamma gene effect on lipid peroxidation and antioxidant defense in patients with type 2 diabetes

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In this research, we discover effect of polymorphism rs1801282 of the PPARG gene on the formation oxidative stress in patients with type 2 diabetes, depending on the duration of the disease: till 5 years, 5-10 years and more than 10 years. Activity of lipid peroxidation was assess in terms of diene conjugates (DC) and malonic dealdehid (MDA) and antioxidant system status - the activity of superoxide dismutase (SOD), catalase and the level of α-tocopherol (α-TF). Molecular genetic studies conducted by the method of polymerase chain reaction (PCR) in real time. During disease 5-10 years, found increase levels of DC and MDA in patients with polymorphism Pro_{12}→Ala for 34.9 and 34.7%, in compared with Pro_{12}→Pro (P=0.01). Availability Pro_{12}→Pro stipulated reduction of catalase activity during disease 5-10 years at 75% (P=0.001), and for those, who are ill for more than 10 years at 2,04 times (P=0.01), which is not different from the reference level (F=1.19; P=0.600), but in the case Pro_{12}→Ala, this figure was in 2 times higher. The main conclusion is that the type Pro_{12}→Ala of polymorphism rs1801282 of the PPARG gene causes the development of oxidative stress in patients with type 2 diabetes with 5-10 years durations, and Pro_{12}→Pro - deficiency the enzyme catalase level of antioxidant system in patients with durations of disease more than 5 years.

Key words: polymorphism Pro_{12}→Ala of the PPARG gene; rs1801282; type 2 diabetes; oxidative stress.

INTRODUCTION

The incidence of diabetes mellitus (DM) worldwide was 9% in the adult population, including 90% of patients have type 2 diabetes. A similar situation exists as to Ukraine, where nowadays are more than 1.3 million patients with type 2 diabetes and control epidemiological studies argue, that the true prevalence of the disease at least are in three times higher [1].

Medical and social severity of type 2 diabetes is caused not only by prevalence of this disease, but also by the development of a large number of complications, primarily related to the impairment of endothelial microvasculature vessels, which is based on hyperglycemia and intensification of lipid peroxidation (LPO) [2]. In the pathogenesis of type 2 diabetes is activated by excessive formation of reactive oxygen species, leading to an intensification of LPO and oxidative stress, which takes a leading role in the development of complications [8-10]. Proved, that type 2 diabetes - a free radical pathology [3]. Intensification of LPO and oxidative stress development starts before the clinical manifestation of type 2 diabetes and during early years of the disease [4], but in patients with more than 10 years - is reduced. The degree of oxidative stress is closely associated with the weakening of enzymatic antioxidant system (AOS), disease duration and degree of decompensating of carbohydrate metabolism.

Gene PPARG is a major factor in the regulation of proliferation of adipocytes, as it increases the expression of the protein transporter of fatty acids, expression and activity of acetyl-CoA synthase, fosfatidilinozitol-3-kinase, gene expres-

sion adiponectin, glucose transporter (GLUT-4), inhibits expression leptin gene, is involved in the regulation of protein oxidative phosphorylation, inhibits the expression in adipose tissue tumor necrosis factor-alpha, which is accompanied by a decrease in insulin resistance and an increase in insulin secretion β-cells. Associative impact polymorphic marker rs1801282 gene PPARG on the development of type 2 diabetes was confirmed in studies at European and Russian populations [5]. According to our previous studies, we found an association 12Pro allele of polymorphism rs1801282 gene PPARG with type 2 diabetes [6].

Localization polymorphism rs1801282 of gene PPARG - Chr.3: 12393125 on NCBI Build 37. Sikvens areas analyzed AACTCTGGGAGATTCTCCTATTGAC [C/G] CAGAAAGCGATTCCTTCACTGATAC, polymorphic codon CCA / GCA. This polymorphism is a Single nucleotide polymorphism C to G, as a result, there is a replacement of the amino acid proline to alanine at position 12 in the protein gamma receptor, which 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.

Publication of results of studies on Chinese populations indicate, that the variant Pro_{12}→Pro of polymorphism rs1801282 of gene PPARG promotes oxidative stress, and patients with genotype Pro_{12}→Ala were less prone to complications of type 2 diabetes [7]. Although, studies on cardiomyocytes show that cells with overexpressing PPARG, more resistant to oxidative stress [8]. Today, the influence of polymorphisms rs1801282 of gene PPARG on oxidative processes and antioxidant is not in doubt. A recent study conducted by Chia-Ter Chao (2016) demonstrated Pro_{12}→Ala association with increasing SOD activity in renal disease ($P<0.028$) [9]. We also found the intensification of LPO in carriers of alleles 12Pro in polymorphism Pro_{12}→Ala of gene PPARG, whereas a significant decrease in activity of the enzyme catalase links AOC in patients with type 2 diabetes, was found as in the case of polymorphism Pro_{12}→Pro of gene PPARG, and as in the presence of the allele 12Pro in this genotype [10]. Thus, the main purpose of this study was to determine the stages of formation in terms of oxidative stress, by levels of LPO and AOC in patients with type 2 diabetes, depending on the duration of the disease.

METHODS

In this study involved 138 patients, of which 88 were patients with type 2 diabetes (experimental group) and 50 not suffered from this pathology (control group). Patients with type 2 diabetes were divide into three groups for disease duration: 5 years, 5-10 years and more than 10 years. The material for the study was the blood of patients. Blood sampling was perform of patients in the morning on an empty stomach. To assess the activity of LPO indices defined diene conjugates (DC) and malonic dealdehid (MDA). DC content of unsaturated fatty acids in plasma were determine by Z. Placer method, in VB Gavrilov et al. modifications. MDA concentration was determine by its reaction with tiobarbitur acid with further quantitative determination of colored product on a spectrophotometer «Specord» (Germany), the MDA level expressed in mmol/g protein. Status AOC was evaluate by the activity of superoxide dismutase (SOD), catalase and the level of α-tocopherol (α-TF). To determine the level of α-TF, was used method of J. Biery and SOD - with Makarevich's method. In determining, the activity of catalase was used method for spectrophotometric measurement, catalase activity per unit of blood taken, mkkat/l.

Bold genomic DNA was performe-using reagents PureLink® Genomic DNA Kits For purification of genomic DNA, manufacturer INVITROGEN (USA), and the analysis of polymorphic DNA loci using a standardized test system TaqMan Mutation Detection Assays Life-Technology (USA). Data analysis was

performe using the statistical package MedCalc v.15.11.0 (MedCalc Software bvba, 1993-2015 years) and MedStat.

RESULTS AND DISCUSSION

During disease 5-10 years, found increased levels of DC in patients with genotype Pro_{12}→Ala at 34.9%, in compared with homozygotes with allele 12Pro (P=0.01). When used univariate analysis of variance, revealed the likely decrease level of DC (P=0.029) between the groups of patients suffering from type 2 diabetes to 5 years, 5-10 years and more than 10 years wich have a gene polymorphism Pro_{12}→Pro , while time, both carriers of genotype Pro_{12}→Ala such a decrease was not found (P=0.204) (Table 1).

The level of MDA in patients suffered with type 2 diabetes for 5 years and 10 years, depending on the Pro_{12}→Ala polymorphism of gene PPARG was not statistically different. And when disease duration was 5-10 years, have seen a probable increase in the level of MDA by 34.7% in patients with genotype Pro_{12}→Ala, compared with native polymorphism Pro_{12}→Pro (P=0.01). The study revealed the likely reduction of MDA by 21.5% in patients with genotype Pro_{12}→Pro, which affected up to 5 years, 5-10 years and more than 10 years (P=0.048).

Analyzing the indicators of lipid peroxidation products (DC and MDA) in patients with type 2 diabetes for 5 years, 5-10 years and more than 10 years, depending on the genotype Pro_{12}→Ala of the gene PPARG see (Figure 1), in patients with polymorphisms Pro_{12}→Pro intensification of LPO more pronounced during the first 5 years of disease and decreases gradually in the course of the disease (DK P=0.029; MDA P=0.048). Patients with genotype Pro_{12}→Ala, front, intensification of LPO in the first 5 years and more than 10 years of disease, expressed to a lesser extent, as the MDA level significantly was not differed from the control group (P=0.635 and P=0.067 for the 5 and 10 years disease, respectively), while in 5-10 years has increased. The data is shown in Table 1 and Figure 1.

Fig.1. LPO activity (the level of DC and MDA) in patients with type 2 diabetes suffering to 5 years, 5-10 years and more than 10 years, depending on the Pro_{12}→Ala polymorphism of the PPARG gene

Based on the fact, that oxidative stress is - an imbalance between processes that are characterized by excessive formation of reactive oxygen and free radicals on the one hand, and AOS activity on the other, there is a need to investigate the performance of AOC.

Effect of polymorphism Pro_{12}→Ala of the PPARG gene on the AOC was studied by analyzing the activity of SOD, catalase and α-TF levels in patients with type 2 diabetes for 5 years, 5-10 years and more than 10 years, depending on the genotype of the gene, obtained data presented in table 2 and figures 2 and 3.

In patients with type 2 diabetes for 5 years, significant differences between the activity of SOD was not found (P=0.12). As shown in Table 2 and Figure 2, we found a probable decrease of

Table 1. Effect of polymorphism Pro12Ala of the PPARG gene indicators for DC and MDA depending on the duration of the disease in type 2 diabetes (M ± m)

Polymorphism Pro_{12}→Ala of the PPARG	Duration of the disease		
	To 5 years	5-10 years	More than 10 years
	DC, U/ml		
Pro_{12}→Pro	3.79±0.195	3.32±0.17	3.12±0.15**
Pro_{12}→Ala	2.93±0.36	4.48±1.015*	2.74±0.94
	MDA, mmol/g protein		
Pro_{12}→Pro	10.84±0.68	9.53±0.55	8.51±0.69**
Pro_{12}→Ala	8.39±0.79	14.59±4.17*	6.87±0.43

For table 1, 2 and figure 1: * - P<0.005 (comparison inside duration group between different polymorphisms);** - P<0.005 (comparison between groups of patients depending on the duration of the disease).

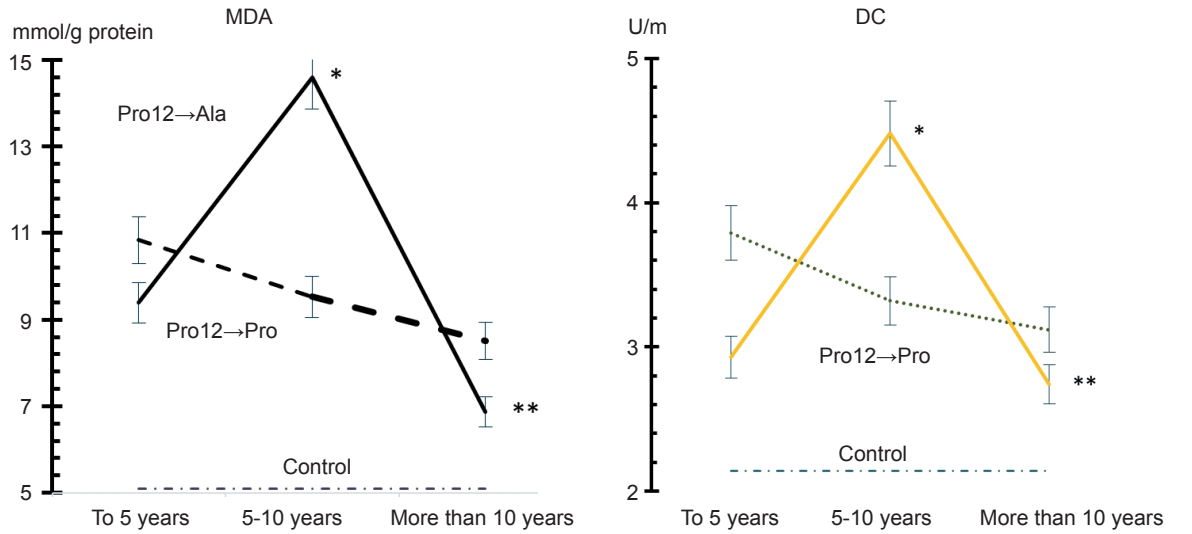

Fig.1. LPO activity (the level of DC and MDA) in patients with type 2 diabetes suffering to 5 years, 5-10 years and more than 10 years, depending on the Pro_{12}→Ala polymorphism of the PPARG gene

SOD activity between patients who had genotype Pro_{12}→Pro, with disease duration of 5 years, 5-10 years and more than 10 years (P=0.001), whereas patients with genotype Pro_{12}→Ala such difference was not found (P=0.462). It should also pay attention to the fact, that SOD activity in patients with genotype Pro_{12}→Ala, suffering from type 2 diabetes 5-10 years, was higher than in the first 5 years of the disease, though in patients, suffering over 10 years, this figure was in 2 times lower (P=0.04).

Thus, the importance of polymorphism rs1801282 of the PPARG gene is that in case of Pro_{12}→Pro SOD activity decreases uniformly from the first years of disease in type 2 diabetes (P=0.001), whereas the presence of genotype Pro_{12}→Ala observed significant (Student's criterion, bilateral critical region, significance at P=0.004) decrease in this level only after 10 years of disease.

Significant differences between the levels of catalase activity in patients suffering from diabetes for 5 years was not found (P=0.55). In Pro_{12}→Pro genotype carriers were found significant decrease in catalase activity between 5-10 years of disease by 75% (P=0.001), and those who are ill for more than 10 years at 2,04 times (P=0.01), than was not different from

Table 2. Effect of polymorphism Pro12→Ala of the PPARG gene on activity SOD, catalase and α-TF level, depending on the duration of the disease in type 2 diabetes (M ± m)

Polymorphism Pro_{12}→Ala of the PPARG	Duration of the disease		
	To 5 years	5-10 years	More than 10 years
	SOD, U/ml		
Pro_{12}→Pro	0.59±0.041	0.43±0.015	0.27±0.025**
Pro_{12}→Ala	0.41±0.103	0.49±0.029	0.24±0.015
	Catalase, mkkat/l		
Pro_{12}→Pro	27.56±1.42	23.44±0.46	15.57±0.59**
Pro_{12}→Ala	20.96±2.73	41.01±0.45*	31.78±7.17* **
	α-TF mkmol/l		
Pro_{12}→Pro	8.84±0.45	10.02±0.55	5.76±0.55**
Pro_{12}→Ala	9.03±0.82	12.42±2.58	4.32±0.8**

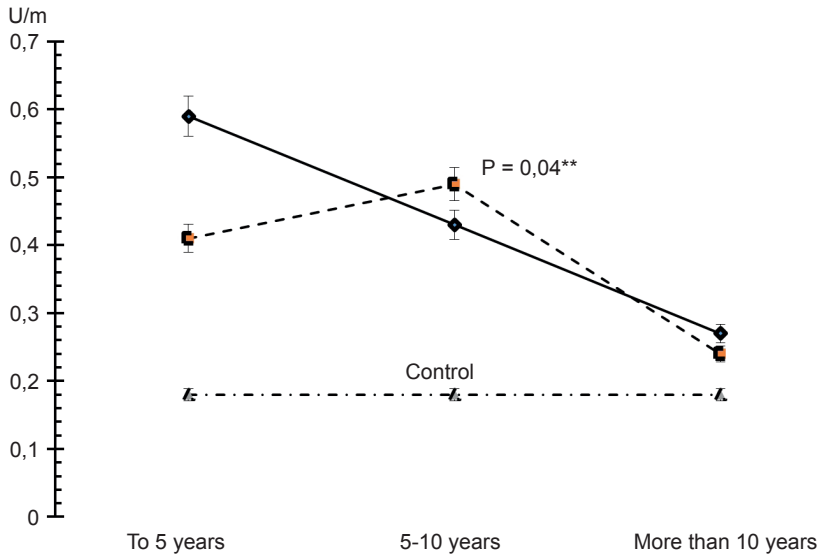

Fig.2. The level of SOD in patients with type 2 diabetes, which affected up to 5 years, 5-10 years and more than 10 years, depending on the polymorphism Pro_{12}→Ala of the PPARG gene. * - P=0.001 when compared with patients suffering from 5-10 and more than 10 years; ** - comparison inside duration 5-10 years group between different polymorphisms

the control (F=1.19; P=0.600). We proved significant decrease of catalase activity between groups of patients with type 2 diabetes suffering to 5 years, 5-10 years and more than 10 years for both cases Pro_{12}→Pro polymorphism (P=0.001) and for Pro_{12}→Ala (P=0.002) (Figure 3).

A similar distribution was obtained from the analysis of α-TF level between patients with different disease duration (Figure 3). It is necessary to point out, that the level of α-TF significantly reduced with the passage of the disease, that evidenced by used univariate analysis of variance between groups of patients with type 2 diabetes for 5 years, 5-10 years and more than 10 years, level of significance for genotype Pro_{12}→Pro was P=0.001, and for

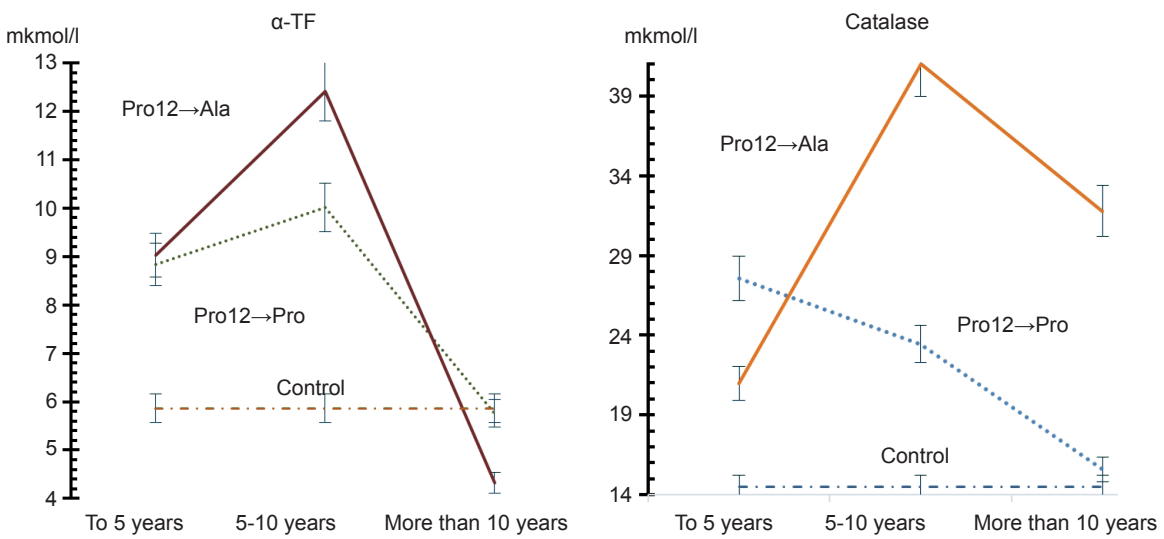

Fig.3. Catalase activity and the level of α-TF in patients with type 2 diabetes, which affected up to 5 years, 5-10 years and 10 years, depending on the polymorphism Pro_{12}→Ala of the PPARG gene. * - F=1.19; P=0.600 when compared with patients in the control group; ** - P=0.018 when compared with patients in the control group

Pro_{12}→Ala - P=0.046. During the 10 years of the disease in carriers of genotype Pro_{12}→Pro level of α-TF was no different from the control group (P=0.87) and in the case Pro_{12}→Ala - was statistically lower (F=1,19; P=0.018).

The highest intensification of LPO observed during disease in type 2 diabetes till 5 years, Pro_{12}→Pro genotype carriers with disease duration of 5-10 and over 10 years levels of LPO linear and significantly decreased, although compared with the control, remained at significantly high levels. In case of Pro_{12}→Ala found a significant increase of LPO intensification during disease 5-10 years, whereas after 10 years of disease indices of lipid peroxidation products significantly reduced. AOS activity was significantly higher in carriers of genotype Pro_{12}→Ala, suffering from type 2 diabetes 5-10 years, compared with Pro_{12}→Pro. In patients with type 2 diabetes over 10 years, the presence of polymorphisms Pro_{12}→Pro found catalase enzyme deficiency of AOC, against the background of a significantly high rates of lipid peroxidation, catalase activity significantly does not differ from the control level (F=1.19; P=0.600), instead in carriers of genotype Pro_{12}→Ala this level was 2 times higher.

Probably the ligand-dependent activation of gene PPARG2 more pronounced in the presence of polymorphisms Pro_{12}→Pro, as evidenced by the linear and the significantly reduction of oxidative stress (as indicators of lipid peroxidation, and both activities AOC) in the course of the disease, whereas carriers of genotype Pro_{12}→Ala, had significantly higher levels of oxidative stress during 5-10 years of disease, but after 10 years of disease - significant reduction, which may be due to the depletion of metabolic processes. The impact of polymorphisms rs1801282 of the PPARG gene for the enzyme catalase link of AOC is somewhat different, because in carriers of the genotype Pro_{12}→Ala, catalase activity is significantly higher.

CONCLUSIONS

1. Type Pro_{12}→Ala of polymorphism rs1801282 of the PPARG gene causes the development of oxidative stress in patients during disease in type 2 diabetes 5-10 years.

2. Polymorphism Pro_{12}→Pro of the PPARG gene causes natural deficiency of enzyme catalase level of antioxidant system in patients with type 2 diabetes, which affects more than 5 years.

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ВПЛИВ ПОЛІМОРФІЗМУ PRO_{12}→ALA ГЕНА PEROXISOME PROLIFERATOR-ACTIVATED RECEPTORS НА ПЕРЕКИСНЕ ОКИСНЕННЯ ЛІПІДІВ ТА АНТИОКСИДАНТНУ СИСТЕМУ У ХВОРИХ НА ЦУКРОВИЙ ДІАБЕТ 2-ГО ТИПУ

Досліджували ефект поліморфізму rs1801282 гена PPARG на формування окисного стресу у хворих на цукровий діабет 2-го типу залежно від тривалості захворювання: до 5 років, 5-10 років та понад 10 років. Активність перекисного окиснення ліпідів оцінювали за концентрацією у крові дієнових кон'югатів (ДК) і малонового діальдегіду (МДА), а стан антиоксидантної системи – за активністю супероксиддисмутази, каталази та вмістом α-токоферолу. Молекулярно-генетичне дослідження було проведене методом полімеразно-ланцюгової реакції в реальному часі. Під час тривалості хвороби 5-10 років виявлено збільшення вмісту ДК та МДА у хворих з поліморфізмом Pro_{12}→Ala на 34,9 і 34,7% відповідно порівняно з носіями Pro_{12}→Pro (P=0,01). Наявність поліморфізму Pro_{12}→Pro зумовлювало зниження активності каталази в перебіг захворювання 5-10 років на 75% (P=0,001), а у тих, хто хворіє більше ніж 10 років в 2,04 раза (P=0,01), що статистично не відрізняється від контрольного рівня (F=1,19; P=0,600), тоді як у разі Pro_{12}→Ala, ці показники були у двічі вищими. Отже, нами виявлено, що варіант Pro_{12}→Ala поліморфізму rs1801282 гена PPARG призводить до максимального розвитку окисного стресу у хворих на цукровий діабет 2-го типу з тривалістю захворювання 5-10 років, а наявність Pro_{12}→Pro зумовлює недостатність активності ферментативної каталазної ланки антиоксидантної системи у пацієнтів, що хворіють понад 5 років.

Ключові слова: поліморфізм Pro_{12}→Ala гена PPARG; rs1801282; цукровий діабет 2-го типу; окисний стрес.

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ВЛИЯНИЕ ПОЛИМОРФИЗМА PRO_{12}→ALA ГЕНА PEROXISOME PROLIFERATOR-ACTIVATED RECEPTORS НА ПЕРЕКИСНОЕ ОКИСЛЕНИЕ ЛИПИДОВ И АНТИОКСИДАНТНУЮ СИСТЕМУ У БОЛЬНЫХ САХАРНЫМ ДИАБЕТОМ 2-ГО ТИПА

Исследовали эффект полиморфизма rs1801282 гена PPARG на формирование окислительного стресса у больных сахарным диабетом 2-го типа в зависимости от длительности заболевания: до 5 лет, 5-10 лет и более 10 лет. Активность перекисного окисления липидов оценивали по концентрации в крови диеновых конъюгатов (ДК) и малонового диальдегида (МДА), а состояние антиоксидантной системы - по активности супероксиддисмутазы, каталазы и содержания α-токоферола. Молекулярно-генетическое исследование было проведено методом полимеразной цепной реакции в реальном времени. В течение срока болезни 5-10 лет выявлено увеличение содержания ДК и МДА у больных с полиморфизмом Pro_{12}→Ala на 34,9 и 34,7% соответственно в сравнении с носителями Pro_{12}→Pro (P=0,01). Наличие полиморфизма Pro_{12}→Pro приводило к снижению активности каталазы в период заболевания 5-10 лет на 75% (P=0,001), а в тех кто болеет более 10 лет в 2,04 раза (P=0,01), что статистически не отличается от контрольного уровня (F=1,19; P=0,600), тогда как в случае Pro_{12}→Ala, эти показатели были в два раза выше. Таким образом, нами выявлено, что вариант Pro_{12}→Ala-полиморфизма rs1801282 гена PPARG приводит к максимальному развитию окислительного стресса у больных сахарным диабетом 2-го типа с длительностью заболевания 5-10 лет, а наличие Pro_{12}→Pro обусловливает недостаточность активности ферментативного каталазного звена антиоксидантной системы у пациентов, страдающих более 5 лет.

Ключевые слова: полиморфизм Pro_{12}→Ala гена PPARG; rs1801282; сахарный диабет 2-го типа; окислительный стресс

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Received 23.09.2016