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РАСПРЕДЕЛЕНИЕ ПОЛИМОРФИЗМА ГЕНА TNF-A (G-308A) И ЕГО СВЯЗЬ С РАЗВИТИЕМ И ТЯЖЕСТЬЮ АПЛАСТИЧЕСКОЙ АНЕМИИ В ПОПУЛЯЦИИ УЗБЕКИСТАНА

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Резюме. *Цель. Оценить распределение полиморфизма гена TNF-α (G-308A) и его связь с риском и тяжестью АА в популяции Узбекистана. Методы. В исследование "случай-контроль" были включены 86 пациентов с приобретенным АА и 98 здоровых лиц контрольной группы. Полиморфизм гена TNF-α (G-308A) был генотипирован с помощью молекулярно-генетического анализа. Были статистически оценены равновесие Харди-Вайнберга (ХВ), аллельные и генотипические частоты и связи с тяжестью АА.*

У всех участников был взят образец периферической крови объемом четыре миллилитра для исследования однонуклеотидного полиморфизма TNF-α (G-308A) с использованием полимеразной цепной реакции полиморфизма длины рестрикционного фрагмента (ПЦР-ПДРФ). Образцы хранились при температуре -80 °C до выделения ДНК. Результаты подвергались статистической обработке с применением пакета прикладных ПК программ «OpenEpi 2009, Version 2.3». Выводы. Результаты последующего математического анализа различий между изученным геном TNF-α (G-308A) среди выборки пациентов с АА как по сравнению со здоровыми не было обнаружено существенных различий в частотах аллелей (G против A) и генотипов (GG, GA, AA) (P>0,05). Стратификация по тяжести заболевания также не выявила статистически значимых различий (P>0,05). Аллель А оказался редким во всех группах (5,0% у пациентов против 5,6% у контроля). HWE сохранялся как в группе пациентов, так и в контрольной группе. Мутантный генотип АА не был обнаружен ни в одной из групп. Полиморфизм гена TNF-α (G-308A) независимо не связан с риском или тяжестью АА у узбекской популяции. При проведении генетических исследований АА следует учитывать этническую и региональную генетическую изменчивость.

Ключевые слова: *апластическая анемия, TNF-α (G-308A), полиморфизм, цитокины, генетическая ассоциация, Узбекистан.*

DISTRIBUTION OF TNF-A (G-308A) GENE POLYMORPHISM AND ITS ASSOCIATION WITH THE DEVELOPMENT AND SEVERITY OF APLASTIC ANEMIA IN THE UZBEK POPULATION

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Summary. Purpose of the study. *To assess the distribution of TNF-α (G-308A) gene polymorphism and its association with the risk and severity of AA in an Uzbek population. Methods. A case-control study included 86 patients with acquired AA and 98 healthy controls. TNF-α (G-308A) polymorphism was genotyped by molecular genetic analysis. Hardy-Weinberg equilibrium (HWE), allelic and genotypic frequencies, and associations with AA severity were statistically evaluated. A four milliliter peripheral blood sample was taken from all participants to study single nucleotide polymorphism TNF-α (G-308A) using polymerase chain reaction of restriction fragment length polymorphism (PCR-PDRF). The samples were stored at a temperature of -80 °C until DNA was isolated. The results were statistically processed using the PC application package "OpenEpi 2009, Version 2.3". Conclusions. The results of subsequent statistical analysis of differences in the studied TNF-α (G-308A) gene among the sample of patients with AA compared to healthy controls revealed no significant differences in the frequencies of alleles (G vs. A) and genotypes (GG, GA, AA) (P>0.05). Stratification by disease severity also did not show any statistically significant differences (P>0.05). The A allele was rare in all groups (5.0% in patients vs. 5.6% in the control group). The Hardy–Weinberg equilibrium (HWE) was maintained in both the patient and control groups. The mutant AA genotype was not detected in any of the groups. The TNF-α (G-308A) gene polymorphism was not independently associated with either the risk or severity of AA in the Uzbek population. When conducting genetic studies on AA, ethnic and regional genetic variability should be taken into account.*

Key words: *aplastic anemia, TNF-α (G-308A), polymorphism, cytokines, genetic association, Uzbekistan.*

Introduction. Aplastic anemia (AA) is a rare but life-threatening hematological disorder characterized by pancytopenia and hypocellular bone marrow in the absence of malignancy or fibrosis [4].

Although the exact pathophysiology of anemia remains unclear, it is associated with abnormalities of hematopoietic stem/progenitor cells (HSPCs), dysfunction of the hematopoietic microenvironment, immune mechanism disorders, and genetic-level changes. Firstly, HSPCs play a crucial role in maintaining hematopoiesis. The mechanism of HSPC abnormalities involves the following: cytotoxic cells directly destroy HSPCs through cytotoxicity and hematopoietic inhibitory factors via well-known Fas/FasL and NO pathways, leading to HSPC apoptosis [7].

Recent studies have shown that defective functioning of regulatory T cells leads to increased production of interferon-gamma (IFN-γ) and tumor necrosis factor-alpha (TNF-α), causing stem cell damage and resulting in bone marrow aplasia [9,10]. Among cytokines, TNF-α is of particular interest due to its cytotoxic and immunomodulatory properties [2, 6]. TNF–α is encoded by the *TNF–α* gene located on chromosome 6, in close proximity to a highly polymorphic region of the class III HLA–B locus of human major histocompatibility complex. It is a well-known fact that the gene products are affected by polymorphic markers in this region. A single-nucleotide polymorphism (SNP) of TNF–α promotor gene results in two types of alleles, i.e., TNF1 and TNF2. TNF1 is a common allele defined by the presence of guanine, whereas TNF2 is a rarer allele characterized by the substitution of guanine with adenosine, located at position –308 (NC_000006.11:g.31543031 G>A, rs1800629), which influences the expression of TNF–α [1,5]. There is increasing evidence that the G/A polymorphism in the –308 promoter region of the TNF-α gene may influence the transcription, expression, and subsequent biological activity of TNF-α, suggesting that an individual's genetic profile may play a determining role in its clinical behavior [3,8].

To date, no investigations have examined the distribution or potential association of TNF-α (G-308A) polymorphism with AA in the Uzbek population — a region with unique genetic and environmental characteristics. Considering known inter-ethnic variability in allele frequencies and disease associations, this study aimed to investigate the distribution of TNF-α (G-308A) polymorphism and its association with the development and severity of AA in Uzbekistan.

Purpose of the study. To assess the distribution of TNF-α (G-308A) gene polymorphism and its association with the risk and severity of AA in an Uzbek population.

Methods. A case-control study included 86 patients with acquired AA and 98 healthy controls. TNF-α (G-308A) polymorphism was genotyped by molecular genetic analysis. Hardy-Weinberg equilibrium (HWE), allelic and genotypic frequencies, and associations with AA severity were statistically evaluated.

To carry out molecular genetic analyses of immune modulator genes, 4.0 ml venous blood samples were taken from AA and healthy patients into test tubes containing EDTA TNF-α (G-308A), which were stored in freezers at a temperature of -80 °C until the biomaterial was completely collected.

After blood collection, DNA of patients and healthy people was isolated using AmpliPrime RIBOT-prep reagents (Russia) on a NanoDrop 2000 spectrophotometer (NanoDropTechnologies, USA) with a wavelength equal to A260/280 nm with a determination of DNA concentration by a ratio of 1.7/1.8.

Polymorphic gene TNF-α (G-308A) was detected by RT PCR using a thermal cycler (Applied Biosystems 2720, USA; RotorGeneQ, QUAGEN Germany and Corbett Research - CG1-96, QUAGEN Germany).

The amplification procedure was carried out by pre-denaturation of DNA at a temperature of 94 ° C for 3 minutes; 30 denaturation cycles at a temperature of 94 ° C for 20 seconds; annealing of primers at a temperature of 58 ° C for 20 seconds; elongation process at a temperature of 61 ° C for 30 seconds; DNA synthesis at a temperature of 61 °C for 7 minutes; electrophoresis on 2% agarose gel with final analysis of the results using a UV transilluminator with an integrated imaging camera.

LITECH test systems (Russia) were used to detect the studied genetic markers. The SNPs were tested for deviations from the Hardy-Weinberg equilibrium using the Chi-square test. The relative risk associated with alleles and genotypes was calculated as the odds ratio (OR) with a 95% confidence interval (CI), which were performed using the PC program "OpenEpi 2009, Version 2.3".

Results. Analyzing the structure of the TNFα (G-308A) gene polymorphism in the main group of patients with aplastic anemia (AA) (n=86), no significant differences were found compared to the corresponding values in the control group. The predominant variants in the patient group for the TNFα (G-308A) gene were also the major G allele (95.0%) and the G/G genotype (89.5%), while the mutant A allele (5.0%) and the heterozygous G/A genotype (10.5%) occupied recessive positions in terms of frequency (see Table 1).

With a slightly higher frequency in patients with non-severe AA (n=16) compared to both the control and the main patient groups, the major G allele (96.9%) and the G/G genotype (93.8%) were observed for the studied gene. In accordance with this, the mutant A allele (3.1%) and the heterozygous G/A genotype (6.2%) showed lower frequencies (see Table 1).

Table 1.

Structural Analysis of TNFα (G-308A) Gene Polymorphism in Healthy Control and Aplastic Anemia Patient Groups

№	Group	Alleles (n/%)				Genotypes (n/%)					
		G		A		G/G		G/A		A/A	
		N	%	N	%	N	%	n	%	N	%
1	Main AA group, n=86	163	95.0	9	5.0	77	89.5	9	10.5	0	0.0
2	Non-severe AA, n=16	31	96.9	1	3.1	15	93.8	1	6.2	0	0.0
3	Severe AA, n=46	87	94.6	5	5.4	41	89.1	5	10.9	0	0.0
4	Very severe AA, n=24	45	93.8	3	6.2	21	87.5	3	12.5	0	0.0
5	Comparative control group, n=98	185	94.4	11	5.6	87	88.8	11	11.2	0	0.0

Among patients with the severe form of aplastic anemia (n=46), the major G allele (94.6%) and the G/G genotype (89.1%) of the TNFα (G-308A) gene were also observed at higher frequencies, whereas the mutant A allele (5.4%) and the G/A heterozygote genotype (10.9%) showed lower frequencies. Meanwhile, in the group with very severe aplastic anemia (n=24), compared to the control group and the other patient groups described above, the frequency of the major G allele (93.8%) and the G/G genotype (87.5%) was relatively lower, while the frequencies of the unfavorable A allele (6.2%) and the G/A genotype (12.5%) were comparatively higher in accordance with the distribution pattern (see Table 1).

When analyzing the significance of differences in the frequencies of alleles and genotypes of the TNFα (G-308A) gene polymorphism in the group of patients with non-severe aplastic anemia (AA) compared to those with severe and very severe forms of AA, no statistically significant differences were found in the carriage of the mutant A allele (3.1% vs. 5.4%; χ^2=0.3; P=0.6; RR=1.0; CI: 0.47–2.03; OR=0.6; CI: 0.06–4.86 and 3.1% vs. 6.3%; χ^2=0.4; P=0.6; RR=1.0; CI: 0.3–3.11; OR=0.5; CI: 0.05–4.66), in the predominant G/G genotype (93.8% vs. 89.1%; χ^2=0.3; P=0.6; RR=1.1; CI: 0.03–38.79; OR=1.8; CI: 0.2–16.49 and 93.8% vs. 87.5%; χ^2=0.4; P=0.6; RR=1.1; CI: 0.04–32.48; OR=2.1; CI: 0.21–21.68), or in the heterozygous G/A genotype (6.3% vs. 10.9%; χ^2=0.3; P=0.6; RR=0.6; CI: 0.02–21.2; OR=0.5; CI: 0.06–4.93 and 6.3% vs. 12.5%; χ^2=0.4; P=0.6; RR=0.5; CI: 0.02–15.16; OR=0.5; CI: 0.05–4.72).

In addition, when comparing the differences in allele and genotype frequencies of the TNFα (G-308A) polymorphism between the severe and very severe forms of AA, no statistically significant differences were identified in the carriage of the mutant A allele (5.4% vs. 6.3%; χ^2<3.84; P=0.9; RR=1.0; CI: 0.16–6.08; OR=0.9; CI: 0.2–3.77), in the predominant G/G genotype (89.1% vs. 87.5%; χ^2<3.84; P=0.9; RR=1.0; CI: 0.34–3.09; OR=1.2; CI: 0.26–5.38), or in the heterozygous G/A genotype (10.9% vs. 12.5%; χ^2<3.84; P=0.9; RR=0.9; CI: 0.29–2.63; OR=0.9; CI: 0.19–3.92).

Conclusion. In this study, we analyzed the distribution of the TNF-α gene promoter polymorphism (G-308A, rs1800629) in patients with aplastic anemia (AA) of varying severity compared to healthy controls in an Uzbek population cohort. The results of subsequent statistical analysis of differences in the studied TNF-α (G-308A) gene among the sample of patients with AA compared to healthy controls revealed no significant differences in the frequencies of alleles (G vs. A) and genotypes (GG, GA, AA) (P>0.05). Stratification by disease severity also did not show any statistically significant differences (P>0.05). The A allele was rare in all groups (5.0% in patients vs. 5.6% in the control group). The Hardy–Weinberg equilibrium (HWE) was maintained in both the patient and control groups. The mutant AA genotype was not detected in any of the groups. The TNF-α (G-308A) gene polymorphism was not independently associated with either the risk or severity of AA in the Uzbek population. When conducting genetic studies on AA, ethnic and regional genetic variability should be taken into account.

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