Experimental and Applied Medical Science, 5, 5: 230-237, 2024. DOI: 10.46871/eams.1521348
Clinical Study

Evaluation of the Correlation Between Brucella Serum Agglutination Titre and Liver Involvement

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Abstract

Objective: *Brucellosis is a zoonotic infectious disease transmitted to humans. Typical symptoms of brucellosis are undulant fever, malaise and night sweats. It can lead to complications with involvement of many organs. Early and effective treatment strategies can be effective in preventing these complications. However, different complications can be seen in some cases. Liver involvement is one of these complications.*

Methods: *In this retrospective study, we aimed to investigate the relationship between serum agglutination titre and liver involvement in patients with brucellosis in a city in Turkey. Liver involvement was considered absent if ALT and AST <35 U/L and present if ALT and/or AST ≥35 U/L and patients were divided into two groups.*

Results: *A total of 970 patients with a standard tube agglutination test ≥1/20 were included in the study. 658 (67.8%) of the patients were female. Alanine aminotransferase (ALT) median 20 U/L (6-903) and aspartate aminotransferase (AST) median 21 U/L (9-716) were detected in pretreatment tests. The rate of liver involvement was 25.9% among all patients. A positive correlation was found between serum agglutination titres and serum transaminase levels in our study (AST, $r = 0.269$, $p < 0.001$; ALT, $r = 0.249$, $p < 0.001$).*

Conclusion: *The results of our study indicate that a positive correlation between high serum agglutination titre and liver involvement. In this study, we aimed to contribute to the literature on this subject.*

Key words: *Liver, Serum agglutination titre, Brucella*

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Introduction

Brucellosis is a zoonotic infectious disease transmitted from infected animals (sheep, goats, cattle, camels, pigs, or other animals) to humans. It is also known as undulant fever, Mediterranean fever, or Maltese fever. It is the most common zoonosis worldwide (1). The endemic areas of brucellosis include the Mediterranean basin countries, Central Asia, China, the Middle East, the Indian subcontinent, sub-Saharan Africa, Mexico, and parts of Central and South America. Approximately 500,000 cases are reported worldwide annually (2). Brucellosis is an endemic disease in Turkey. It is especially more common in the Southeastern and Eastern Anatolia regions of the country (3).

Typical symptoms in patients with brucellosis are insidious onset of undulant fever, malaise, and nocturnal sweating. Accompanying symptoms include arthralgia, chills, anorexia, weight loss, depression, headache, low back pain, myalgia, fatigue, and concomitant localised infection-related symptoms. Asymptomatic course may be observed in some patients. One or more focal involvement complications of brucellosis are observed in approximately 30% of cases with a range of 6-92% according to literature data (4, 5). Osteoarticular involvement (spondylitis, sacroiliitis and peripheral arthritis) is the most common form of involvement in focal brucellosis and is observed around 40-50 % (1). Genitourinary involvement is the second most common form of focal involvement due to brucellosis. It may lead to orchitis, epididymitis, prostatitis and testicular involvement in males and tubo-ovarian abscess in females. It is observed in approximately 10 % of cases. Neurological involvement (encephalitis, brain abscess, neuritis, myelitis, etc.), pulmonary involvement (interstitial pneumonia, bronchitis, lobar pneumonia, etc.), intra-abdominal involvement (hepatic or splenic abscess, cholecystitis, pancreatitis, etc.), ocular involvement (keratoconjunctivitis, corneal ulcer, iridocyclitis, etc.), cardiovascular involvement (endocarditis, myocariditis, pericarditis, thrombophilebitis, etc.), dermatological involvement (maculopapular, papulonodular or erythema nodosum-like rashes, granulomatous vasculitis, etc.) are complications caused by brucellosis. Brucellosis may affect any organ system (6).

Brucellosis can be transmitted to humans by consumption of unpasteurised infected milk and meat products, contact of skin or mucous membranes with infected animal tissue (placenta, etc.), contact with infected animal fluids (milk, blood, urine, etc.),

inhalation of infected aerosols or inoculation into the eye. Consumption of unpasteurised dairy products (raw milk, butter, cheese and ice cream) is the most common route of transmission (7).

The definitive diagnosis of brucellosis can be made,

1) by culture of the bacteria from blood, body fluids (cerebrospinal fluid, synovial fluid, urine, pleural fluid, etc.) or tissue (bone marrow or liver biopsy) or

2) in the presence of a quadrupling or more in *Brucella* antibody titre between serum samples taken ≥2 weeks apart during the acute and convalescent phase. Possible diagnosis of brucellosis can be made by standard tube agglutination test ≥1:160 in a serum sample taken after the onset of symptoms or by detection of *Brucella* DNA in a clinical sample by polymerase chain reaction test (8).

In the literature, there are some studies investigating the relationship between serum agglutination titre and brucellosis complications. The study included not only cases with brucella serum agglutination titre >1/160 but also those with <1/160 according to the diagnostic criteria because our aim was to evaluate the relationship between serum agglutination titre and liver involvement.

Materials and Methods

Study design and sample size: In our study, the data of patients who were asked for brucella test (Rose-Bengal, standard tube agglutination, Coombs standard tube agglutination) between 01.01.2013 and 10.04.2023 in a tertiary education and research hospital in Turkey were scanned. Those with negative results of all three tests and only those with positive Rose-Bengal test were excluded from the study. The data of those with positive Rose-Bengal test, standard tube agglutination and Coombs standard tube agglutination tests were analysed. Patients with incomplete data, patients with previous brucellosis, patients who were started brucellosis treatment, hepatitis B surface antigen positive, hepatitis C antibody positive, patients with known chronic liver disease, alcohol users, drug users for any reason and patients with comorbid diseases were not included in the study. In this retrospective study, a total of 970 individuals with brucella standard tube agglutination titre ≥1/20 were included.

Statistical analysis: Median, minimum, and maximum values were given for the quantitative variables used in the study. Liver involvement was considered absent if alanine aminotransferase (ALT) and aspartate aminotransferase (AST) <35 U/L and present if ALT and/or AST ≥35 U/L and the patients were divided into two

groups. The conformity of serum agglutination titre measurement of patients with brucellosis to normal distribution was analysed by Kolmogorov-Smirnov test and it was found that it did not conform to normal distribution ($p > 0.05$). Mann-Whitney U test was used to compare the variables according to liver involvement. Logistic regression analysis was performed to determine whether gender, age and serum agglutination titre variables affected liver involvement, and the reference value was taken as male (first).Spearman correlation analysis was used to determine the correlation between ALT and AST quantitative values and serum agglutination titre measurement. Statistical analyses were performed using IBM SPSS v25 (IBM SPSS, Inc., Chicago, IL, USA) and statistical significance level $p < 0.05$ was accepted.

Ethical approval: This study complied with the standards of medical ethics as so endorsed by decision 300.30.09, dated 21.09.2023, of the Ethics Committee of Gaziantep Islam Science and Technology University.

Results

Brucella tests were performed in 151335 patients. 147622 patients had negative results of all three tests. Among the remaining 3713 patients, patients with positive Rose-Bengal test but negative standard tube agglutination test were excluded from the study and the data of 1644 patients were analysed. Patients with incomplete data, hepatitis B surface antigen positive, hepatitis C antibody positive, previously known chronic liver disease, alcohol users, drug users for any reason and patients with comorbid diseases were excluded. A total of 970 patients with standard tube agglutination test ≥1/20 were included in the study .

658 (67.8%) of the patients were female and 312 (32.2%) were male.AST median 21 U/L (9-716), ALT median 20 U/L (6-903) were found. Liver involvement was not detected in 719 patients (74.1%), while liver involvement was present in 251 patients (25.9%). No statistically significant difference was found in terms of age of the patients according to liver involvement status ($p = 0.070$). When serum agglutination titre values are compared according to the liver involvement status of the patients, there is a statistically significant difference ($p<0.05$) (Table 1).

Table 1. Mann-Whitney U results by liver involvement.

	Liver involvement (n=251)	No-liver involvement (n=719)	p-value
Age	41 (7-79)	44 (4-93)	0.070
Serum agglutination titre	320 (20-5120)	80 (20-2560)	**<0.001**

It is observed that serum agglutination titre value is high in patients with liver involvement. A positive correlation was found between serum agglutination titres and ALT levels, r = 0.249, p < 0.001) (Figure 1).

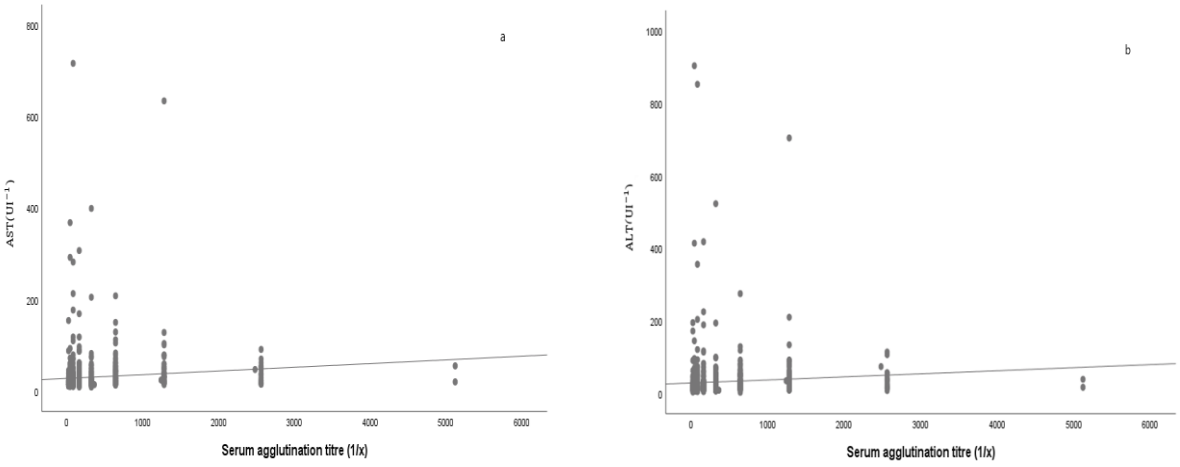

Figure 1. A positive correlation was found between serum agglutination titres and serum transaminase levels. (a) AST, r = 0.269, p < 0.001. (b) ALT, r = 0.249, p < 0.001.

According to the results of logistic regression analysis, the effects of age, serum agglutination titre and gender variables on liver involvement were found to be statistically significant (p < 0.05). A one-year increase in age reduces the risk of liver involvement by 2%. A one time increase in serum agglutination titre variable increases the risk of liver involvement by 1.001 times (95% CI 1.000-1.0001). The risk of liver involvement in males was 2.087 times higher than in females, in other words, the risk of liver involvement in females was 0.521 times lower than in males (Table 2).

Table 2. Logistic regression analysis of factors affecting liver involvement.

	B	S.E.	Wald	p-value	Exp(B)	95% CI
Age	-0.020	0.003	52.463	<**0.001**	0.980	0.975-0.985
Serum agglutination titre	0.001	0,000	44.905	<**0.001**	1.001	1.001-1.001
Gender	-0.736	0.146	25.449	<**0.001**	0.479	0.360-0.637

S.E: Standard error, **CI:** confidence interval, **Exp (B):** Odds ratio.

Discussion

The liver is the most commonly affected organ in patients with active brucellosis. Approximately 50% of patients have clinical and biochemical data related to liver involvement. The most common clinical presentation of liver involvement is hepatomegaly. Liver biopsies from patients with brucellosis showed necrosis of liver cells, parenchymal lesions and granuloma in liver tissue caused by inflammation. In brucella-related hepatitis, an increase in serum aminotransferase levels is observed in 5-40% of patients (9, 10).

There are studies showing that the increase in serum aminotransferase levels in brucellosis patients is related with age. In the pediatric age group, the increase in serum aminotransferase levels has been observed at 60% and even higher rates in older adults, while in the young adult group it has been observed at a rate of 25% (11-13). In the study by Sahinturk et al. no effect of age and gender on liver involvement was demonstrated (p = 0.46) (14).

Various serological methods are used in the diagnosis of brucellosis that enable the detection of antibodies against lipopolysaccharide or other antigens. The most commonly used serological methods are serum agglutination titre and enzyme-linked immunosorbent assays (ELISAs). The risk of clinical progression and complications can be predicted with symptoms and signs such as myalgia, low back pain, weakness/fatigue, weight loss, splenomegaly, and easily accessible laboratory parameters such as serum agglutination titre (15).

The correlation between serum agglutination titre and complications has been researched for years. There are mostly related case reports in the literature. In a meta-analysis of *Brucella* spp. endocarditis cases, it was determined that a Wright agglutination titre above 1/1280 at the time

of diagnosis significantly increased the risk of mortality (16). Brucellosis-related complications have been reported in patients even at low titre standard tube agglutination values. In the case report by Khorvash et al., a 26-year-old male patient had a standard tube agglutination test result of 1/80 and liver involvement was observed (17). Kayaaslan et al. analysed long-term osteoarticular, neurobrucellosis, epididymo-orchitis and hepatic complications in 700 brucellosis patients. Patients were divided into two groups as those with and without complications, and the standard tube agglutination titre was ≥1/160 in 97.1% of patients with complications and 97.8% of those without complications ($p = 0.584$) (18). In a study of 195 patients, it was found that high standard tube agglutination titre was an independent risk factor for liver involvement. In the study, the mean standard tube agglutination titre was found to be higher in patients with liver involvement compared to those without (1/485 and 1/306, respectively) ($p = 0.001$). A positive correlation was found between serum transaminase levels and serum agglutination titres (AST, $r = 0.164$, $p = 0.022$; ALT, $r = 0.138$, $p = 0.054$) (14). Similarly, a positive correlation was found between serum agglutination titres and serum transaminase levels in our study (AST, $r = 0.269$, $p < 0.001$; ALT, $r = 0.249$, $p < 0.001$).

In the study of Akritidis et al. no effect of gender and age on liver involvement was observed in 14 patients with brucellosis, 9 of whom were isolated from blood culture and 5 of whom were serologically diagnosed (9). In our study, liver involvement was found to be more common in males than females.

Limitations

The limitation of the study was the lack of physical examination and ultrasonographic hepatomegaly and splenomegaly data. We did not have radiologic and histopathologic data of our patients.

Conclusion

In conclusion, brucellosis is a zoonotic disease that should be followed closely in terms of diagnosis, treatment, and complications especially in endemic regions. Brucellosis is a public health problem that can be prevented with effective strategies. In this study, a positive correlation between high serum agglutination titre and liver involvement was shown. In the literature, there is a need for multicentre studies in which more patients are evaluated to examine the correlation between serum agglutination titre and complications.

Conflict of interest: The authors declare that they have no relevant conflict of interest.

Acknowledgments: None.

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