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Prevalence of rs4986790 (Asp299Gly) polymorphism of the TLR-4 gene in patients with acute brucellosis depending on the damage to the hepatobiliary system

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Human genetic factors play an important role in the present stage of studying new aspects of the pathogenetic mechanisms of development, course, and outcomes of infectious diseases. Some studies established that the Asp299Gly polymorphism of the TLR-4 gene, which is currently associated with a variety of diseases, is of clinical importance. The article presents the results of an examination of 120 patients with acute brucellosis. The diagnosis of brucellosis was made based on complaints, anamnesis, epidemiologic and clinical data, and the results of specific examinations. The Asp299Gly polymorphism of the TLR-4 gene was determined in all patients. A detailed characterization of patients with brucellosis has been presented. Young, working-age men prevailed among the examined persons. Carriers of the G allele of the polymorphic (Asp299Gly) TLR-4 gene were found to have an increased risk of acute brucellosis with liver damage (OR=12.76, 95% CI [4.25-38. 29]), and in the case of carrying the A allele, on the contrary, they had a decreased risk of acute brucellosis with liver damage (OR=0.0895% CI [0.03-0.24]) model significant at $\chi 2$ =27.87P < 0.0001). Carriage of homozygous genotype A/A had a protective effect against the development of acute brucellosis with signs of liver damage (OR =0.0295% CI [0.01-0.07]).

Keywords: *Acute brucellosis, TLR-4, polymorphism, gene, liver*

INTRODUCTION

Brucellosis is considered one of the most common bacterial zoonoses worldwide and remains a serious problem not only for public health systems in many countries of the world but also for veterinary medicine in particular (Di Bonaventura et al., 2021; Nicoletti and Paul 2010; Laine, 2023; Yu et al., 2024).

The problem of brucellosis is the lack of adequate statistical data on the true incidence of the disease in the human population, as a consequence of the lack of vigilance against this disease, as well as in the delayed diagnosis, untimely and inadequately prescribed specific treatment, which in turn leads to an increase in the number of chronic cases of brucellosis after completion of etiotropic therapy (Franco et al., 2007; Saha, et al., 2013).

Brucellosis is a disease characterized by a wide variety of clinical manifestations with possible involvement of almost any organ of the human body and a wide range of so-called clinical "masks", which leads to the untimely and delayed establishment of the correct diagnosis (Adone and Pasquali 2013; Al Dahouk et al., 2013).

Moreover, Brucella species can persist for extended periods within phagocytes, evade detection by the immune system, and establish chronic infections (Dominguez-Flores et al., 2023; De Jong et al., 2012). The enduring presence of Brucella leads to the suppression of

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mononuclear apoptotic cells, hindrance of dendritic cell maturation, decreased presentation of antigens, and deactivation of T-cells (Ganji et al., 2017). The host's immune system encounters delays in identifying Brucella due to alterations or complete suppression of pathogen-associated molecular patterns (Mazlan et al., 2021). Numerous research studies have been carried out to explore the resistance of B. melitensis to antibiotics like rifampicin and trimethoprim / sulfamethoxazole (Barbosa et al., 2015; Wareth et al., 2022). As a result, the identification of new targets against B. melitensis remains highly significant (Pradeepkiran et al., 2021).

As a result of ingestion and development of brucellosis infection in the human body, immunologic restructuring of the whole organism occurs. At the present stage, there are still many gaps regarding the complete understanding of all pathogenetic links in the formation of postinfection immune responses. First of all, the issues of intercellular relationships in the process of immune response formation remain open (Ahmed, et al., 2016).

Unlike other pathogens, the brucellosis pathogen is not characterized by classical pathogenic factors such as exotoxins, exoproteases, cytolysins, and other exoenzymes that cause direct damage to human cells (Moreno, 2002). Hence, it is logical that in brucellosis, tissue damage may result from indirect mechanisms, probably due to activation of host immune responses following recognition of the brucellosis pathogen by immune system receptors such as TLRs. It was found that TLR-2, TLR-4, and TLR-9 may participate in the recognition of brucellosis by phagocytes (Oliveira 2008; Dominguez-Flores, 2023; Yu et al., 2024).

One of the main reasons influencing any changes in TLR immune response in brucellosis is considered to be genetic polymorphism of these receptor genes (Molteni et al., 2016; Netea et al., 2012).

The role of TLR-4 polymorphism and its association with brucellosis is an ambiguous and poorly studied problem. Currently, there is single data on this issue, so in one study the polymorphism (Asp299Gly) of the TLR-4 gene in brucellosis patients was analyzed. It was concluded that the G allele was more prevalent in patients with brucellosis compared to healthy individuals (33.6% vs. 20.7%, p=0.000003). Thus, this study first established an association between genetic polymorphism of the TLR-4 gene and susceptibility to brucellosis (Rezazadeh et al., 2006).

To determine the frequency of the TLR-4 gene polymorphism rs4986790 (Asp299Gly) in patients with acute brucellosis in the Republic of Azerbaijan, taking into account liver damage.

MATERIALS AND METHODS

We examined 120 patients with clinical brucellosis who sought medical care at Baku Clinic and Baku Central Clinical Hospital. All patients gave written permission to be included in the study.

The diagnosis of acute brucellosis was established based on clinical data, anamnesis, including epidemiologic, and objective examination data, and results of laboratory diagnostics, including specific diagnostics.

The diagnosis of acute brucellosis was established based on clinical and epidemiological data, anamnesis, and data from an objective examination, results of laboratory diagnostics, including specific ones.

Specific research was carried out by ELISA on Awareness and Stat Fax 3200 devices using NovaLisa Brucella IgG, IgM test systems (Germany) with the detection of IgM and IgG.

The criteria for inclusion in the study were a diagnosis of acute brucellosis, and the duration of clinical symptoms was taken into account, namely up to 3 months from the onset of the first complaints.

The main group consisted of 120 individuals who fully met the inclusion criteria for the study. The control group consisted of 30 practically healthy individuals who underwent a scheduled annual examination. The groups were representative by age and gender. Patients of both groups are ethnic Azerbaijanis who permanently reside in the Republic of Azerbaijan. The average age of patients in the main group was 35.9±2.8 years. Among those examined, males predominated – 75.0%.

All 120 patients with acute brucellosis were

divided into three subgroups according to the disease severity. The following symptoms served as severity criteria: fever, sweating, chills, headache, insomnia, decreased blood pressure, tachycardia, hepatosplenomegaly, myocarditis, pericarditis, endocarditis, changes in the general blood count, levels of pro-inflammatory and anti-inflammatory cytokines. Thus, a mild degree was diagnosed in 74 (61.7%) patients, a moderate degree - in 35 (29.1%) patients and only 11 (9.2%) patients had a severe condition.

To assess the liver condition, all patients underwent biochemical studies with the mandatory determination of total bilirubin, ALT, AST, ALP, GGT, and LDH levels, and also ultrasound examination of abdominal cavity organs was performed on a Voluson E8 General Electric apparatus using 4D convex 4-8 MHz RAB 4-8D volumetric multifrequency transducer.

All patients were screened for markers of viral hepatitis A, B, C, D, and E. Hepatitis of non-viral etiology, namely autoimmune, toxic, and alcoholic, was also excluded.

The TLR-4 gene polymorphism (Asp299Gly) was tested in all patients. Genomic DNA was extracted from peripheral blood mononuclear cells using a DNA extraction kit according to the manufacturer's instructions. Amplifications followed by electrophoretic separation of amplification products of the corresponding gene were used to identify polymorphic alleles. Amplification was performed on an iCycler IQ5 amplifier (BioRad, USA).

The amplification mode was as follows: 93°C, 1 min; 35 cycles: 93°C, 10 sec; 64°C, 10 sec, 72°C, 20 sec; 72°C, 1 min.

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

Statistical processing of the study results was performed using the programs "SPSS 20.0" and "STATISTICA 6.0". A Comparison of genotype and allele frequencies was performed by analyzing conjugation tables using Fisher's exact test and the $\chi 2$ criterion depending on the analysis assumptions. The risk of pathology development was assessed by calculating the odds ratio (OR) with a 95% confidence interval (CI) by simple logistic regression.

RESULTS AND DISCUSSION

Among patients with acute brucellosis, the normal serum ALT level was detected much more frequently ($p<0.05$), namely in 64.17±4.38% (77 patients), while elevated in 35.83±4.37% (43 patients). The AST level in serum was found to be above normal in 40.00±4.47% (48 patients), while the normal level was found in 60.00±4.48% (72 patients).

We believe the higher AST level in these patients is attributed to the high frequency of cardiovascular lesions, namely in 77.5% of individuals (Fig.1).

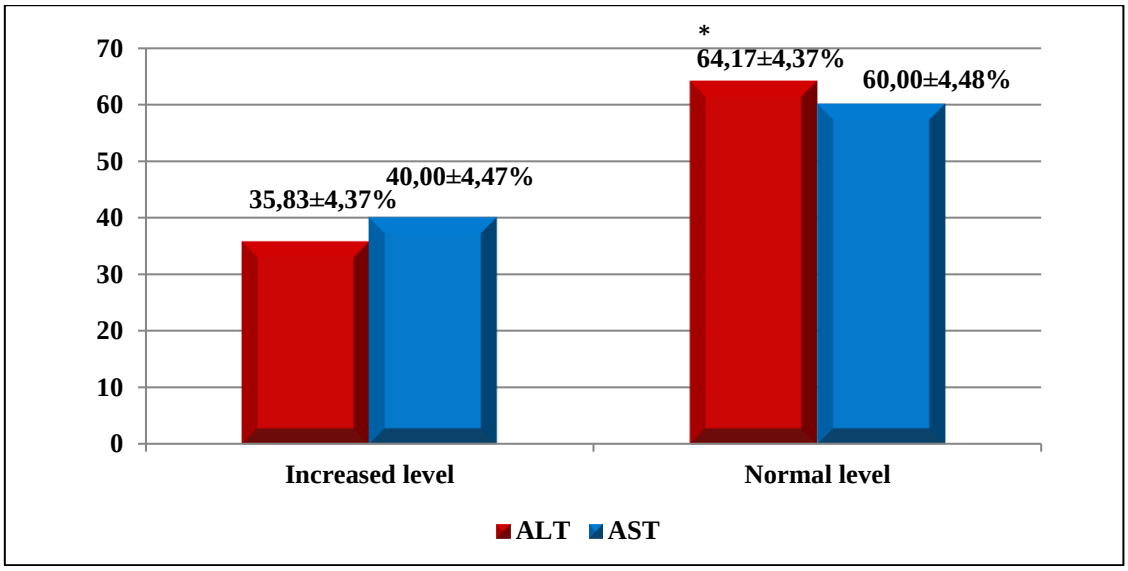

Fig. 1. Distribution of patients with acute brucellosis depending on serum ALT and AST levels.

Table 1. Analysis of the odds ratio for the increase in the level of cytolysis indicators in the serum of patients with acute brucellosis, depending on gender

Patients with acute brucellosis (n=120)	Males (n=90)		Females (n=30)		Differences between groups, p	OR (95% CI)
	Abs.	%	Abs.	%		
Patients with elevated ALT levels (n=43)	38	42.22±5.21	5	16.67±6.80*	0.015	3.65 [1.28-10.41]
Patients with normal ALT levels (n=77)	52	57.78±5.20	25	83.33±6.81		
Patients with elevated AST levels (n=48)	46	51.11±5.27	2	6.67±4.56*	0.0004	14.64 [3.29-65.13]
Patients with normal AST levels (n=72)	44	48.89±5.27	28	93.33±4.55*		

Note. * - p < 0.05 - statistically significant difference between male and female patients with acute brucellosis.

Table 2. The frequency of detection of the rs4986790 (Asp299Gly) polymorphism of the TLR-4 gene in patients with acute brucellosis depends on the severity degree

Genotypes TLR-4 (Asp299Gly)	Patients with acute brucellosis (n=120)					
	Mild degree (n=74)		Medium degree (n=35)		Severe degree (n=11)	
	Abs.	%	Abs.	%	Abs.	%
Genotype A / A	33	44.59±5.77	2	5.71±3.92 *	0	0
Genotype A / G	40	54.06±5.79	33	94.29±3.92*	9	81.82±11.62
Genotype G / G	1	1.35±1.30	0	0	2	18.18±11.62

Note. * p < 0.05 - between patients with acute brucellosis with different degrees of severity.

Gender-dependent peculiarities in patients with acute brucellosis with normal and elevated levels of transferases in serum were established. Thus, males had elevated levels of transferases 2.5 times more frequently compared to females. The same tendency was observed concerning the gender distribution of the AST level. Thus, the AST level elevation was registered only in 2 female patients, which amounted to 6.67±4.56%, compared to 51.11±5.27% male patients.

Male gender among patients with acute brucellosis was found to be associated with more frequent detection of the elevated serum ALT level (OR=3.65; 95% CI [1.28-10.41]; p=0.015). The same association was found for elevated serum AST (OR=14.64; 95% CI [3.29-65.13]; p=0.0004) (Table 1).

The A/G genotype of the TLR-4 gene was detected almost 1.74 times more frequently in patients with moderate acute brucellosis than in

patients with mild acute brucellosis.

Acute brucellosis was significantly more frequent (7.9 times) in carriers of homozygous genotype A/A, compared to patients with acute brucellosis of medium severity ($p<0.05$). No significant differences were found among carriers of the G/G genotype with different degrees of severity (Table 2).

The A/A genotype of the TLR-4 gene was detected in 44.59±5.77% of patients with a mild course of acute brucellosis, whereas among patients with moderate severity, this genotype was detected only in 5.71±3.92%.

No patient with a severe course of acute brucellosis was found among carriers of genotype A/A of the TLR-4 gene. In contrast, acute brucellosis was usually severe or moderately severe in carriers of the A/G genotype of the TLR-4 gene.

When analyzing the rs4986790 (Asp299Gly) polymorphism of the TLR-4 gene, a reliable difference was found only in carriers of homozygous genotype A/A between acute brucellosis patients and practically healthy individuals ($p <0.05$). Thus, genotype A/A of the TLR-4 gene was detected 3.4 times more frequently in patients with acute brucellosis without signs of liver damage than in patients with liver damage.

The A/A genotype of the TLR-4 gene was detected in 11.62±4.88% of individuals with acute brucellosis with liver damage, while in the case of acute brucellosis without signs of liver damage, it was observed in 38.96±5.56% of the patients. Among carriers of other genotypes, no significant difference was found in the groups of patients with and without liver damage.

Table 3. Frequency of detection of the rs4986790 (Asp299Gly) polymorphism of the TLR-4 gene among patients with acute brucellosis, depending on liver damage and in practically healthy individuals

Genotypes and alleles TLR-4 (Asp299Gly)	Acute brucellosis patients with liver damage (n=43)		Acute brucellosis patients without liver damage (n=77)		Healthy individuals (n=30)	
	Abs.	%	Abs.	%	Abs.	%
Genotype A / A	5	11.62±4.88	30	38.96±5.56 *	27	90.00±5.47*
Genotype A / G	35	81.40±5.93	47	61.04±5.56	2	6.67±2.27*
Genotype G / G	3	6.98±3.88	0	0	1	3.33±1.64
Allele A	45	52.33±5.38	107	69.48±3.71	56	93.33±3.22
Allele G	41	47.67±5.38	47	30.52±3.71	4	6.67±3.22 *

Note. * P < 0.05 - between patients with acute brucellosis with and without liver damage and healthy individuals.

Table 4. Association of alleles and genotypes for the rs4986790 (Asp299Gly) polymorphism of the TLR-4 gene and susceptibility to acute brucellosis with liver damage

Alleles and genotypes	Acute brucellosis patients with liver damage (n=43)	Healthy individuals (n=30)	χ^2	p	OR	
					Values	95% CI
Allele A	0.523	0.933	27.87	<0.0001	0.08	0.03 - 0.24
Allele G	0.477	0.067			12.76	4.25 - 38.29
Genotype A/A	0.116	0.900	44.65	0.001	0.02	0.01 - 0.07
Genotype A/G	0.814	0.067			61.25	12.03 - 311.76
Genotype G/G	0.070	0.033			2.18	0.22 - 21.98

Table 5. Dominant inheritance model of variant allele at polymorphism rs4986790 (Asp299Gly) in acute brucellosis patients with liver damage

Genotypes	Acute brucellosis patients with liver damage (n=43)	Healthy individuals (n=30)	χ^2	p	OR	
					Values	95% CI
Genotype A/A	0.116	0.900	44.09	<0.0001	0.02	0.01-0.07
Genotypes A/G +G/G	0.884	0.100			68.40	15.05-310.87

The G allele was significantly more frequent (7.2 times) in acute brucellosis patients with liver damage than in healthy individuals and 4.6 times more frequent in acute brucellosis patients

without liver damage ($p < 0.05$) (Table 3). Thus, in acute brucellosis patients with liver damage, the frequency of allele G was 47.67±5.38%, whereas in healthy individuals, this allele was detected only in 6.67±3.22% of cases.

The next stage was to determine the risks of acute brucellosis with liver damage, taking into account the carriage of polymorphic variants of the TLR-4 gene. When studying the allele frequency distribution in patients with acute brucellosis, it was found that carriers of the G allele of the polymorphic (Asp299Gly) TLR-4 gene had an increased risk of acute brucellosis with liver damage (OR=12.76, 95% CI [4. 25-38.29]), while in the case of carrying the A allele, on the contrary, the risk of acute brucellosis with liver damage was reduced (OR=0.0895% CI [0.03-0.24]), the model is significant at $\chi 2$ =27.87P < 0.0001) (Table 4).

The association of genotypes for polymorphism rs4986790 (Asp299Gly) of the TLR-4 gene and susceptibility to acute brucellosis with liver damage was also established. We found, a significantly increased risk of acute brucellosis with liver damage among carriers of the A/G genotype of the TLR-4 gene ($\chi 2$ =44.65; p=0.001; OR =61.25; 95% CI [12.03-311.76]), whereas carriage of the homozygous A/A genotype, on the contrary, had a protective effect against the development of acute brucellosis with signs of liver damage (OR =0.0295% CI [0.01-0.07]).

When analyzing the total frequency of A/G +G/G genotypes in acute brucellosis patients with liver damage and practically healthy individuals, we used the dominant model of inheritance of acute brucellosis (Table 5).

The combination of A/G +G/G genotypes in acute brucellosis patients with liver damage was detected 8.8 times more frequently than in practically healthy individuals (OR =68.40% CI [15.05-310.87]; $\chi 2$=44.09, p<0.0001).

CONCLUSIONS

1. In patients with acute brucellosis, the male gender was associated with a higher incidence of elevated serum ALT levels (OR=3.65; 95% CI [1.28-10.41]; p=0.015).

2. In carriers of homozygous genotype A/A, acute brucellosis was much more frequently (7.9 times) milder than in patients in whom the course of brucellosis was considered to be of medium severity (p<0.05).

3. Carriers of the G allele of the polymorphic (Asp299Gly) TLR-4 gene had an increased risk of acute brucellosis with liver damage (OR=12.76, 95% CI [4.25-38.29]), and conversely, carriers of the A allele had a decreased risk of acute brucellosis with liver damage (OR=0.0895%, CI [0.03-0.24]) model significant at $\chi 2$ =27.87, P < 0.0001).

4. Carriage of the homozygous genotype A/A had a protective effect against the development of acute brucellosis with signs of liver damage (OR =0.0295% CI [0.01-0.07])

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