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Allelic Polymorphism of Cytokine Genes in the Patients with Acute Myocardial Infarction with Elevated ST-Segment.

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ABSTRACT

The present investigation was performed to determine the risk of morbidity in Acute Myocardial Infarction with elevated ST-segment (AMI-ST). AMI-ST was diagnosed in accordance with the classification of the European Society of Cardiology (ESC). Single nucleotide polymorphisms of the promoter region of SNP-gene of TNF-α (G-308A), IL-10 (G-1082A) and IL-6 (G-174C) were studied. The polymorphism of cytokine genes was examined using restriction analyses of the amplified specific areas of genome of all study participants. AMI-ST study group consisted of 59 men and 22 women of Kazakh ethnicity (72.8% and 27.2%, respectively). This group was divided into two groups by complications in the course of AMI. Thus, the AMI-ST group A consisted of 49 patients without any complication, whether during or post hospitalization period. The AMI-ST group B consisted of 32 patients with complications (60.5% and 39.5%, respectively). One hundred healthy blood donors of Kazakh ethnicity served the Control study group. A minor type A/A homozygous genotype detected in the G-308A position of the TNFα gene promoter region was associated with complicated AMI-ST (OR = 6,89; 95%, $p<0.05$). Also, a minor type homozygous genotype C/C detected in the G-174C position of the IL-6 gene promoter region was associated with complicated AMI-ST (OR = 4,60; 95%, $p <0.05$). No significant difference was found in the allele frequency and genotypes of G-1082A polymorphism of IL-10 gene of AMI-ST groups. Additional studies with a significantly increased sample size are necessary, to investigate whether polymorphisms of cytokine genes could be considered a morbidity genetic risk factor in the Kazakh ethnicity patients with AMI-ST (East Kazakhstan region of the Republic of Kazakhstan).

Keywords: myocardial infarction, cytokines, cytokine gene polymorphisms.

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INTRODUCTION

According to the World Health Organization (WHO), cardiovascular diseases (CVD) are the leading cause of death worldwide. [1] Each year, about 17.5 million people die from CVD worldwide, which is about 30% of all deaths. More than 7 million people of them die from coronary heart disease (CHD), which represents 12.8% of all deaths [1]. Particularly, CHD the main manifestation of which is myocardial infarction (MI) is the main causes of death among CVD [2,3]. The acute MI (AMI) with ST-segment elevation (AMI-ST) dramatically increases the severity of CHD. Therefore, cardiologists worldwide are searching for the new opportunities for primary and secondary AMI-ST prevention [4].

Genetic studies determining the disease susceptibility for AMI and its complications is one of the prevention approaches. The genetic analyses revealed a number of susceptible genes and single nucleotide polymorphisms (SNPs) that predispose to the development of acute CHD [5]. The findings of the recent studies show an important role of cytokine network in the initiation and progression of atherosclerosis and CHD. However, only a limited number of studies investigated the impact of polymorphism of the cytokine genes in the development of MI. Yet, the results of the genetic testing taken into account with the known risk factors can significantly improve the predictability of the development and progression of MI.

Mostly, the pro-inflammatory interleukins tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) are considered to be the cytokines of importance. TNF-α is a major inflammatory cytokine, produced by cardiomyocytes, myocardial macrophages and lymphocytes, which has significant biological activity [6]. A disorder of TNF-α metabolism plays a certain role in the development of cardiovascular disorders [7,8].

The genes interleukins have an extremely high degree of polymorphism. The number of sections of this polymorphism in a single gene may reach a several dozen. Regions of this polymorphism may be located in the coding exons, introns, and which is particularly important in the promoter regulatory areas of the gene structure. For the present study, the genes of TNF-α, pro-inflammatory cytokine IL-6 and anti-inflammatory cytokine interleukin 10 (IL-10) were selected. These three genes are associated with CHD and MI, according to the international databases. Also, based on the data above, a polymorphic marker (G-1082A) of IL-10 gene located in its promoter and probably associated with the intensity of synthesis of interleukins [9] and polymorphic marker (G-174C) of IL-6 gene were selected. Although, many possible gene polymorphisms of TNF-α have been described, only a replacement at the G-308A position affects the change of transcription and cytokine production [10]. The allelic polymorphism in the promoter regions of the interleukin genes provides a various degree of cytokine production upon antigen stimulation, i.e, various cellular inflammatory responses, including AMI. Developing the above concept we have analyzed the incidence of certain alleles of a number of genes of interleukins, located exactly in the promoter sites of the genes.

OBJECTIVE

To study the incidence of allelic polymorphism of genes and genotypes of TNF-α, IL-10 and IL-6 cytokines in the AMI-ST patients.

MATERIALS AND METHODS

We examined 81 AMI-ST patients, who received treatment in the cardiology department of the Medical Center of Semey State Medical University. The study included 59 men and 22 women (72.8% and 27.2%, respectively) of Kazakh ethnicity, at the average age of 62.8 years (40 - 91 years). The control group consisted of 100 healthy blood donors of Kazakh ethnicity and nationality, similarly with the study group of AMI-ST patients. In addition, considering that the external factors are significant in the development of CVD, the control group was recruited from the persons living in the East Kazakhstan region, matching the AMI-ST patients by sex and age. AMI-ST was diagnosed in accordance with the classification of the European Society of Cardiology (ESC). All the AMI-ST patients received in the hospital standard medical therapy. An SNP of promoter region of TNF-α (G-308A), IL-10 (G-1082A) and IL-6 (G-174C) genes was studied in all patients. Genotyping of promoter regions of cytokines was performed on the basis of the nucleotide sequence of the studied genes from Gen Bank www.ncbi.nlm.nih.gov/genbank/ database using the registration numbers of TNF-α G-308A (rs1800629), IL-10, G-1082A (rs1800896) and IL-6, G-174C (rs1800795). The detection of polymorphisms of the genes of IL-10 and TNF-α was performed using fluorescence pattern of real time

polymerase chain reaction (real-time PCR, "Liteh", Russia). The detection of polymorphisms of the gene of IL-6 was performed using PCR of electrophoresis pattern with synthesized allele-specific primers ("Liteh", Russia). The DNA was isolated from peripheral blood leucocytes using standard techniques.

The written Informed Consent was obtained from all the participants in the study. The study excluded patients with acute inflammation, immunopathology, cancer, and chronic diseases at the stage of exacerbation. The study was performed in accordance with the Declaration of Helsinki. The approval for performance of the study was granted by the ethics committee of Semey State Medical University on 13.11.2013. During the next 12 months from the date of the approval, the presence of endpoints was evaluated and included the following: alive / dead, congestive heart failure stage III-IV according to Killip, decompensated heart failure, post-MI left ventricular aneurysm, early post-MI angina, recurrent MI, progressive angina (with restenosis in the stent area), acute cardiac rhythm and conduction disturbances.

All AMI-ST patients were divided into A and B groups. AMI-ST A group consisted of 49 (60.5%) patients who did not have complications, whether during or post hospitalization period. AMI-ST B group consisted of 32 (39.5%) patients with complications (Table 1).

Table 1. The incidence of end points within 12 months

End points	Hospitalization period	Post-hospitalization period
Early post-MI angina	17,2% (14)	12,3% (10)
Acute heart failure stage III-IV according to Killip	12,3% (10)	-
Congestive heart failure functional class III-IV by NYHA	-	22,2% (18)
Development of sub-acute left ventricular aneurysm	4,9% (4)	4,9% (4)
Death	8,6% (7)	7,4% (6)
Recurrent MI	-	11,11% (9)
Acute cardiac rhythm and conduction disturbances	6% (5)	17% (14)
Progressive angina (with restenosis in the stent area)	-	4,9% (4)

The prevalence of AMI-ST complications throughout 12 months follow-up was recorded both during or post hospitalization period. The obtained data were processed using SPSS Inc., version 20.0. Statistical analysis included the calculation frequency of genotypes and alleles of genes, their combinations and the odds ratio (OR - odds ratio) with the calculation of the 95% confidence interval (95% Confidence Interval - 95% CI). The distribution of genotypes for the studied polymorphic loci was tested for compliance with the Hardy-Weinberg equilibrium. The significant difference in the studied traits of allocation incidence among the groups was determined using χ 2 (two-sided Fisher's exact test).

RESULTS AND DISCUSSION

The following results were obtained by the analyses of the molecular genetic studies (Table 2). In the analyses of incidence of genetic polymorphism of G-308A site of the TNF-α gene, G/G genotype was observed in 78.0%, G/A in 21.0% and A/A in 1% of the participants of the Control group. In the analyses of incidence of genetic polymorphism of G-1082A site of the IL-10 gene, G/G genotype was observed in 52,0%, G/A in 46.0% and A/A in 2% of the participants of the Control group. In the analyses of incidence of genetic polymorphism of G-174C site of the IL-6 gene, G/G genotype was observed in 64,0%, G/C in 35.0% and C/C in 1% of the participants of the Control group. Respectively, G/G genotype was regarded as the wild-type homozygote, G/A – heterozygote and A/A – minor-type homozygote.

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Table 2. Distribution of genotype properties and allele polymorphism of TGF-α (G-308A), IL-10 (G-1082A) and IL-6 (G-174C) genes of AMI-ST patients and healthy individuals. Relation of polymorphisms to AMI-ST.

Polymorphism loci	Genotype	Groups								χ 2	p-value
		AMI-ST A (n-49)			AMI-ST B (n-32)			Control (n-100)			
		n	incidence %	OR*	n	incidence %	OR*	n	incidence %		
TNF-α G-308A	G/G	31	63,26	1,84	11	34,37	0,54	78	78,0	30,52	0,001
	G/A	16	32,65	0,87	12	37,50	1,14	21	21,0		
	A/A	2	4,08	0,14	9	28,12	6,89	1	1,0		
	G	78	79,59	1,50	34	53,12	0,66	177	88,5		
	A	20	20,41	0,43	30	46,87	2,29	23	11,5		
IL-10 G-1082A	G/G	19	38,77	1,24	10	31,25	0,80	52	52,0	15,40	0,003
	G/A	24	48,98	1,04	15	46,87	0,95	46	46,0		
	A/A	6	12,24	0,55	7	21,87	1,79	2	2,0		
	G	62	63,26	1,16	35	54,69	0,86	150	75,0		
	A	36	36,73	0,81	29	45,31	1,23	50	25,0		
IL-6 G-174C	G/G	38	77,55	1,65	15	46,87	0,60	64	64,0	17,83	0,001
	G/C	9	18,37	0,53	11	34,37	1,87	35	35,0		
	C/C	2	4,08	0,21	6	18,75	4,60	1	1,0		
	G	85	86,73	1,35	41	64,06	0,73	163	81,5		
	C	13	13,26	0,36	23	35,94	2,71	37	18,5		

Note: * comparison between AMI-ST groups

Analyses of distribution of G-308A genotypes of the TNF-α gene revealed a wild-type homozygous incidence of 63.26% in the AMI-ST A group and 34.37% in the AMI-ST B group. As it can be seen from the data, the incidence of homozygous 308 G/G genotype was found higher in the AMI-ST A group, as compared to AMI-ST B group (Table 2). The occurrence of the homozygous 308 A/A genotype was 28.12% in the AMI-ST B group, which was significantly higher as compared to 4,08% in the AMI-ST B group (OR = 6,89, 95%, p <0.05) (Table 2). Our results are supported by the works of several authors [11]. The 308 A/A and 308 A/G genotypes of the TNF-α gene were shown to be associated with an increased risk of adverse outcome in the AMI patients [11].

Analyses of the distribution of G-1082A genotypes of the IL-10 gene in the AMI-ST A group have shown incidence of 38,77% of wild-type homozygous 1082 G/G, compared to 12.24% of minor type homozygous 1082 A/A genotype. Similarly, in the AMI-ST B group incidence of wild-type homozygous 1082 G/G genotype was 31,25%, which was higher than 21.87% of occurrence of minor type homozygous 1082 A/A genotype.

The analyses of occurrence of G-174C genotypes of IL-6 revealed in the AMI-ST B group 34,37% of incidence of heterozygous 174 G/C, which was higher than 18.75% of incidence of the homozygous minor type 174C/C genotype. In the AMI-ST A group the incidence of the wild-type homozygous 174G/G was 77,55%, while the incidence of heterozygous 174G/C genotype was 18,37% and the incidence of the minor type homozygous 174C/C genotype was 4.08%.

Data analyses of polymorphism incidence in the promoter region of IL-6 gene, in AMI-ST patients, have shown that the occurrence of the minor type homozygous 174C/C genotype was associated with development of adverse outcome (OR = 4,60; 95%, p<0.05). It was established that wild-type homozygous 174G/G genotype was associated with reduction of development of adverse outcome in AMI-ST patients (OR = 1,65; 95%, p <0.05).

Our data are supported be the work of several investigators, who revealed the association of functional G-174S polymorphisms of IL6 gene as the CVD and particularly MI risk factor [12].

According to our data, the incidence of A allele polymorphic G-308A genotype of TNFα gene in the AMI-ST B group was approximately twice higher than in the AMI-ST A group (46.87% vs. 20.41%, respectively) and 4 times higher than in the control group (46.87% vs. 11.5%, respectively), which is apparently associated

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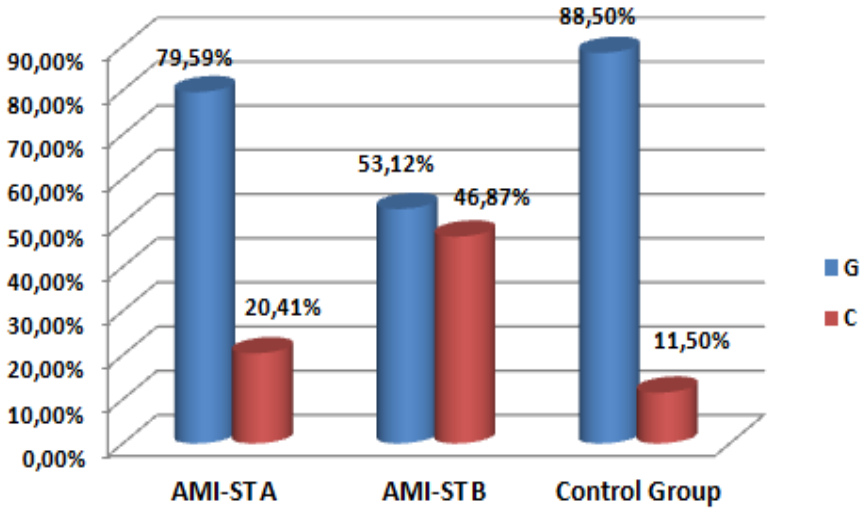
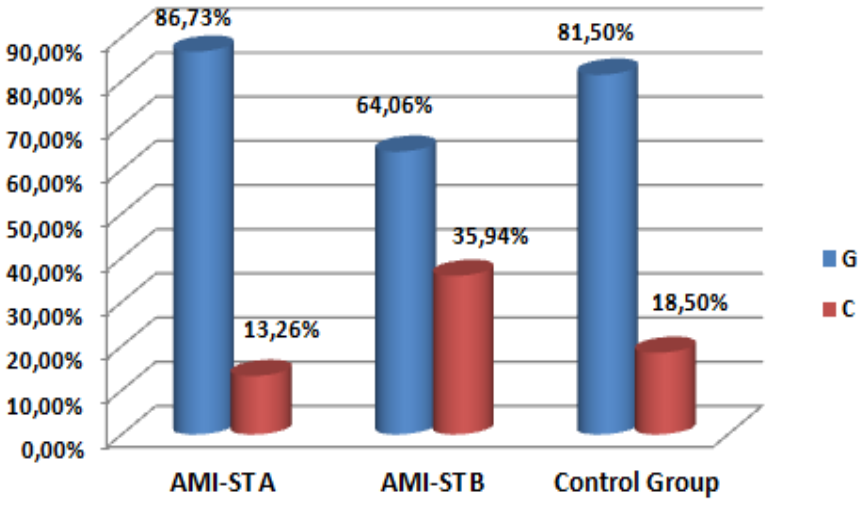
with adverse outcome of the disease. Nevertheless, the incidence of G allele polymorphic G-308A genotype of TNFα gene was not significantly different among the study groups (p = 0.083) Figure 1.

Figure 1. The incidence of allele polymorphism of G-308A genotype of TNF-α gene



The incidence C of allele polymorphic G-174C genotype of IL-6 gene in the AMI-ST B group was approximately 3 times higher than in the AMI-ST A group (35.94% vs. 13.26%, respectively) and twice higher than in the control group (35.94% vs. 18,50%, respectively), which was apparently associated with adverse outcome of the disease. Nevertheless, the incidence of G allele polymorphic G-308A genotype of TNFα gene was not significantly different among the study groups (p = 0.174) Figure 2.

Figure 2. The incidence of allele polymorphism of G-174C of IL-6 gene



No statistical difference was found in the incidence of allele polymorphism of G-1082A genotype of IL-10 gene of all study groups (p = 0.253) Figure 3.

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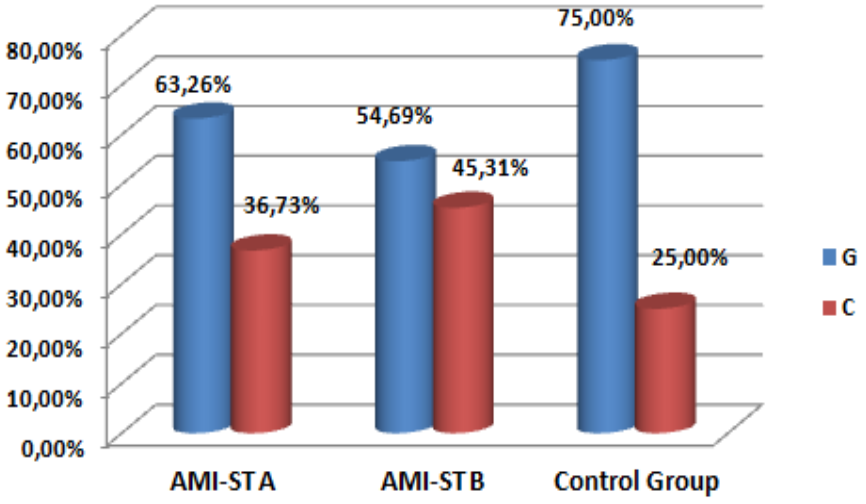
Figure 3. The incidence of allele polymorphism of G-1082A of IL-10 gene



Thus, minor type homozygous 308A/A genotype of TNFα gene, minor type homozygous 174C/C genotype of IL-6 gene, apparently could be considered as a possible genetic risk factor of adverse outcome in the AMI-ST patients of Kazakh ethnicity. However, these data have to be confirmed in the future studies performed with substantially larger groups of patients.

According to the study results, no significant difference between the AMI-ST groups was found in the occurrence of alleles and genotypes of G-1082A polymorphism of IL-10 gene. These data have to be confirmed in the future studies performed with substantially larger groups of patients.

The analysis of incidence of allele distribution revealed that G allele of the polymorphic genotypes are found significantly more often in all the investigated groups (figures 1,2,3).

Also, it should be noted that A alleles of the polymorphic G-308A genotype of TNFα gene, polymorphic G-1082A genotype of gene IL-10, and allele C of a the polymorphic G-174C genotype of IL-6 gene were found more frequently, but not statistically different ($p > 0.05$) in the AMI-ST B group.

CONCLUSIONS

Thus, the main result of the present study is identifying, A/A polymorphic G-308A genotype of TNFα gene and C/C of polymorphic G-174C genotype of IL-6 gene, which are associated with complications of AMI-ST. These polymorphic genotypes could have a prognostic value for AMI-ST patients of Kazakh ethnicity of the East Kazakhstan Region of Kazakhstan. However, at this stage of such a forecast is possible only after further study with a significant increase in the sample size. Further investigation with substantially larger groups of patients, is warranted to confirm these data.

Limitations of the study. The limited number of patients in the study groups, which weakens statistical patterns, is the limitation of the present study.

Conflict of interest. The authors declare no potential conflict of interests requiring disclosure in this article.

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