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# Prevalence of polymorphism (-174 G / C) of IL-6 gene in patients with acute brucellosis and hepatobiliary system damage

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## Abstract

Modern brucellosis is characterized by high variability of clinical symptoms. Its evolution is going in the direction of acute forms increasing, with an early development of focal metastatic and infectious-allergic lesions, which, in their turn, increase the severity of the disease's course. This testifies to early sensitization development in patients in endemic regions already in the acute phase of brucellosis. The objective: to determine the prevalence of the polymorphism (-174 G / C) of the IL-6 gene in patients with hepatic damages in acute brucellosis in the Republic of Azerbaijan. 178 patients were examined, 120 of them fully met all the criteria for inclusion in the study, so they formed the treatment group. The definition of polymorphism (-174 G / C) of the IL-6 gene was made to all patients. Statistical analysis of the results obtained was performed with "SPSS 20.0" and "STATISTICA 6.0" programs. Among 120 patients with acute brucellosis, the disease of the hepatobiliary system had 43 (35.8%) persons according to the generalization of laboratory and instrumental methods of examination. Genotype C / C IL-6 gene was 4.2 times more frequent in patients with acute brucellosis and liver damage than in hepatic lesions-free persons. It was found that among the carriers of C / C genotype, risk of brucellosis prevalence with hepatic damage is significantly

higher ($\chi 2$ = 12.26; p = 0.002; OR = 0.97; 95% CI [0.28 - 3.41]), while the carriership of the homozygous genotype G / G, on the contrary, has protective effect on the development of brucellosis (OR = 0.16, 95% CI [0.05 - 0.50]).

**Key words**: **acute brucellosis, hepatitis, polymorphism, IL-6.**

**Urgency of the problem.** High variability of clinical symptoms is typical for the modern brucellosis course. It is a systemic disease in which various organs and systems disaster is possible due to its pathogenesis - the presence of bacteremia in acute brucellosis, and endotoxicosis and systemic inflammation with involvement of osteoarticular, cardiovascular, G-U, nervous and endocrine systems, significant patient's quality of life deterioration [1, 2]. Brucellosis evolution under modern conditions is going in the direction of its acute forms prevalence, with an early development of focal metastatic and infectious-allergic lesions, which, in their turn, increase the severity of the disease's course. This testifies to early sensitization development in patients in endemic regions already in the acute phase of brucellosis.

Liver, as the largest organ of the reticuloendothelial system, is affected practically all brucellosis patients. Liver injury may be accompanied by an increase in its enzymes from mild to moderate [4, 5]. According to the literary data, the frequency of liver damages ranges from 2-3% to 40% [6, 7]. Also, hepatobiliary system damages may be manifested by liver and spleen abscesses, although the frequency of such complications is not high and according to various researchers data amount from 1% to 3% [8, 9]. Dean A. S. et al. reported that splenomegaly is registered in 15-60% of cases [10].

Particular attention should be paid to IL-6, which contributes to immune response implementation and inflammation in pathological processes, and various genesis conditions, including brucellosis [11]. IL-6 is a multifunctional cytokine that plays an important role in inflammatory responses. It is produced by several types of cells, such as T - lymphocytes, macrophages, fibroblasts and endothelial cells [12]. Previous studies have shown that levels of IFN-γ, IL-6 and TNF-α increase in brucellosis patients [13, 14]. As of today, the role of gene IL-6 polymorphism in pathogenesis of various diseases has been proved. Specifically, different genotypes of the IL-6 gene may be associated with more frequent cardiovascular damage, as well as a higher risk of complications in diabetes mellitus [15, 16].

Similar studies have also been conducted among patients with brucellosis in various ethnic groups. The data on the relationship between IL-6 polymorphism (-174 G / C) and

susceptibility to brucellosis are available. At the same time, a number of studies refute these data [17, 18].

**The objective:** to determine the prevalence and features of IL-6 gene (-174 G / C) in patients with hepatic injury in acute brucellosis in the Republic of Azerbaijan.

**Materials and methods**: 178 patients with suspected brucellosis were examined. They applied for medical aid to Baku Clinic and Central Clinical Hospital in Baku. PICs were obtained in all cases.

The diagnosis was established on the basis of clinical data, anamnesis, including epidemiological, data of objective examination, results of specific and nonspecific laboratory diagnostics.

To evaluate liver state, all patients were subjected to biochemical studies with mandatory determination of total bilirubin, SGPT, AST, ALP, GGT, LDH. Ultrasound examination of the abdominal cavity on the Voluson E8 GeneralElectric device was performed using a 4- to 8-MD 4-D Convex Multi-frequency Sensor RAB 4-8D.

All patients were examined for markers of viral hepatitis A, B, C, D, E. Hepatitis of non-viral etiology, namely, autoimmune, toxic, alcohol were excluded.

Specific methods of examination were conducted on the Awareness and StatFax 3200 apparatus by ELISA using NovaLisaBrusella IgG, IgM (Germany) test systems for detecting IgM and IgG to brucella.

In addition to specific diagnostic data, the duration of clinical symptoms up to 3 months after the appearance of the first complaints was taken into account to establish the severity of the process.

In 178 patients examined, 120 persons, who made treatment group (TG), completely met all the criteria for inclusion in the study. The control group (CG) consisted of 30 practically healthy persons, who underwent a planned annual medical examination. Both groups were age and gender representative. Patients of both groups are ethnic Azerbaijanii, permanently dwelling in the Republic of Azerbaijan. The average age of patients in the main group was 35.9 ± 2.8 years old. Male subjects predominated (75.0%).

Polymorphism IL-6 (-174C / G) was determined in all patients. DNA amplification of the loci under investigation was carried out automatically using the structure of the following primers: F 5'-TGACTTCAGCTTTACTCTTTGT-3 'and R 5'-CTGATTGGAAACCTTATTAAG-3'. After initial denaturation at 94°C for 5 minutes each stage took place in 3 stages: denaturation - 94°C (30 s), annealing of primers (30-45 s),

elongation - 72°C (30-40 s). Final elongation lasted 7 minutes at 72°C. For identification of IL-6 gene alleles restriction analysis of amplicons was performed using NlaIII endonuclease restriction. It was restrained for 12 hours at a 37°C. The products of the hydrolysis of the amplified sequences were analyzed by electrophoresis in a 2% agarose gel.

The reliability of the differences in the distribution of genotypes by polymorphic loci between the groups was verified to match the Hardy-Weinberg equilibrium (http://gen-exp.ru/calculator_or.php).

Statistical processing of the results obtained was carried out using "SPSS 20.0" and "STATISTICA 6.0" programs. The comparison of the frequencies of genotypes and alleles was carried out by analyzing the conjugation tables using Fischer's exact test and the $\chi 2$ criterion, depending on the assumptions of the analysis. The risk of pathology development was estimated by calculating the odds ratio (OR) with 95% confidence interval (CI) by simple logistic regression.

**Results and discussion**

An increase in the level of transaminases occurred in almost a third of patients with acute brucellosis. The increase in ALT level was determined in 43 (35.8%) patients, AST above the upper limit was found in 48 (40.0%). In our opinion, a higher level of AST compared with ALT in these patients may be due to the high incidence of cardiovascular damage in people over 45 years of age, namely 77.5% of patients (Fig. 1).

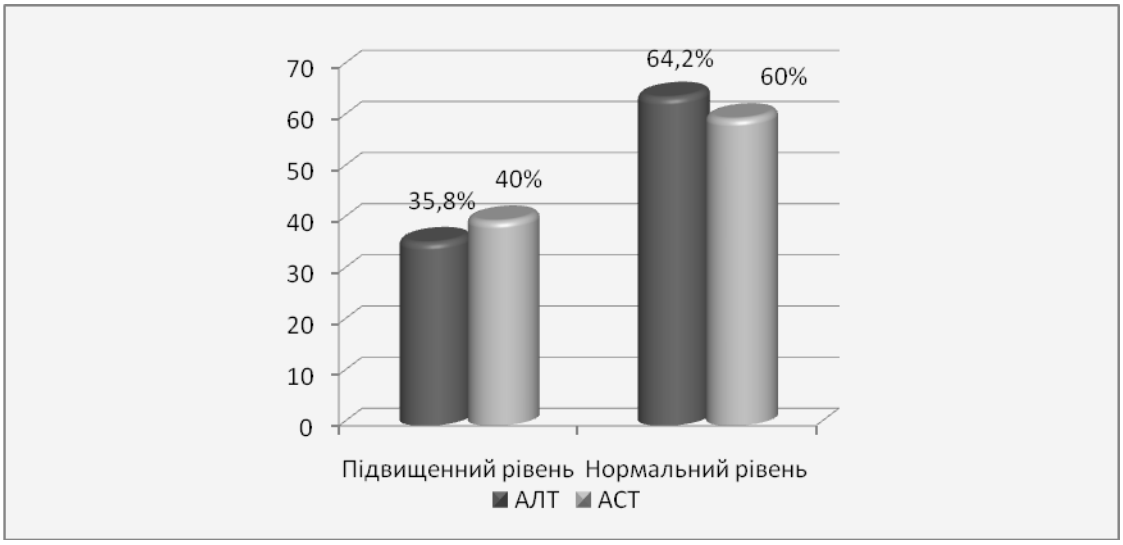


Fig. 1. Distribution of patients with brucellosis depending on the levels of transaminases

In ultrasound examination of the abdominal cavity hepatomegaly was detected in 49 people (40.8%). Thus, out of 120 patients with acute brucellosis, the disease of the

hepatobiliary system was 43, which was 35.8%, according to the generalization of laboratory and instrumental methods of investigation.

In the analysis of the polymorphism of IL-6 (-174 G / C), the genotype C / C of the IL-6 gene was 4.2 times more frequent in patients with acute brucellosis with liver disease than without a defeat ($p < 0.05$). The genotype G / G was more common in individuals without signs of liver injury, namely 5.8 times more likely than in patients with acute brucellosis, which have liver damage. Mutant allele C was significantly higher in 4.4 times among patients with brucellosis with liver disease ($p < 0.05$) (Table 1).

Table 1

Frequency of detection of polymorphism (-174 G / C) of IL-6 gene in patients with acute brucellosis with and without liver injury and healthy persons

| Genotypes and alleles IL-6 (-174 G / C) | Br patients with liver injury, n=43 | | Br patients without liver injury, n=77 | | Healthy persons, n=30 | |
|---|---|---|---|---|---|---|
| | Abs | % | Abs | % | Abs | % |
| Genotype G / G | 6 | 13.9 | 62 | 80.5 | 15 | 50.0 |
| Genotype G / G | 30 | 69.8 | 12 | 15.6 | 10 | 33.3 |
| Genotype C / C | 7 | 16.3 | 3 | 3.9 | 5 | 16.7 |
| Alle G | 42 | 48.8 | 136 | 88.3 | 40 | 66.7 |
| Alle C | 44 | 51.2 | 18 | 11.7 | 20 | 33.3 |

*Note:* * $p < 0,05$ - between patients with brucellosis without liver injury and healthy persons

The next stage in our study was to identify the risk of brucellosis prevalence with liver injury, taking into account the carrier of polymorphic variants of IL-6 gene. In studying the peculiarities of the distribution of the alleles frequency in brucellosis patients, it was found that carriers of allele C polymorphic (-174 G / C) IL-6 gene have an increased prevalence risk with liver injury (OR = 2.10, 95% CI [1.06 - 4.15]), whereas in the case of allele G carrier, on the contrary, the risk of brucellosis development (OR = 0.48, 95% CI [0.24 - 0.95]) is lower. The model is reliable at $\chi 2 = 4.56$, $p = 0.03$ (Table 2).

We established that among C / C genotype carriers a significantly increased risk of brucellosis with liver injury exsists ($\chi 2 = 12.26$; $p = 0.002$; OR = 0.97; 95% CI [0.28 - 3.41]), whereas the carriership of the homozygous genotype G / G, on the contrary, has protective effect against the development of brucellosis (OR = 0.16, 95% CI [0.05 - 0.50]).

Table 2

Association of alleles and genotypes for polymorphism (-174 G / C) of IL-6 gene with vulnerability to brucellosis with liver injury

| Alleles & genotypes | Br patients with liver injury, n=43 | Healthy persons, n=30 | $\chi 2$ | P | OR | |
|---|---|---|---|---|---|---|
| | | | | | Mean | 95% CI |
| Allel G | 0.488 | 0.667 | 4.56 | 0.03 | 0.48 | 0.24-0.95 |
| Allel C | 0.512 | 0.333 | | | 2.10 | 1.06-4.15 |
| Genotype G/G | 0.140 | 0.500 | 12.26 | 0.002 | 0.16 | 0.05-0.50 |
| Genotype G/C | 0.698 | 0.333 | | | 4.62 | 1.70-12.54 |
| Genotype C/C | 0.163 | 0.167 | | | 0.97 | 0.28-3.41 |

When analyzing the total frequency of G / C + C / C in brucellosis patients with liver injury aand practically healthy persons (Table 3), we used heridity recessive model of brucellosis. It has been established that the combination of genotypes G / C + C / C among brucellosis patients with liver injury was determined 1.8 times more frequent than in practically healthy subjects (OR = 6.17, 95% CI [2.01 - 18.92], $\chi 2 = 11.21$, $p = 0.0008$).

Table 3

Distribution of genotype frequencies in adults by the recessive polymorphic marker (-174 G / C) of IL-6 gene

| genotypes | Br patients with liver injury, n=43 | Healthy persons, n=30 | X2 | P | OR | |
|---|---|---|---|---|---|---|
| | | | | | Mean | 95% CI |
| Genotype G/G | 0.140 | 0.500 | | | 0.16 | 0.05-0.50 |
| Genotype G/C +C/C | 0.860 | 0.500 | 11.21 | 0.0008 | 6.17 | 2.01-18.92 |

Brucellosis remains a significant health problem for endemic countries Factor in symptoms' polymorphism, multisystematicity of this disease, difficulties arise in its early diagnosis and verification of complications [19]. The most common clinical manifestation of liver damage is hepatomegaly, which, according to literature data, is seen in the limits 6% -

88% [20]. According to various data, the increased transaminases are observed in 5 - 40% of individuals.

Available data suggest an increase in serum transaminases, depending on age, e.g. in pediatric population they reach 60%, and percentage is higher among the elderly persons, while among young working age people this symptom constitutes only 25% [21].

The role of IL-6 polymorphism and its association with brucellosis is ambiguous. Thus, in most researches conducted among Turkish and Iranian population there were no associations between IL-6 gene polymorphism and susceptibility to brucellosis. Sadaf Asaei et al. (2013) found that IL-8 gene polymorphism, and not IL-6, is brucellosis sensitivity factor [12]. The same data on the lack of correlation between IL-6 gene and brucellosis polymorphism was documented by Ozgur Gunal et al. (2017) [22].

Data different from the ones mentioned were obtained by Budak et al. (2009). So, they were able to establish a connection between IL-6 polymorphism (-174 G / C) and brucellosis. Specifically, genotypes (C / C and G / C) IL-6 were more common in brucellosis patients than in the control group of healthy individuals. The authors concluded that polymorphism of the IL-6 gene could affect susceptibility to brucellosis and be regarded as a genetic risk factor for the development of fulminant form of the disease. Ilkay Karaoglan et al. (2009) received the following results, namely genotype G / C IL-6 (-174) may be a risk factor for the development of brucellosis focal complications, whereas genotype G / G may be a protective factor against this infection [23].

**Conclusions:**

1. Genotype of C / C gene IL-6 was 4.2 times more frequent in patients with acute brucellosis and liver injuriese than in those without lesions.

2. Among carriers of genotype C / C, a significantly increased risk of liver brucellosis ($\chi 2 = 12.26$; $p = 0.002$; OR = 0.97; 95% CI [0.28 - 3.41]) was determined.

3. Genotypes G / C + C / C among patients with brucellosis with liver injury were determined 1.8 times more than in practically healthy subjects (OR = 6.17, 95% CI [2.01 - 18.92], $\chi 2 = 11.21$, $p = 0.0008$).

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