

THE POLYMORPHISM -308 G/A OF THE TNF GENE AND METABOLIC IMBALANCE IN PATIENTS WITH TYPE 2 DIABETES MELLITUS AND NON-ALCOHOLIC FATTY LIVER DISEASE, TAKING INTO ACCOUNT CARDIOVASCULAR COMPLICATIONS*

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Non-alcoholic fatty liver disease (NAFLD), the most common form of liver disease, is now recognized as a major public health problem worldwide [1-5].

NAFLD is a potentially serious liver disease that affects approximately one-quarter of the global adult population, causing a substantial burden of ill health with wide-ranging social and economic implications. It is a mul-

tisystem disease and is considered the hepatic component of metabolic syndrome [6]. The prevalence of liver disease has risen rapidly in Western countries, with a worldwide prevalence of 25%. NAFLD is becoming more common chronic liver disease in Western industrialized countries, particularly in patients with central obesity, type 2 diabetes mellitus (T2DM), dyslipidaemia, and metabolic syndrome [7].

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It should also be noted that insulin resistance (IR) — the main etiological factor in the development of non-alcoholic steatosis — can lead to the development of T2DM. In addition, obesity, as one of the cardiovascular risk factors, occurs in most cases in patients with NAFLD. Thus, patients with NAFLD have an increased risk of cardiovascular complications and mortality [8], which in turn provides grounds for considering NAFLD as an additional independent risk factor for cardiovascular diseases (CVD) [9-11]. It is known, that NAFLD can be a risk factor for the development of type 2 diabetes and CVD. It has been shown that NAFLD increases the risk of CVD regardless of other predictors and manifestations of metabolic syndrome (MS) [12, 13]. Undoubtedly, the liver plays the most important role in the development of atherogenic dyslipidemia, being at the same time a target organ. There are data in the literature that atherogenic dyslipidemia is found in 20–80% of patients with NAFLD [14].

One of the most productive modern technologies for genomic studies of T2DM and its complications is the analysis of associations of polymorphic markers of candidate genes with the development of the disease.

Tumor necrosis factor alpha (TNF- α), a member of the TNF/TNFR cytokine family, is an intercellular transmission molecule that has been reported in a wide range of human infections. TNF- α is one of the most important adipocytokines, the role of this molecule in the pathogenesis of NAFLD has not been identified and its relationship with genetic factors, such as single nucleotide polymorphism remains unknown [15].

DNA variations in the promoter region of the TNF- α gene can directly contribute to the

transcription of the TNF gene. The TNF- α gene is located within the highly polymorphic region of the head the major histocompatibility complex class III region on the short arm of chromosome 6p21.3 [16, 17]. The TNF- α gene is one of the largest polymorphic cytokine genes. The promoter-enhancer region contains 9 to 13 polymorphic sites of the SNPs type. However, the most significant for humans is the replacement of guanine for adenine in positions -308 and -238. Some polymorphisms in the promoter region of the TNF- α gene were identified. Positions -308 and -238 fall on the promoter, which is indicated by the possibility of transcription factors linking with the central part of the gene and, in this way, to add to the transcription rate [17]. Among them, a polymorphism at position -308 in the promoter region, which develops from guanine (G) to adenine (A) substitution, seems to be associated with an increase in the variability of TNF- α in vivo and in vitro [18, 19]. Given nucleotide changes — the appearance is wider, for example, among Europeans, close to 27–33% of their genotype have a polymorphic TNF α -308A allele and close to 7–10% — a rare TNF α -238A allele. The TNF α -308 A allele is the strongest transcription activator with a 6-7-fold shift in the induced transcription level of the TNF- α gene [16, 17].

Therefore, the **aim** of our work was to determine the circulating levels of TNF- α and the nature of its relationships with the components of insulin resistance, both metabolic and hormonal, in patients with type 2 diabetes; and establishing the nature of cardiovascular complications (taking into account TNF- α gene polymorphism) in the presence and absence of NAFLD.

MATERIAL AND METHODS

Case-control study included information about 50 practically healthy people from the city of Kharkiv and the region. The examined population (except control subjects) consisted exclusively of patients with type 2 diabetes mellitus with a long-term existence of the disease against the background of MS, with varying degrees of glycemic control and violations of liver homeostasis in the absence of re-

nal failure. 117 people were selected for analysis: 63 of them with type 2 diabetes in the presence of NAFLD and 54 patients with type 2 diabetes without NAFLD. The data were collected through a standard questionnaire. All patients were interviewed regarding a full medical history that included age, sex, occupation, duration of diabetes, mode and duration of treatment, presence of any associated

illness, surgical history, personal history of smoking/alcohol/drug abuse, dietary habit and family history of diabetes. All cases and controls signed an informed consent for clinical, biochemical and genetic studies and the protocols were approved by the institutional review board (IRB) of SI «V. Danilevsky Institute for Endocrine Pathology Problems of the NAMS of Ukraine». The cases were clinically and biochemically confirmed as type 2 diabetes. Diagnosis NAFLD were verified in accordance with the recommendations of the American Gastroenterological Association (AGA) and the American Association for the Study of liver disease (AASLD) based on the clinical course of the disease [20].

Genotyping of the T2DM patients was carried out by the method of polymerase chain reaction and restriction fragment length poly-

morphism using primers (TNF- α -308 G > A, rs 1800629: forward: gcaataggttttgaggccatg; reverse: gggacacacaagcatcaaggat) and *NcoI* endonuclease. Restriction products were analyzed by electrophoresis in a 2% agarose gel. pUC19 DNA hydrolyzed by *MspI* endonuclease was used as a molecular weight marker. Total TNF- α concentration, insulin resistance (HOMA-IR) and insulin sensibility (QUICKI), lipid profile were determined. Statistical analysis was performed using parametric and non-parametric methods. To compare the indices with normal distribution Student's t-test was used and for comparison variables with abnormal distribution Mann-Whitney's U-test was used. The data are presented as mean \pm SEM. All statistical tests were two tailed and a probability (p) value of 5% or less was considered statistically significant [21].

RESULTS AND THEIR DISCUSSION

The group of patients with type 2 diabetes differs from the control group by greater heterozygosity. The specific weight of heterozygous genotypes is one third higher ($p < 0.05$) than in the control group. When comparing the distribution of genotypes for the -308 G > A polymorphism of the TNF- α gene in patients with type 2 diabetes and practically healthy individuals, it was found that the percentage of heterozygotes in patients with type 2 diabetes is 1.53 times higher than among practically healthy individuals. While among the individuals of the control group, we did not detect homozygous carriers of the AA genotype (Table 1). The frequency of carriers of the A allele in patients with type 2 diabetes in total in

the homozygous (-308 AA TNF- α) and heterozygous (-308 GA TNF- α) variants was 24.79%, and in the population control group — 14.00% ($p < 0.05$).

According to the obtained data, the frequencies of genotypes among patients with type 2 diabetes differ from those of practically healthy individuals ($\chi^2 = 5.15$; $df = 1$; $p < 0.05$). The following fact draws attention: carriers of the A allele (AA + GA) in the group of patients are about a third (24.79%) more than in the control group (14.00%).

Therefore, the obtained data allow us to make an assumption about the presence of the -308G > A polymorphism of the TNF- α gene with the development of type 2 diabetes. This

Table 1

The ratio of genotypes of 308 G > A of the TNF- α gene in patients with type 2 diabetes and healthy people

SNP	Group	n	Genotype %			Statistics for allele frequencies (control – T2D)
			GG	AG	AA	
- 308 G > A TNF α	Control	50	86,00	14,00	0,00	$\chi^2 = 5,15$; $df = 1$; $\chi^2_{st} = 3,84$; $p < 0,05$
	Patients with T2D	118	75,42	21,18	3,39	

Notes:

T2D — type 2 diabetes;

n — the number of examinees;

χ^2 and χ^2_{st} — the actual and threshold value of the criterion;

df — number of degrees of freedom; p is the level of significance.

gave reason to assume that AA homozygosity and GA heterozygosity for the -308G > A polymorphism of the TNF- α gene is a factor of increased risk for type 2 diabetes.

Genotyping of 55 patients with type 2 diabetes without NAFLD and 63 patients with type 2 diabetes with NAFLD was analyzed for the -308G > A single nucleotide polymorphism of the TNF- α gene. In patients with type 2 diabetes with NAFLD, the frequencies of genotypes for the -308G > A polymorphism of the TNF- α gene were distributed as follows: 2 individuals were homozygous AA, 12 were homozygous AG, and 49 had the heterozygous genotype GG. Allele frequencies were also calculated, which are $p_G = 0.873$, $p_A = 0.127$. Statistically significant differences in the frequency of alleles between control groups and patients with type 2 diabetes in the presence of NAFLD were not detected ($p > 0.05$). In patients with type 2 diabetes in the absence of NAFLD, the frequencies of genotypes for the -308G > A polymorphism of the TNF- α gene were distributed as follows: 2 individuals were homozygous AA, 13 were homozygous AG, and 40 had the heterozygous genotype GG. There were also no statistically significant differences in the frequency of alleles between groups of patients with type 2 diabetes in the presence and absence of NAFLD ($p_G = 0.861$, $p_A = 0.157$) ($p > 0.05$).

The frequencies calculated by us for the polymorphism -308 G > A of the TNF- α gene are consistent with literature data. For example, the frequency of the A allele for African women in the study by Yael T. Joffe et al. (2010) [22] was 14 and 20% (for groups with normal weight and obesity, respectively). The authors of this work indicate that the frequencies obtained by polymorphism -308 G > A of the TNF α gene do not differ from those reported for Caucasians (13–23%) [23], African Americans (14.4%). The data of these researchers correspond to the results obtained by us for patients with type 2 diabetes. In the general studied population of Iranian Azeri Turkish patients, genotypes according to the polymorphism -308 G > A of the TNF- α gene were distributed as follows: 73.1% 308 GG, 24.8% 308 GA and 2.1% 308 AA. The frequencies of the 308G and 308A alleles were 85.4% and 14.6%, re-

spectively, and they were in Hardy–Weinberg equilibrium [25]. Bhushan B. et al. (2009) indicate in their work that the frequency of allele A according to polymorphism -308 G > A of the TNF- α gene in Asian Indians with obesity was 12.6% [26], which also coincides with our data.

When comparing the frequency distribution of genotypes in our study, a rather interesting fact was revealed. It is noteworthy that among patients with type 2 diabetes (excluding liver damage) and among patients with type 2 diabetes in the absence of NAFLD, carriers of the rare A allele are 2 times more common than among practically healthy individuals ($p < 0.05$). At the same time, when comparing the frequencies of genotypes among control subjects and patients with type 2 diabetes in the presence of NAFLD, the frequency of carriers of the A allele is 1.5 times higher than in controls, but the statistical significance of this difference is leveled ($p > 0.05$).

From the molecular genetic point of view, T2D is being studied quite actively both abroad and also in Ukraine [27]. However, the results of studies on the relationship of candidate tumor necrosis factor genes and their receptors with the development of T2D are ambiguous in different populations. For example, it has been shown that the A allele *rs1800629* TNF- α is a risk factor for the development of T2D in Iranian (OR = 2.34) [28], Indian (OR = 3.21) [29] and Brazilian (OR = 1.82) [30] populations. Among the population of Croatia and Hungary, these associations were not found [31], and in the population of Mexico it was found that the GG *rs1800629* TNF- α genotype (OR = 3.64) is a risk factor for the development of T2D (OR = 3.64) [32]. Given the pilot nature of our study, a preliminary estimate of the odds ratio was made (Table 2.).

Our primary data showed that carrying the A allele in homo- or heterozygous states (AA + GA) increases the risk of type 2 diabetes by approximately two times (OR = 1.97, 95% CI 0.96–4.03), and in the absence in the genotype of this allele (GG), the risk is approximately halved (OR = 0.50, 95% CI 0.25–1.04) compared to the average value for the population as a whole. However, the significance of this odds ratio was not statistically proven. Despite of the fact that the statistical significance of the

Table 2

Odds ratio (OR) of to have a type 2 diabetes with different genotypes		
Genotype	OR	95% CI _{OR}
AA + AG	1,97	0,96–4,03
GG	0,50	0,25–1,04

Notes:

OR — odds ratio;

CI_{OR} — odds ratio confidence interval.

above odds ratios was not proven, the obtained data suggest that the studied tumor necrosis factor gene polymorphism is more associated with the risk of developing type 2 diabetes mellitus. Perhaps, the reason is that the limited number of samples did not allow to achieve the minimum necessary power of the criterion, and this justifies the feasibility of further research under the conditions of increasing the sample.

Our data showed that carrying the A allele in homo- or heterozygous states (AA + GA) in patients without NAFLD increases the risk of diabetes (OR = 2.35, 95% CI 1.15–4.79), and the absence of this allele (GG) in the genotype decreases the risk (OR = 0.43, 95% CI 0.21–0.87), $p < 0.05$.

Compared to controls, patients with type 2 diabetes with impaired glucose homeostasis were characterized by a marked increase ($p < 0.001$) in body mass index (BMI). In patients with type 2 diabetes, there was also a significant ($p < 0.01$) increase in the levels of FFA, TG, TNF- α , HOMA-IR indices, fasting insulin and a decrease in insulin sensitivity (QUICKI). It was found that the average level of TNF- α in patients with type 2 diabetes is 3 times higher than in the control group ($p < 0.01$). A positive correlation was found between TNF- α and the level of TG ($r = 0.33$, $p < 0.05$), which may indicate the contribution of TNF- α to the development of nonalcoholic steatohepatitis. It is possible that in NAFLD it is TNF- α , activating intracellular signaling molecules, that forms the resistance of hepatocytes to the action of insulin. In patients with a combined course of NAFLD with type 2 diabetes, an additional indicator of the progression of metabolic changes is hyperproduction of TNF- α .

The stratification of the diabetic population in the presence and absence of NAFLD proved a more pronounced increase in the circulating

levels of TNF- α in the presence of NAFLD ($p < 0.05$), which substantiates the feasibility of using this indicator for further use as a diagnostic parameter of the aforementioned complication. It was established that the level of TNF- α in this category of patients with NAFLD increased significantly with increasing body weight. Thus, in the group with increased BMI (26–29 kg/m²) its level was (17.98 \pm 5.34) pg/ml, and in the group with BMI \geq 30 (33.58 \pm 5.90) pg/ml, in the control group (5.47 \pm 3.44) pg/ml ($p < 0.05$). It is known that with the development of obesity, there is not only an increase in the size and number of adipocytes (hypertrophic/hyperplastic expansion of adipose tissue), but also a change in their functional activity, which contributes to the development of clinical and metabolic changes associated with obesity. Correlation between BMI and TNF- α ($r = 0.18$; $p = 0.06$) may indicate the mutually potentiating role of these factors in the progression of NAFLD, which deepens under conditions of increased body weight. Thus, the search for ways of pathogenetic correction of the mechanisms of progression of NAFLD is very urgent and requires improvement of treatment schemes for fatty liver dystrophy in patients with type 2 diabetes.

The increase in expression of IR in patients with type 2 diabetes complicated by NAFLD is accompanied by an increase in the activity of the pro-inflammatory chain of cytokines represented by TNF- α , which can be considered as a trigger of complications. Despite of a fairly large number of works studying the mechanisms of NAFLD development, there is no clear understanding of the dependence of pro-inflammatory cytokines on the stage of development of NAFLD and the activity of the pathological process. Thus, studying the significance of TNF- α in the pathogenesis and progression of NAFLD is of undeniable interest.

When comparing the groups examined in the presence and absence of NAFLD, we found statistically significant differences in indicators of body weight, BMI, waist-to-hip ratio. Statistically significant differences in the distribution of patients with and without NAFLD according to the degree of obesity were revealed ($p < 0.001$). The obtained data are consistent with the results of studies showing that the prevalence of NAFLD increases with increasing BMI, and a high degree of obesity increases the risk of developing this disease [33].

NAFLD is common in patients with hypertension but controversy exists as to whether screening for this condition should be performed [34]. There is an opinion that patients with NAFLD have higher systolic and diastolic blood pressure [35] but in our work we did not find a statistically significant difference between the groups with and without NAFLD (systolic pressure (142.20 ± 3.84) mm Hg, diastolic pressure (92.30 ± 3.24) mm Hg for NAFLD vs. systolic pressure (142.86 ± 1.86) mm Hg, diastolic pressure (87.17 ± 2.86) mm Hg without NAFLD, $p > 0.05$).

NAFLD is associated with an increased risk of cardiovascular diseases [36]. In our study, the presence of arterial hypertension (AH) was found in more than half of obese patients. AH was also significantly more common in the group of patients with NAFLD (76.56% versus 64.71% in the group without NAFLD), however, the statistical significance of this difference was not proven, perhaps due to the small size of the sample. Several studies published in recent years have documented a high risk of coronary heart disease (CHD) in patients with NAFLD [37, 38].

In our study, 67.37% of patients with type 2 diabetes were diagnosed with CAD. It turned out that CAD was observed in 63.2% of patients with NAFLD and in 68.6% of patients who did not have concomitant liver pathology ($p > 0.05$).

In our study, we determined the level of high-SRP, which is a protein of the acute phase of the inflammatory process and is synthesized mainly in the liver. Among patients with type 2 diabetes, its level was 4.95 ± 0.75 mg/L. In the group of patients with type 2 diabetes, a significant correlation was shown between high-SRP

and TG level ($r = 0.29$; $p < 0.047$), glycemia ($r = 0.29$; $p < 0.01$), IRI ($r = 0.39$; $p < 0.005$), HOMA-IR ($r = 0.41$; $p < 0.01$), and FFA ($r = 0.28$; $p < 0.05$). Our results emphasize the large number of adverse factors and the potentiation of their effects in patients with type 2 diabetes, namely, the determining role of IR and associated hormonal and metabolic shifts. Among patients with type 2 diabetes with NAFLD, the level of highly sensitive C-reactive protein was higher than in patients with type 2 diabetes without NAFLD: (5.97 ± 0.89) versus (4.17 ± 0.86) mg/l, $p < 0.05$. The odds ratio indicator (OR = 6.19; 95% CI 2.47–16.53; $p < 0.05$) confirms that a high level of highly sensitive C-reactive protein is a predictor of the risk of developing NAFLD. In the group of patients with type 2 diabetes in the presence of NAFLD, a significant correlation relationship was shown between highly sensitive C-reactive protein and waist-to-hip ratio ($r = 0.62$; $p < 0.008$) and glucose ($r = 0.43$; $p < 0.01$). It should be noted, that the obtained results do not exclude the pathogenetic contribution of low-intensity chronic inflammation to the formation of atherogenic cardiovascular disorders, especially their early stages. The obtained data indicate that NAFLD makes a significant contribution to the increase in cardiovascular risk. For the purpose of early identification and correction of CVD risk factors, a comprehensive examination of patients with obesity and type 2 diabetes is necessary.

The distribution of sick individuals by polymorphism -308 G > A of the TNF- α gene in the presence and absence of NAFLD, taking into account cardiovascular complications, is presented in Tables 3 and Table 4. Our data shown that carrying the A allele in homo- or heterozygous states (AA + GA) in patients with NAFLD decreases the risk of CHD, and decreases the risk of retinopathy, *vice versa* the presence of GG genotype increases the risk of CHD more than four times (OR = 4.74, 95% CI 1.31–17.09), $p < 0.05$, and this genotype increases the risk of retinopathy more than five times (OR = 5.58, 95% CI 1.08–28.60), $p < 0.05$ in patients with damaged liver (Table 5).

In group of patients without injured liver there are no significant risks associated with any allele or genotypes of TNF- α gene (Table 6).

Table 3

**Results of genotyping by polymorphism – 308 G > A
of the TNF- α gene in patients with type 2 diabetes complicated
by non-alcoholic fatty liver disease, in the presence
and absence of macro-/microvascular complications**

Complication	A-308G TNF- α	
	AA + AG (n = 15)	GG (n = 49)
Neuropathy –	5	7
Neuropathy +	10	42
Retinopathy –	4	3
Retinopathy +	11	46
Hypertension –	4	9
Hypertension +	9	40
Coronary heart disease –	11	18
Coronary heart disease +	4	31
Coronary heart disease + Hypertension	2	29

Table 4

**Results of genotyping by polymorphism – 308 G > A
of the TNF- α gene in patients with type 2 diabetes mellitus,
not burdened by non-alcoholic fatty liver disease, in the presence
and absence of macro-/microvascular complications**

Complication	A-308G TNF- α	
	AA + AG (n = 9)	GG (n = 42)
Neuropathy –	1	20
Neuropathy +	8	22
Retinopathy –	3	13
Retinopathy +	6	29
Hypertension –	1	17
Hypertension +	8	25
Coronary heart disease –	3	22
Coronary heart disease +	6	20
Coronary heart disease + Hypertension	6	18

These facts confirmed that NAFLD increases the risk of cardiovascular complications. Patients with NAFLD had increased odds of coronary heart disease and Retinopathy compared to patients without NAFLD. Cardiovascular disease and NAFLD share many risk factors, an individual with NAFLD is estimated to have a 57 to 69% increased risk of cardiovascular disease, independent of known common risk factors [39]. A growing body of evidence also indicates that NAFLD is strongly associated with an increased risk of major CVD events and other cardiac complications (i.e., cardiomyopathy, cardiac valvular calcification and

cardiac arrhythmias), independently of traditional cardiovascular risk factors. Importantly, liver inflammation is accompanied by hepatic accumulation of inflammatory leukocytes and increased hepatic and extrahepatic cytokine production [40].

When comparing the distribution of genotypes for the -308 G > A polymorphism of the TNF- α gene in patients with type 2 diabetes with neuropathy and practically healthy individuals, it was found that the percentage of heterozygotes in patients with type 2 diabetes with neuropathy is 1.43 times higher than among practically healthy individuals (among

Table 5

Odds ratio (OR) of genotypes of -308 G>A of the TNF- α gene (AA + AG vs GG) in patients with different genotypes with type 2 diabetes mellitus complicated by non-alcoholic fatty liver disease, in the presence and absence of macro-/microvascular complications (with allele A as a risk factor)

Complication	OR	S _(OR)	CI _(OR)	P
Neuropathy	0.33	0.68	0.087–1.27	> 0,05
Retinopathy	0.18	0.83	0.04–0.920	< 0,05
Hypertension	0.51	0.71	0.12–2.01	> 0,05
Coronary heart disease	0.21	0.65	0.06–0.76	< 0,05
Coronary heart disease + Hypertension	0.53	0.90	0.09–3.14	> 0,05

Notes:

OR — odds ratio;

S_(OR) — standard error of odds ratio;CI_(OR) — odds ratio confidence interval;

p — is the level of significance.

Table 6

Odds ratio (OR) of genotypes of -308 G > A of the TNF- α gene (AA + AG vs GG) in patients with different genotypes with type 2 diabetes mellitus, not burdened by non-alcoholic fatty liver disease, in the presence and absence of macro-/microvascular complications (with allele A as a risk factor)

Complication	OR	S _(OR)	CI _(OR)	p
Neuropathy	7.273	1.105	0.83–63.39	> 0,05
Retinopathy	0.897	0.782	0.194–4.151	> 0,05
Hypertension	5.440	1.106	0.622–47.607	> 0,05
Coronary hearth disease	2.200	0.772	0.485–9.983	> 0,05
Coronary heart disease + Hypertension	1.111	0.662	0.303–4.071	> 0,05

Notes:

OR — odds ratio;

S_(OR) — standard error of odds ratio;CI_(OR) — odds ratio confidence interval;

p — is the level of significance.

the individuals of the control group, we did not detect homozygous carriers of the AA genotype). The frequency of carriers of the A allele in patients with type 2 diabetes mellitus with neuropathy in total in the homozygous (-308 AA TNF- α) and heterozygous (-308 GA TNF- α) variants was 26.66%, and in the population control group — 14.00% ($p < 0.05$).

As a result of genotyping by polymorphism -308 G > A of the TNF- α gene in patients with type 2 diabetes in the presence and absence of NAFLD, taking into account micro-/macrovascular complications, no statistically significant differences in the frequency of alleles between groups of patients were found ($p > 0.05$). Our

study did not prove the contribution of the genetic component to the formation of cardiovascular complications due to SNP -308 G > A of the TNF- α gene in patients with type 2 diabetes in the presence and absence of NAFLD. Perhaps the power of the selected criterion did not allow us to achieve a statistically significant difference under the conditions of the available sample size. Studies examining the relationship between TNF- α polymorphisms and coronary artery disease risk have yielded conflicting results [41, 42]. In some works, variants of TNF- α were indeed associated with the development, progression [42], while, for example,

such an association was absent in of the Chinese population [43].

The results obtained by us regarding SNP -308 G > A of the TNF- α gene and their comparison with the data of other clinical studies prove the specific importance of the studied population.

A large number of polymorphisms have a statistically significant effect on cardiovascular risk at the population level. However, due to

the polygenic and multifactorial determination of most cardiovascular factors, the contribution of a single polymorphism is most likely moderate. Genetic testing can identify variants associated with increased individual cardiovascular risk, coronary atherosclerosis risk, or stroke. Recently, commercial genetic testing is available to determine individual risk, but the clinical effectiveness of such an examination has not been proven [44].

CONCLUSIONS

Non-alcoholic fatty liver disease is closely related to hormonal and metabolic risk factors and markers of cardiovascular disease and type 2 diabetes and may increase the risk of developing and progressing cardiovascular complications.

- Patients with type 2 diabetes with obesity and NAFLD are characterized by an increase in the blood content of the pro-inflammatory cytokine TNF- α , a marker of inflammation of the vasculature, which indicates the chronicity of systemic subacute inflammation in this category of patients.
- Patients with type 2 diabetes with excess body weight and an insulin-resistant pattern of hormonal and metabolic disorders were diagnosed with pronounced hyper-TNF- α -nemia due to the modulating effect of non-alcoholic fatty liver disease.
- The contribution of the genetic component to the formation of the predisposition to the development of type 2 diabetes mellitus based on the single-nucleotide polymorphism -308 G > A of the TNF α gene was determined, which makes it possible to consider the carrier of the A allele as a factor of increased risk for the development of type 2 diabetes mellitus.
- No association of the studied polymorphism with the risk of developing NAFLD was found. The obtained data make it possible to assume that the studied polymorphism -308 G > A of the TNF α gene is more associated with the risk of deve-

loping type 2 diabetes, and the occurrence or progression of NAFLD primarily depends on metabolic imbalance, and not on the contribution of the studied polymorphism.

- The absence of reliable differences in the concentration of circulating TNF- α under the conditions of macro-/microvascular complications due to NAFLD was proven, which proves the leading effect of insulin resistance and dysglycemia on the above-mentioned parameters of the chronic inflammatory process in patients with type 2 diabetes complicated by NAFLD. The conducted study suggests the presence of additional factors that modulate the general level of the pro-inflammatory cytokine TNF- α in the circulation of patients with type 2 diabetes, burdened by NAFLD, against the background of cardiovascular complications.
- In our study, the contribution of the genetic component to the formation of cardiovascular complications by single nucleotide polymorphism -308 G > A of the TNF- α gene in patients with type 2 diabetes in the presence and absence of NAFLD was not revealed.
- Our study confirmed that NAFLD increases the risk of cardiovascular complications. The presence of GG genotype increases the risk of CHD more than four times, and this genotype increases the risk of retinopathy more than five times in patients with damaged liver.

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**THE POLYMORPHISM -308 G/A OF THE TNF GENE
AND METABOLIC IMBALANCE IN PATIENTS WITH TYPE 2 DIABETES MELLITUS
AND NON-ALCOHOLIC FATTY LIVER DISEASE,
TAKING INTO ACCOUNT CARDIOVASCULAR COMPLICATIONS**

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Non-alcoholic fatty liver disease (NAFLD), the most common form of liver disease, is now recognized as a major public health problem worldwide. Tumor necrosis factor alpha (TNF- α), a member of the TNF/TNFR cytokine family, is an intercellular transmission molecule that has been reported in a wide range of human noninfection diseases. The **aim** of the study was to determine the circulating levels of TNF- α and the nature of its relationships with the components of insulin resistance, both metabolic and hormonal, in patients with type 2 diabetes; and establishing the nature of cardiovascular complications (taking into account TNF- α gene polymorphism) in the presence and absence of NAFLD.

Materials and methods. Case-control study included information about 50 practically healthy people from the city of Kharkiv and the region. The examined population (except control subjects) consisted exclusively of patients with type 2 diabetes mellitus with a long-term existence of the disease against the background of metabolic syndrome, with varying degrees of glycemic control and violations of liver homeostasis in the absence of renal failure. 117 people were selected for analysis: 63 of them with type 2 diabetes in the presence of NAFLD and 54 patients with type 2 diabetes without NAFLD. Genotyping for single nucleotide polymorphism -308 G > A of the TNF- α was performed by the method of polymerase chain reaction with appropriate primers and *NcoI* endonuclease. Testing of statistical hypotheses was carried out using the odds ratio and χ^2 criteria at the significance level of $P \leq 0.05$.

Results. The contribution of the genetic component to the formation of the predisposition to the development of type 2 diabetes mellitus based on the single-nucleotide polymorphism -308 G > A of the TNF α gene was determined, which makes it possible to consider the carrier of the A allele as a factor of increased risk for the development of type 2 diabetes mellitus.

No association of the studied polymorphism with the risk of developing NAFLD was found. The obtained data make it possible to assume that the studied polymorphism -308 G > A of the TNF α gene is more associated with the risk of developing type 2 diabetes, and the occurrence or progression of NAFLD primarily depends on metabolic imbalance, and not on the contribution of the studied polymorphism.

Conclusions. Non-alcoholic fatty liver disease is closely related to hormonal and metabolic risk factors and markers of cardiovascular disease and type 2 diabetes and may increase the risk of developing and progressing cardiovascular complications. The contribution of the genetic component to the formation of the predisposition to the development of type 2 diabetes mellitus based on the single-nucleotide polymorphism -308 G > A of the TNF α gene was determined, which makes it possible to consider the carrier of the A allele as a factor of increased risk for the development of type 2 diabetes mellitus.

Keywords: type 2 diabetes mellitus, TNF- α , single nucleotide polymorphism, non-alcoholic fatty liver disease, cardiovascular complications.

**ПОЛІМОРФІЗМ -308 G > A ГЕНА TNF I МЕТАБОЛІЧНИЙ ДИСБАЛАНС
У ХВОРИХ НА ЦУКРОВИЙ ДІАБЕТ 2 ТИПУ ТА НЕАЛКОГОЛЬНУ ЖИРОВУ ХВОРОБУ
ПЕЧІНКИ З УРАХУВАННЯМ СЕРЦЕВО-СУДИННИХ УСКЛАДНЕНЬ**

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Неалкогольна жирова хвороба печінки (НАЖХП), найбільш розповсюджена форма захворювань печінки, на тепер визнана головною проблемою у сфері охорони здоров'я у світі. Фактор некрозу пухлин ФНП- α представник родини цитокінів TNF/TNFR — являє собою молекулу міжклітинної передачі, що задіяна в широкому спектрі захворювань, в тому числі неінфекційних.

Метою дослідження було визначення рівня циркулюючого фактору некрозу пухлин α (ФНП- α) та характеру його взаємодії із складовими метаболічного синдрому: інсулінорезистентності, як метаболічними, так і гормональними, у хворих на цукровий діабет (ЦД) 2 типу; та встановлення характеру серцево-судинних ускладнень (з урахуванням поліморфізму гена ФНП- α) за наявності та відсутності НАЖХП.

Матеріали та методи. До контрольної групи увійшли 50 практично здорових осіб з м. Харкова та області. Обстежену групу (окрім контрольної) склали виключно хворі на цукровий діабет 2 типу з тривалим перебігом захворювання на тлі метаболічного синдрому, з різним ступенем глікемічного контролю та порушеннями гомеостазу печінки за відсутності ниркової недостатності. Для аналізу було відібрано 117 осіб: 63 з них з ЦД 2 типу за наявності НАЖХП та 54 пацієнти з ЦД 2 типу без НАЖХП. Генотипування однонуклеотидного поліморфізму 308 G > A ФНП- α проводили методом полімеразної ланцюгової реакції з відповідними праймерами та ендонуклеазою *NcoI*. Перевірку статистичних гіпотез проводили за допомогою відношення шансів та критерія χ^2 за рівня значущості $P \leq 0,05$.

Результати. Визначено внесок генетичної складової у формування схильності до розвитку цукрового діабету 2 типу на основі однонуклеотидного поліморфізму — 308 G > A гена ФНП- α , що дає змогу вважати алель А фактором підвищеного ризику розвитку цукрового діабету 2 типу. Не виявлено зв'язку досліджуваного поліморфізму з ризиком розвитку НАЖХП. Отримані дані дають змогу припустити, що досліджуваний поліморфізм — 308 G > A гена ФНП- α більшою мірою асоціюється з ризиком розвитку ЦД 2 типу, а виникнення чи прогресування НАЖХП в першу чергу залежить від метаболічного дисбалансу, а не від впливу досліджуваного поліморфізму.

Висновки. Неалкогольна жирова хвороба печінки тісно пов'язана з гормональними та метаболічними факторами ризику, маркерами серцево-судинних захворювань і цукровим діабетом 2 типу та може підвищити ризик розвитку та прогресування серцево-судинних ускладнень. Визначено внесок генетичної складової у формування схильності до розвитку цукрового діабету 2 типу на основі однонуклеотидного поліморфізму -308 G > A гена ФНП- α .

Ключові слова: цукровий діабет 2 типу, фактор некрозу пухлин- α , однонуклеотидний поліморфізм, неалкогольна жирова хвороба печінки, серцево-судинні ускладнення.