Brucellosis as a rare cause of granulomatous hepatitis with hepatic and bone marrow granulomas: A case report

Kendal Yalçın[1], 0000-0003-2137-7934
Elif Tuğba Tuncel[2], 0000-0002-0908-1949
Feyzullah Uçmak[1], 0000-0002-2677-3971
Remzi Beştaş[3], 0000-0001-5302-8953

[1] Division of Gastroenterology and Hepatology, Department of Internal Medicine, Dicle University School of Medicine, Diyarbakır, Turkey
[2] Manisa City Hospital, Department of Gastroenterology, Manisa, Turkey
[3] Dicle Memorial Hospital, Department of Gastroenterology, Diyarbakır, Turkey

Corresponding Author: Kendal Yalcin, MD
Division of Gastroenterology and Hepatology, Department of Internal Medicine
Dicle University School of Medicine, 21280, Diyarbakır, Turkey
Fax: 0 412 2488523
Phone: 0 532 3724782
E-mail: kendalyalcin@hotmail.com

ABSTRACT

Brucellosis may involve the liver and data on histopathology of the liver in brucellosis are limited. Brucellosis is characterized by high fever, joint-back pains and hepatosplenomegaly. In this report, a case of granulomatous hepatitis with granulomas in liver and bone marrow, who admitted to our clinic with non-specific symptoms, hepatomegaly, splenomegaly, digital clubbing, along with laboratory signs of intrahepatic cholestasis, is presented. Granulomas were detected in bone marrow and hepatic specimens of the patient. The diagnosis of brucellosis was based on isolation of *Brucella mellitensis* from blood culture accompanied by serum agglutination titers of 1:640. By the specific brucellosis treatment, laboratory and clinical findings were improved. Especially in endemic areas, in cases of elevated transaminase levels with the presence of related clinical findings, brucellosis should be considered . Also brucellosis should be considered in differential diagnosis of intrahepatic cholestasis and/or granulomas in hepatic and bone marrow biopsies. This case report presents various histopathological features and provides detailed information in the liver involvement of brucellosis.

Key words: Brucellosis, granulomatous disease, liver, bone marrow

INTRODUCTION

Hepatic granulomas are chronic inflammatory lesions consist of enlarged, modified macrophages (epitheloid histiocytes), which are generally surrounded by fibroblasts and lymphocytes. Hepatic granulomas are detected in 2-15% of hepatic biopsies in general. There are many causative factors among etiologies of hepatic granuloma cases in which infectious factors being the leading cause (tuberculosis, cyst hydatic, syphilis, brucellosis, typhoid fever, chronic hepatitis B and C, Epstein Barr virus infection, HIV, fungal infections, sarcoidosis, primary biliary cirrhosis, Hodgkin's disease, drug reactions, polymyalgia rheumatica etc.).[1,2]

Brucellosis is transmitted to human beings by occupational contact or by contaminated milk from animals.[3,4] Its clinical appearance may mimic many diseases. It may cause localized disease involvement in nearly all organs. It is most frequently localized in the bones, joints, central nervous system, heart, lung, spleen, testes, liver, gallbladder, kidneys, prostate and skin. It may lead to granulomatous hepatitis in the cases of liver involvement.[5,6]

Our aim to present this case is to emphasize that reactive hepatitis is not always the cause of elevations in liver functional tests observed in brucellosis, and that rarely

encountered granulomatous hepatitis should also be considered. In addition, we aimed to investigate the histopathological features of liver involvement in brucellosis, in which there is limited available data.

CASE REPORT

Fifty-four years old, freelance worker, male patient admitted to our institution with the complaints of abdominal pain, headache, knee pain, fatigue and weight loss, which were increased in the last one and a half months. The patient indicated that he lost weight (10 kg) in the last 4 month-period. There was no significant finding in his medical history other than smoking habbit for 2 pacs/day.

His general status was good, conscious and cooperative. His vital signs were as follows: blood pressure 80/60 mmHg, heart rate 98/minute, respiratory rate 14/minute and fever 370C. In his physical examination conjunctivas were pale, there were both hepatomegaly and splenomegaly, which were passing the costal margin by 5-6 cm in the midline, and digital clubbing.

Laboratory tests at the time of his admission were: hemoglobin 9 gr/dl, hematocrit 26%, leukocyte count 3600/mm3, platelet count 151,000/mm3, MCV 82 fl, erythrocyte sedimentation rate (ESR) 41 mm/hour, serum iron 19 ug/dl, serum iron binding capacity 153 ug/dl, C-reactive protein 34 mg/dl, and rheumatoid factor 71. Baseline biochemical findings were: ALT 41 U/L, AST 72 U/L, alkaline phosphatase 523 U/L, gamma-glutamyl transferase 62 U/L, albumin 2.6 gr/dl, globulin 4.1 gr/dl, total bilirubin 0.8 mg/dl, fasting blood glucose 83 mg/dl, urea 28 mg/dl, creatinine 0.8 mg/dl, LDH 394 U/L, total cholesterol 135 mg/dl, sodium 132 mmol/L, potassium 4.8 mmol/L, chloride 103 mmol/L and calcium 7.6 mg/dl. In the upper abdominal ultrasonography, the liver was enlarged (190 mm), its parenchyma was hypoechoic and spleen was in normal structure, but enlarged (150 mm). In Doppler ultrasonography, vascular structures were within normal limits. Upper gastrointestinal system endoscopy and PA chest roentgenogram were normal.

There were physical and laboratory findings related to hepatomegaly, splenomegaly, digital clubbing, anemia, leukopenia, high ESR and intrahepatic cholestasis. The patient was evaluated for hematological diseases (lymphoma, leukemia) and intrahepatic cholestasis (of infiltrative and infectious causes). Peripheral blood smear and bone marrow aspiration were performed. Hypochromatic microcytic anemia was diagnosed by the peripheral blood smear with no sign of hemolysis. Examination of bone marrow aspiration was non-diagnostic. Anti-Toxoplasma IgM, anti-cytomegalovirus IgM, anti-Epstein Barr virus IgM, anti-rubella IgM,

HBsAg, anti-HBs, anti-HBc IgG, anti-HCV, anti-HAV IgM, VDRL, and anti-HIV were all negative in serological analysis. Tuberculin skin test was also negative.

Hemoglobin electrophoresis, hemolysis tests, and thyroid function tests were all normal. There was only a mild increase in the tumor marker CA 19.9 (77 U/ml). In protein electrophoresis, gamma globulin band was increased to a value of 24.2%. Autoimmune markers including ANA, ASMA, LKM-1, AMA, AMA-M2, P-ANCA and C-ANCA were negative.

The patient underwent bone marrow biopsy, which revealed signs of granulomatous myelitis (Figure 1). The patient had no dilatation in intra and extrahepatic bile ducts with elevated levels of transaminases and cholestatic enzymes. The rationale for performing liver biopsy was first to rule out a hepatic disease and then to exclude other causes of liver pathology in the setting of intrahepatic cholestasis. Written informed consent for liver biopsy was obtained from the patient.

The biopsy was reported as granulomatous hepatitis without caseous necrosis and Langerhans type giant cells (Figure 2a-b). Other biopsy findings were patchy proliferation areas in bile ducts (signs of intrahepatic cholestasis), dense mononuclear inflammatory cell infiltrations in portal areas, bile pigment accumulation, mild mononuclear cell infiltration in local areas of the parenchyma (focal lytic necrosis) and minimal interface hepatitis. Hepatic biopsy specimen was evaluated by Ziehl-Neelsen staining and no acid resistant bacilli was detected. Meanwhile, Rose Bengal and brucellosis agglutination test results were reported to be positive (1:640). *Brucella melitensis* was grown in the blood cultures. Lumbosacral and pelvis graphies demonstrated signs of sacroileitis. The patient was administered rifampicin 600 mg/day and doxycycline 200 mg/day, for six weeks duration. There were improvements by the therapy, in the clinical, hematological and biochemical parameters with resolution of the laboratory signs of cholestasis. The patient was in complete clinical remssion and free of symptoms, during the 6 months of follow-up period.

DISCUSSION

Hepatic granulomas are chronic inflammatory lesions, which developed due to late type hypersensitivity reactions against antigenic stimulation. Hepatic granulomas are detected in 12-15% of hepatic biopsies generally.[1,2] Sarcoidosis, Hodgkin's disease, drug reactions, polymyalgia rheumatica, tuberculosis, brucellosis, typhoid fever, hydatid cyst, syphilis, chronic hepatitis B and C, EBV, HIV and fungal infections should be considered in the

differential diagnosis in patients with granulomatous hepatitis and hepatic granuloma in their hepatic histologies. 10%-15% of the cases are still idiopathic.

Primarily involved laboratory tests in the differential diagnosis are skin tuberculine test, serum ACE level, AMA, PA chest graphy and computed tomography (if necessary), VDRL, HIV, *Brucella* agglutination tests, anti-HCV and HBsAg. Additionally, it is important to ask for the possible hepatotoxic agents. In the present case, after the appropriate tests for differential diagnosis were performed, the patient was diagnosed as brucellosis and he was cured with appropriate treatment. Patient in this case report admitted to our institution with complaints of non-specific symptoms like headache, abdominal pain and knee pain and fatigue, rather than classical signs of brucellosis like fever and sweating.

Brucellosis, primarily being a disease of wild and domestic animals, is transmitted to human beings by animal breeding, butchering or through contaminated dairy products.3,4 Brucellosis is characterized by high fever, night-sweats, fatigue, joint-back pains and hepatosplenomegaly. Although morbidity of brucellosis in our country is considerably high, it is a disease with very low mortality. Brucellosis is widespread and a serious public health problem in eastern and western Anatolia of Turkey.[7-9]

Definitive diagnosis of brucellosis is made by culturing the agent in blood, bone marrow or infected tissue cultures. Although growth in blood cultures show variations (15-70%), as it is an intracellular microorganism, the culture growth rate is around 90% in the bone marrow. The disease is mainly diagnosed by indirect methods as patients used various antibiotics before the brucellosis diagnosis, and there are no culturing facilities in healthcare units at rural areas. For this reason, various serological tests, including serum agglutination tests (SAT), Rose Bengal, immunofluorescence antibody (IFA) tests and ELISA, are being used.[3,4] However, SAT, when compared to others, seems to be a standard method. While values of 1:160 and above are diagnostic in this test, false negative results may also be seen. Ozkurt et al. reported SAT positivity rate of 96% in their series of 50 patients. This reported rate was 100% in 30 patients with acute brucellosis, %94.1 in 17 patients with subacute brucellosis, and 66.6% in 3 patients with chronic brucellosis.[10]

Hepatic involvement is variable in brucellosis.[11] In some papers, hepatic involvement in brucellosis is reported up to 10%. However, this is generally in reactive hepatitis form. Hepatitis, expressed as a modest increase in serum transaminase levels, is observed in 25% of the patients, ranging from 5% to 40%.[11,12] Involvement of the liver is common in human brucellosis but liver brucelloma or pseudotumor necrotizing granuloma is a very uncommon negative effect of this infection, observed only in 1.7 % of individuals. Sometimes there may

be non-caseified granulomas, whereas pyogenic *Brucella* abcess is also rarely reported. Although a progressive hepatic disease is defined, this situation is very rare.[11]

Granulomas are frequently encountered in the liver because of its rich blood supply and dense reticuloendothelial cell content. Granulomas develop as a response of immune system against indigestible foreign substances by inflammatory cells and macrophages. Substances, which can not be digested by macrophages, may be typically infectious microorganisms (mycobacterias, cystosomas, etc) and materials, which are injected or taken by oral route (talk, rock oil, drugs, etc).[13]

Sarcoidosis and tuberculosis constitute over one third of the granulomatous hepatic diseases. Despite detailed examinations, 10% of the cases are remained undiagnosed.6 In Turkey, it has been demonstrated that infectious causes are responsible for over the half of hepatic granuloma cases (tuberculosis, hydatid disease, brucellosis and typhoid fever).[14] Additionally, hepatic granuloma presence in variable proportions are reported in histological examination of chronic viral hepatitis.[15,16] It is reported in two studies conducted in our country that hepatic granulomas are rarely encountered in chronic hepatitis B (1.5%) and C (1.3%) patients.[17,18] Brucellosis may present itself in different clinical forms by involving various organs; even atypical cases with peritonitis, meningitis and ascites are also reported.[19,20] The present case is a patient, who admitted with non-specific symptoms and intrahepatic cholestasis signs in addition to hepatic and bone marrow involvements. Since this patient's intra- and extrahepatic bile ducts were not dilated, but his transaminases and cholestatic enzymes were elevated, intrahepatic cholestasis pattern was suspected. Hepatic biopsy to differentiate infiltrative and infectious causes was performed. Differential diagnosis for various autoimmune diseases (primary sclerosing cholangitis), infiltrative diseases (amyloidosis, sarcoidosis, lymphoma and granulomatous diseases) and Stauffer syndrome (Hodgkin's disease, renal cell carcinoma) is possible by hepatic biopsy, which is accepted as the gold standard in intrahepatic cholestasis. Brucella abortus is the most common species that can cause hepatic granulomas. Histopathologically, histiocytic granulomas are present, often with central necrosis, portal and peripheral infiltration, and hyperplasia of the Kupffer cells. These granulomas result from the caseation of a granulomatous reaction by persistent Brucellae within macrophages.Although B. abortus tends to establish a granulomatous form of hepatitis, B. melitensis may cause both diffuse and granulomatous lesions in the liver. Apart from progressive clinical improvement, improvements in biochemical and hematological parameters were also observed in this patient by the treatment after the diagnosis and the laboratory signs of cholestasis were disappeared. Reactive hepatitis signs,

granulomatous hepatitis, hepatic granuloma formation, parenchymal necroses, Kupffer's cell hyperplasia, and cholestasis pattern may be observed in hepatic injury due to Brusella melitensis agent.[11,13] Despite of many reported cases of human brucellosis, the extent, severity and the histopathological features of liver involvement are still unclear.[11,21] This case supplies additional information in this area.

Brucellosis, as a cause of intrahepatic cholestasis, should be considered in cases with mildly increased transaminases along with prominently increased cholestasis enzymes. In such cases, improvements both in clinical outcome and hepatic injury can be achieved by administration of early and effective chemotherapy. Moreover, this case report presents different histological patterns and provides detailed information in liver involvement of brucellosis[22].

REFERENCES

1. Dourakis SP, Saramadou R, Alexopoulou A, Kafiri G, Deutsch M, Koskinas J, et al. Hepatic granulomas: a 6- year experience in a single center in Greece. Eur J Gastroenterol Hepatol 2007;19(2):101–4.

2. Mugahi S, Nashibi R, Alavi SM, Gharkholu S, Najafi K. Epidemiological Features, Clinical Manifestation and Laboratory Findings of Patients With Brucellosis. *Arch Clin Infect Dis.* 2014; **9**.

3. Pappas G, Akritidis N, Bosilkovski M, Tsianos E. Brucellosis. N Engl J Med 2005;352(22):2325-36.

4. O'Callaghan, D. Human brucellosis: Recent advances and future challenges. Infect. Dis. Poverty 2020, 9, 101.

5. Erdem I, Ali RK, Elbasan S, Ozcaglayan O, Degirmenci PO, Sedef S, et al. An unusual presentation of brucellosis: hepatic microabscesses. Clin Case Rep. 2017;5(3):229.

6. Aziz S, Al-Anazi AR, Al-Aska AI. A review of gastrointestinal manifestations of Brucellosis. *Saudi J Gastroenterol.* 2005; **11**(1) : 20 -7.

7. Vancelik S, Guraksin A, Ayyildiz A. Seroprevalence of human brucellosis in rural endemic areas in eastern Turkey. Trop Doct 2008;38(1):42-3.

8. Cetinkaya Z, Aktepe OC, Ciftci IH, Demirel R. Seroprevalence of human brucellosis in a rural area of Western Anatolia, Turkey. J Health Popul Nutr 2005;23(2):137-41.

9. Kose S, Smits HL, Abdoel TH, Ozbel Y. Prevalence of *Brucella* antibodies in rural and suburban communities in three provinces of Turkey: need for improved diagnosis and prevention. J Infect 2006;53(5):308-14.
10. Ozkurt Z, Erol S, Tasayran MA, Kaya A. Detection of *Brucella mellitensis* by the BacT/Alert automated system and Brusella broth culture. Clin Microbiol Infect 2002;8(11):749-52.
11. Akritidis N, Tzivras M, Delladetsima I, Stefanaki S, Moutsopoulos HM, Pappas G. The liver in brucellosis. Clin Gastroenterol Hepatol 2007;5(9):1109-12.
12. Gur A, Geyik MF, Dikici B, Nas K, Cevik R, Sarac J, et al. Complications of brucellosis in different age groups: a study of 283 cases in southeastern Anatolia of Turkey. Yonsei Med J 2003;44(1):33-44.
13. Zheng, R.; Xie, S.; Lu, X.; Sun, L.; Zhou, Y.; Zhang, Y.; Wang, K. A Systematic Review and Meta-Analysis of Epidemiology and Clinical Manifestations of Human Brucellosis in China. Biomed. Res. Int. 2018:1–10.
14. Mert A, Ozaras R, Bilir M, Tahan V, Cetinkaya A, Yirmibescik S, et al. The etiology of hepatic granulomas. J Clin Gastroenterol 2001;32(3):275-6.
15. Yamamoto S, Iguchi Y, Ohomoto K, Mitsui Y, Shimabara M, Mikami Y. Epitheloid granuloma formation in type C chronic hepatitis: report of two cases. Hepatogastroenterology 1995;42(3):291-3.
16. Emile JF, Sebagh M, Féray C, David F, David F, Reynès M. The presence of epithelioid granulomas in hepatitis C virus-related cirrhosis. Hum Pathol 1993;24(10):1095-7.
17. Tahan V, Ozaras R, Lacevic N, Ozden E, Yemisen M, Ozdogan O, et al. Prevalence of hepatic granulomas in chronic hepatitis B. Dig Dis Sci 2004;49(10):1575-7.
18. Ozaras R, Tahan V, Mert A, Uraz S, Kanat M, Tabak F, et al. The prevalence of hepatic granulomas in chronic hepatitis C. J Clin Gastroenterol 2004;38(5):449-52.
19. Demir MI, Kader Ç, Çolak NY, Kocabiyik O, Erbay A, Şebnem ER. Evaluation of brucellosis cases. Bozok Tıp Dergisi. 2017;7(3):47–51.
20. Mugahi S, Nashibi R, Alavi SM, Gharkholu S, Najafi K. Epidemiological Features, Clinical Manifestation and Laboratory Findings of Patients With Brucellosis. *Arch Clin Infect Dis.* 2014; **9**
21. Kuntz E, Kuntz HD. Bacterial infections and the liver. Hepatology: textbook and atlas: history, morphology, biochemistry, diagnostics, clinic, therapy. Wetzlar, Germany: Springer Verlag; 2008;3:490-1.

22. Gul, H. C., and H. Erdem. Brucellosis in J. E. Bennett, R. Dolin and M. J. Blaser, eds. Mandell, Douglas, and Bennett's principles and practice of infectious diseases. Elsevier Saunders, Philadelphia;2015;8:2584–2588.

FIGURE LEGENDS

Figure 1 (Hematoxyline-Eosine, X20). Bone marrow biopsy: Granulomatous myelitis; granuloma consisted of multinuclear giant cells, epitheloid histiocytes, fibroblasts, and lymphocytes between bone lamelles.

Figure 2a (Hematoxyline-Eosine, X10) - 2b (Hematoxyline-Eosine, X20). Granulomatous hepatitis consisted of epitheloid histiocytes, fibroblasts, lymphocytes, and multinuclear giant cells without caseous necrosis. Other biopsy findings are patchy proliferation areas in bile ducts (signs of intrahepatic cholestasis), periportal bile pigment accumulation, dense mononuclear inflammatory cell infiltrations in portal areas, mild mononuclear cell infiltration in local areas of the parenchyma (focal lytic necrosis) and minimal interface hepatitis.