Liver involvement is associated with blood culture positivity and high agglutination titer in patients with brucellosis in Turkey

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List of abbreviations

LI, liver involvement

BC, blood cultures

SAT, serum agglutination titer

ALT, alanine aminotransferase

AST, aspartate aminotransferase

OR, odds ratio

CI, confidence interval

SD, standard deviation

Abstract

We investigated the incidence and risk factors for liver involvement (LI) in patients with brucellosis in Turkey. Diagnosis was established by blood cultures (BC) or compatible clinical presentation accompanied by serological evidence. Patients received either a combination of doxycycline plus rifampin for 6 weeks or doxycycline for 6 weeks plus streptomycin intramuscularly for the first 2-weeks. Liver function tests including alanine (ALT) and aspartate aminotransferase (AST) were monitored before and at the end of treatment. 195 patients were included in the study. 70 patients had elevated transaminases before treatment. 26 patients had a positive BC for brucellosis. In multivariate analysis, BC positivity (OR=2.44, 95%CI 1.03-5.78, p=0.043) and serum agglutination titer (SAT) (OR=1.001, 95%CI 1.00-1.002, p=0.018) was found to be associated with LI. Serum aminotransferase levels were normalized in all patients with brucellosis. BC positivity and high SAT are independent factors associated with LI in patients with brucellosis.

Brucellosis is a chronic zoonotic infection and among the most widespread zoonoses globally (1). It is still endemic, especially in the eastern and southeastern regions of Turkey (2). The disease has a wide variety of clinical manifestations. It has a well-known predilection for reticuloendothelial system which may manifest as hepatitis, hepatomegaly, splenomegaly and peripheral lymphadenopathy (3). There is no universal definition to determine the liver involvement (LI) in brucellosis. Depending on the study and the definition of LI, the reported frequency of hepatic manifestations is variable. Yet, because the liver is the largest reticuloendothelial system organ of the human body, its involvement in brucellosis is common in a wide clinicopathologic spectrum from mild and transient transaminitis to a severe disease as hepatic abscess. In studies that have liver biopsy to detect hepatic involvement, some degree of histopathological changes at least can be attributed to brucellosis almost invariably. Common histopathological findings after liver biopsy include inflammatory infiltration, parenchymal necrosis, Kuppfer's cell hyperplasia, and granulomas (4). In different studies, LI has been reported between 25-40% of the patients with brucellosis (5, 6), however factors associated with LI are not well-established. In the present study, we investigated the incidence and predictor factors of LI in patients with brucellosis.

Patients with a diagnosis of brucellosis are recorded prospectively in a patient registry in the Department of Gastroenterology, Van Training and Research Hospital, Van, Turkey. The city of Van is located in the easternmost part of Turkey, on the Iranian border with a high reported prevalence of brucellosis in humans (7). Consecutive patients who were diagnosed with brucellosis between January 2012 and June 2013 were included in this single center, retrospective study. An institutional ethics committee approval was not required, due to retrospective analysis of the patient records, in accordance with the National Code on Clinical Research published in Official Gazette numbered with 28617 at Apr 13. 2013 (8). The diagnosis of brucellosis was established by isolation of the pathogen from blood cultures or compatible clinical presentation accompanied by serological evidence which is defined as a serum agglutination titer $\geq$1/320 or seroconversion at any titer during the work-up of the patient. All patients underwent physical examination, and laboratory investigations including alanine aminotransferase (ALT), aspartate aminotransferase (AST) using standard laboratory techniques. *Brucella* serology and blood culture with automated system (BACTEC, Becton–Dickson Diagnostic Instrument System, Franklin Lakes, NJ, USA) were obtained before initiating any anti-bacterial treatment. Brucella

serologic tests were carried out using the standard tube agglutination test as described previously (9).

Patients received either a combination of doxycycline 100mg twice daily plus rifampin 600 mg/day orally for 6 weeks or doxycycline 100mg orally twice daily for 6 weeks plus streptomycin 1 g/day intramuscularly for the first 14 days. Liver function tests including ALT and AST were monitored before and at the end of antibiotic therapy. The presence of LI was defined as persistently having AST and/or ALT levels higher than the upper limit of normal (40 U/l) in at least 2 consecutive measurements. In every patient with an elevated AST and/or ALT level, hepatitis B surface antigen and hepatitis C antibody were obtained to rule out chronic viral hepatitis and ultrasonography was done to detect possible biliary and/or neoplastic disease. Drug toxicity and recent or chronic alcohol use was excluded in all patients with elevated transaminases.

Statistical analyses were performed using IBM SPSS v22 (IBM SPSS Inc., Chicago, IL, USA). Continuous variables are presented as mean ± standard deviation (SD) or median (range) while categorical variables were expressed as frequencies (%). Comparisons of continuous variables between groups were performed by independent samples Student's t-test. Differences between frequencies were evaluated using Pearson chi-square or Fisher's exact test when necessary. Univariate and multivariate logistic regression analyses were performed to identify predictors of LI in patients with brucellosis. A two tailed *P*-value <0.05 was considered statistically significant.

A total of 195 patients (mean age 34±16 years, 127 male) with a diagnosis of brucellosis were included in the study. The number patients with a positive blood culture for brucellosis was 26 (13%). Median AST and ALT levels were 30 (range, 10-240) and 28 (9-253) U/l, respectively. 70 patients (36%) had an elevated AST and/or ALT level before the treatment. None of those patients with elevated transaminases had a positive serology for hepatitis B or C virus. Examination of liver by ultrasonography excluded mass lesion, abscess or biliary pathology in all patients. The frequency of LI was significantly higher in patients with a positive blood culture (14/26, 54% vs. 56/169, 33%, p=0.04) (Figure 1). 128 patients received combination of doxycycline plus rifampin, and 67 patients received doxycycline plus streptomycin. All patients

successfully completed the course of antibiotic treatment and none of them experienced any serious adverse events that require interruption of antibiotic therapy. Adverse events reported by the patients were either mild in severity or were unattributable to the antibiotic treatment. Most common adverse events were non-specific and gastrointestinal in origin.

Univariate analyses revealed that age and gender have no influence on the presence of LI in patients with brucellosis. There was no difference in mean age of patients with or without LI (32.7 ± 16.0 vs 34.5 ± 15.7 years, p=0.46). Mean AST and ALT levels were comparable between male and female patients (AST; 38 vs 40 U/l, p=0.62; ALT; 39 vs 37 U/l, p=0.67; respectively). The number of patients with LI were 46/127 (36%) and 24/68 (35%) in male and female patients, respectively (p=0.89).

Mean serum agglutination titer in patients with LI was significantly higher than in patients without LI (1/485 vs. 1/306, respectively, p=0.001). A positive correlation was found between serum agglutination titers and serum transaminase levels (AST, r=0.164, p=0.022; ALT, r=0.138, p=0.054) (Figure 2). In multivariate analysis, culture positivity (OR=2.44, 95% CI 1.03-5.78, p=0.043) and high serum agglutination titer (OR=1.001, 95% CI 1.00-1.002, p=0.018) were found to be independently associated with presence of LI (Table 1). All patients had a complete clinical response at the end of antibiotic therapy. During the follow-up after treatment cessation no relapse was observed. At the end of antibiotic therapy serum aminotransferase levels were normalized in all patients with brucellosis.

LI is a common clinical manifestation of brucellosis and most patients do not need a liver biopsy to diagnose liver disease. Compatible clinical presentation accompanied by serological evidence is almost always sufficient for a timely and accurate diagnosis. Isolation of the pathogen from blood culture is generally rare, as it is in our study. Most patients with brucellosis and LI either have mild transaminase elevation or liver-related symptoms are generally missing. In rare severe cases a liver abscess, which is mostly associated with *Brucella suis* infection, can develop which may require drainage in addition to antibiotics treatment. In our series, there were no single case of liver abscess which can attributed to that *Brucella melitensis* and *Brucella abortus* are the species usually isolated from cases in Turkey (10-12). In our study, we excluded most common liver diseases including viral hepatitis, biliary and neoplastic diseases that can be associated with elevated liver enzymes. We did not obtain tests for rare diseases such as autoimmune liver

diseases, Wilson's disease and hereditary hemochromatosis. We think that exclusion of those liver diseases is not necessary in the clinical setting of brucellosis, especially when clinical suspicion of a primary liver disease is absent considering the unnecessary expenses of advanced test. Nevertheless, in some cases further tests with a liver biopsy can be required to exclude coexistence of a primary liver disease, especially when transaminases are very high, there is evidence of a liver disease, or liver enzymes do not normalize after appropriate antibiotic treatment. If a liver biopsy is performed, histopathological findings are generally mild and always non-specific. In earlier preclinical studies, it was reported that the type of inflammatory cell infiltration is predictive of liver outcome. The authors reported that there is a relationship between monocytic infiltration and granuloma in liver histology, versus polymorphonuclear cell infiltration and liver abscess (13-15).

In the present study, one-third of the patients with brucellosis were detected to have elevated transaminases considered as LI. Positive blood culture and high agglutination titer were independently associated with LI in patients with brucellosis, which can be interpreted as the duration, severity and immune response against the disease is associated with LI. In a study that included 14 patients with brucellosis who underwent liver biopsy, all patients exhibited granuloma formation and varying degrees of parenchymal inflammatory cellular infiltration (4). In the absence of liver biopsy, we cannot exclude that patients with normal transaminases did not have some degree of LI, which is a limitation in our study. Although elevated transaminases are not sufficiently sensitive and specific for diagnosis of LI in patients with brucellosis, it would be clinically unnecessary and unethical to perform liver biopsy due its invasive nature. The diagnosis of LI would be more accurate if we could perform transient elastography before and after treatment, which can be considered in a prospective study. None of the patients had a severe clinical presentation that can be attributed to relatively early diagnosis of cases. This is most likely due to higher physician awareness because of decades of high incidence in that region. The retrospective setting and lack of long-term follow-up are other limitations of the present study.

In conclusion, we showed that positive blood culture and a high serum agglutination titer are independent factors associated with the presence of LI in patients with brucellosis. The complete resolution of LI is universal in patients receiving proper antibiotic regimen for an optimum duration.

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Conflicts of interest

All authors declare that there is no conflict of interest.

Funding statement

No funding was received for this study.

Ethical statement

There was no experimental work with humans in the present study and the study conforms to the ethical guidelines of the 1975 Declaration of Helsinki. An institutional ethics committee approval was not required, due to retrospective analysis of the patient records, in accordance with the National Code on Clinical Research published in Official Gazette of Turkey numbered with 28617 at Apr 13. 2013.

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Table 1. Univariate and multivariate logistic regression analysis **of factors associated with liver involvement in patients with brucellosis** (N=195, R^2=0.084)**.**

	Univariate			Multivariate		
	OR	95% CI	*p* value	OR	95% CI	*p* value
Age	0.99	0.97-1.01	0.460			
Gender	1.04	0.56-1.93	0.898			
Blood culture positivity	2.35	1.02-5.43	***0.044***	2.44	1.03-5.78	***0.043***
Serum agglutination titer	1.001	1.000-1.002	***0.013***	1.001	1.000-1.002	***0.018***

OR, odds ratio (exp*B);* CI, confidence interval

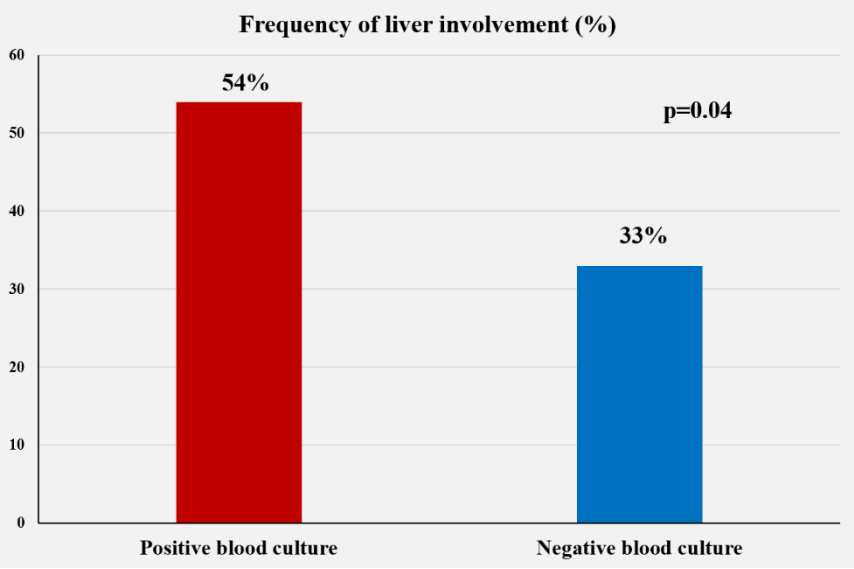
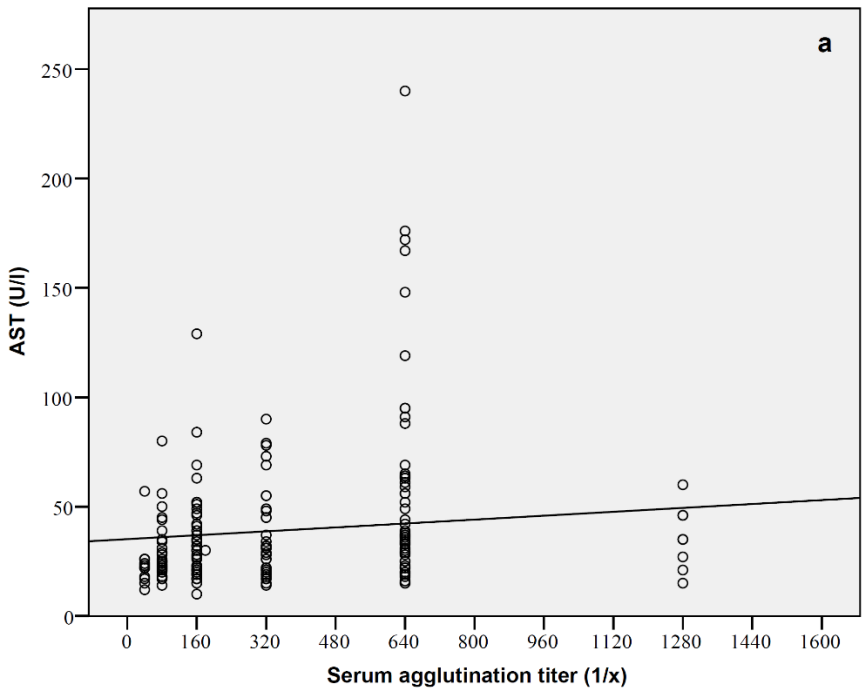


Figure 1. The frequency of liver involvement was significantly higher in patients with a positive blood culture (14/26, 54% vs. 56/169, 33%, p=0.04).



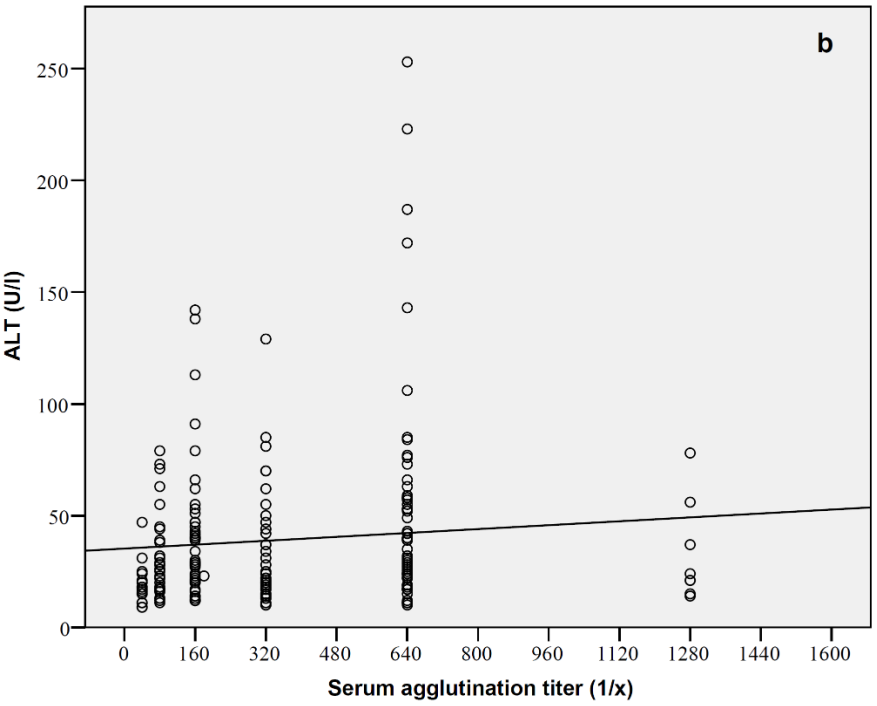


Figure 2. A positive correlation was found between serum agglutination titers and serum transaminase levels (2a, AST, r=0.164, p=0.022; 2b, ALT, r=0.138, p=0.054).