JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Case Report

Secondary hemophagocytic lymphohistiocytosis in children with brucellosis: report of three cases

Yöntem Yaman, Salih Gözmen, Ahmet Kağan Özkaya, Yeşim Oymak, Hurşit Apa, Canan Vergin, İlker Devrim

Department of Pediatric Hematology, Sütçü Imam University Medical School, Kahramanmaraş, Turkey

Abstract

Brucellosis is a systemic zoonotic infectious disease that may cause fever, fatigue, sweating, arthritis, hepatosplenomegaly, cytopenia, and lymphadenopathy. It continues to be an important health problem worldwide. Hemophagocytic lymphohistiocytosis (HLH) is characterized by fever, hepatosplenomegaly, cytopenias, high serum levels of ferritin and triglycerides, low serum fibrinogen levels, and hemophagocytosis in bone marrow, lymph nodes, spleen, or liver. Hemophagocytic lymphohistiocytosis associated with brucellosis is a very rare condition in the pediatric age group. Here, three pediatric cases of secondary HLH associated with brucellosis are reported. Hemophagocytic lymphohistiocytosis should be considered in patients with brucellosis having cytopenias. Hemophagocytosis in brucellosis seems to be cured with appropriate antibiotics and intravenous immunoglobulin.

Key words: brucellosis; hemophagocytic lymphohistiocytosis; pancytopenia.

J Infect Dev Ctries 2015; 9(10):1172-1176. doi:10.3855/jidc.6090

(Received 16 October 2014 – Accepted 23 March 2015)

Introduction

Human brucellosis remains the most common zoonotic disease worldwide, with more than 500,000 new cases annually. In Turkey, brucellosis is common, especially in middle, east and southeast Anatolia [1]. The main symptoms and findings in children with brucellosis are high fever, fatigue, sweating, myalgia, hepatosplenomegaly, lymphadenopathy, arthralgia, and arthritis of the large joints [2]. Anemia, leukopenia, leukocytosis, thrombocytopenia, elevated liver enzymes, increased C-reactive protein (CRP), and erytrocyte sedimentation rate (ESR) are the most prominent laboratory abnormalities.

Hemophagocytic lymphohistocytosis (HLH) is characterized by non-malignant generalized proliferation of histiocytes with marked hemophagocytosis in different organs such as the liver, spleen, bone marrow, and the central nervous system [3]. The diagnostic criteria of HLH are fever, hepatosplenomegaly, cytopenia affecting at least two series, ferritin above 500 μg/L, decreased natural killer cell activity, elevated soluble CD25 level above 2,400 U/mL, fasting triglyceride above 265 mg/dL or fibrinogen below 1.5 g/L, and hemophagocytosis in the bone marrow, spleen, or lymph nodes. The diagnosis of HLH requires the presence of at lease five of the criteria mentioned above [3]. HLH can be classified as primary and secondary [3,4]. Secondary HLH (sHLH) is associated with systemic viral, bacterial, fungal, or parasitic infection, malignancies, and autoimmune diseases [4]. Although HLH and brucellosis are different entities, they share some common presentation features such as high fever and presence of hepatosplenomegaly or cytopenia in some cases. Pancytopenia is not a common findings in children with brucellosis, whereas anemia and leukopenia are the most common hematological findings in brucellosis [5]. Moreover, *Brucella*-associated HLH has been rarely reported in the literature, and the mechanisms of sHLH have not been as well understood in brucellosis as they have been in many other bacterial infections [5-7] Regardless of the etiologic cause, cytokine storm and excessive macrophage activation induce hemophagocytosis in primary and secondary HLH.

In this report, we present our experience with patients with sHLH associated with brucellosis.

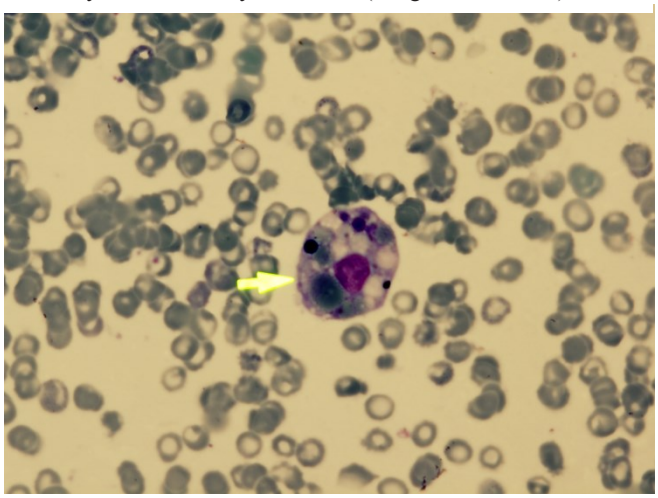
Case reports

Case 1

A 4-year-old boy was admitted to the hospital with fever lasting for four weeks, chills, and fatigue. He

was treated empirically for one week with intravenous ceftriaxone before hospitalization. He had a history of consumption of fresh milk products. On admission, his vital signs were as follows: body temperature was 38.3°C, arterial blood pressure was 90/50 mmHg, heart rate was 114 beats per minute, and respiration rate was 28 breaths per minute. Physical examination revealed no hepatosplenomegaly. Other physical findings were unremarkable. Hemoglobin, white blood cell (WBC) count, and platelet count were 10.8 g/dL, 5,900/mm^3, and 19,5000/mm^3, respectively. The differential leukocyte count on the smear was 7% neutrophils, 76% lymphocytes, 6% reactive lymphocytes, and 11% monocytes. Biochemical analyses were within normal ranges. The ESR and CRP were 50 mm/hour and 6.6 mg/dL, respectively. Serological tests performed for Epstein-Barr virus, cytomegalovirus, herpes simplex virus, human immunodeficiency virus, and *Salmonella* were all negative. The Rose-Bengal test was positive. Coombs anti-*Brucella* test titer was 1:320. Rifampicine (10 mg/kg/day) and trimethoprim-sulfamethoxazole (50 mg/kg/day for sulfamethoxazole) treatment for brucellosis was initiated. Fever subsided on the third day but relapsed on the fourth day of admission, and then gentamicin and doxycycline therapy was initiated instead of trimethoprim-sulfamethoxazole. Due to the persistent fever for more than five days, work-up for sepsis and sHLH was performed. Laboratory findings showed pancytopenia with hemoglobin level of 6.9 g/dL, WBC count of 1,800/mm^3, and platelet count of 95,000/mm^3. Biochemical analysis revealed alanine amino transferase (ALT) of 75 U/L, aspartate aminotransferase (AST) of 120 U/L, serum triglyceride of 275 mg/dL, lactate dehydrogenase (LDH) of 1,371 IU/L, ferritin of 11,173 ng/mL, and fibrinogen of 123 mg/dL. Bone marrow aspiration showed hemophagocytosis (Figure 1). Blood culture, which was taken on admission, was positive for *Brucella* spp., but bone marrow culture was sterile. The patient had intravenous immunoglobuline (IVIG) with a dose of 1 g/kg/day for two days. Fever subsided on the third day, with clinical improvement in the following days. On the seventh day, the laboratory revealed hemoglobin levels of 8.8 g/dL, WBC count of 2,800/mm^3, and a platelet count of 400,000/mm^3. Blood analysis demonstrated complete remission of pancytopenia, and liver function tests, CRP, fibrinogen, and serum ferritin levels returned to normal after six weeks of therapy.

Figure 1. A bone marrow smear showing hemophagocytosis in a patient with brucellosis. The arrow indicates a large histiocyte contained erythroid cells (Wright stain X 100).



Case 2

A 16-year-old previously healthy boy was admitted to the hospital with fever for three weeks, chills, and fatigue. He was treated empirically for one week with intravenously administered ceftriaxone during hospitalization. He had no weight loss or arthralgia. He had a history of consumption of non-pasteurized milk products. On admission, his vital signs were as follows: body temperature was 38.6°C, arterial blood pressure was 90/60 mmHg, heart rate was 112 beats per minute, and respiration rate was 14 breaths per minute. Physical examination revealed splenomegaly (3 cm below the costal margin) and pallor. Other physical findings were unremarkable. Laboratory findings showed pancytopenia with hemoglobin levels of 8.7 g/dL, WBC count of 2,800/mm^3, and a platelet count of 59,000/mm^3. The differential leukocyte count on the peripheral smear was 52% neutrophils, 42% lymphocytes, and 6% monocytes. The ESR and CRP were 60 mm/hour and 7.6 mg/dL, respectively. Biochemical analysis revealed ALT of 48 U/L, AST of 100 U/L, serum triglyceride of 211 mg/dL, LDH of 1,371 IU/L, ferritin > 1,650 ng/mL, and fibrinogen of 87 mg/dL. Other biochemical findings were within normal ranges. Serological tests performed for Epstein-Barr virus, cytomegalovirus, and *Salmonella* were all negative. The Rose-Bengal was positive. Coombs anti-*Brucella* test titer was 1:640. Bone marrow aspiration showed hemophagocytosis. Rifampicine (600 mg/day) and doxycyclin (200 mg/day) treatment was initiated. He received IVIG with a dose of 1 g/kg/day for two days due to sHLH. Fever subsided on day two, with clinical

improvement in the following days. Blood and bone marrow cultures were positive for *Brucella* spp. On day 10, the laboratory revealed hemoglobin levels of 9.4 g/dL, WBC count of 4,300/mm^3, and a platelet count of 32,5000/mm^3. Blood analysis demonstrated complete remission of pancytopenia, and liver function tests, CRP, fibrinogen, and serum ferritin levels returned to normal after six weeks of therapy.

Case 3

An 8-year-old boy was admitted to the hospital with prolonged fever, fatigue, sweating, and abdominal pain. He had fever and fatigue that had lasted for 10 days on admission. He had consumed fresh milk products. Physical examination revealed splenomegaly (5 cm below the costal margin), hepatomegaly (4 cm below the costal margin), and pallor. Laboratory findings showed pancytopenia with hemoglobin levels of 9.2 g/dL, WBC count of 2,700/mm^3, and a platelet count of 62,000/mm^3. The differential leukocyte count on peripheral smear was 33% neutrophils, 66% lymphocytes, and 1% monocytes. The ESR was 75 mm/hour. Biochemical analysis revealed ALT of 35 U/L, AST of 75 U/L, serum triglyceride of 277 mg/dL, LDH of 861 IU/L, ferritin of 543 ng/mL, and fibrinogen of 211 mg/dL. Other biochemical findings were within normal ranges. Serological tests performed for Epstein-Barr virus, cytomegalovirus, and *Salmonella* were all negative. *Brucella* agglutination test was positive with a ratio of 1:2,560. Bone marrow aspiration showed hemophagocytosis. Blood and bone marrow cultures were also positive for *Brucella* spp. Doxycycline and rifampicine treatment was initiated for brucellosis. The patient had 1 g/kg/day IVIG for two consecutive days for sHLH in addition to antimicrobial threapy. The fever dissipated on the third day of therapy. The platelet count increased progressively during treatment. On day seven, the blood count showed hemoglobin level of 10.7 g/dL, WBC count 7,200/mm^3, and a platelet count of 343,000/mm^3. Serum triglyceride was 236 mg/dL, ferritin was 125 ng/mL, ALT was 23 U/L, AST was 45 U/L, and LDH was 465 IU/L. The patient was followed up in the outpatient clinic for six weeks and fully recovered after this period.

Discussion

Brucellosis is a worldwide zoonotic infection that affects the host via ingestion or inhalation, or through conjunctiva or skin abrasions. After infecting the host, the pathogen becomes sequestered within cells of the reticuloendothelial system. Various complications, including neurobrucellosis, endocarditis, osteomyelitis, granulomatosus hepatitis, and pancytopenia may occur in 5% to 10% of patients with brucellosis [1].

Reports on the frequency and diversity of hematologic abnormalities occurring in both adults and children with brucellosis have been reported [5,8]. Anemia and leukopenia have been frequently associated with acute brucellosis, but pancytopenia and thrombocytopenia are less frequently seen. Although pancytopenia is usually due to hypersplenism in brucellosis, the pathogenesis of pancytopenia in brucellosis seems to be multifactorial, including hemophagocytosis, hypersplenism, bone marrow hypoplasia, bone marrow granulomas, and immune destruction [5,9]. The incidence of pancytopenia with brucellosis varies from 3% to 21% in published studies [10]. In the literature, reports related to brucellosis, cytopenia, and hemophagocytosis are limited [5,6,11,12]. We found only one case report describing a child with *Brucella*-associated HLH [7]. In that study, HLH findings resolved after the antibiotic treatment of *Brucella* infection. In additon, Karakulukcu *et al*. described four children with *Brucella*-associated HLH. The authors reported that all of four patients had pancytopenia and recovered fully with specific antimicrobial therapy for *Brucella* infections. We initially gave IVIG treatment to case 2 and case 3 patients, once the diagnosis of sHLH was made. Although antimicrobial therapy for brucellosis was initiated immediately on admission and modified due to relapsing fever for case 1, the patient responded to IVIG treatment after the diagnosis of sHLH. Although the clinical and laboratory findings initially did not seemed to be associated with sHLH in case 1, the patient was reevaulated on the 12th day of hospitalization for relapsing fever, and was diagnosed with sHLH. The use of IVIG in patients with *Brucella*-induced thrombocytopenia has been reported in some cases and series [11,13,14]. The time for normalization of hematological values in patients with pancytopenia due to brucellosis ranged from eight days to three weeks following initiation of appropriate antimicrobial therapy [7,11]. However, the patients with *Brucella*-induced immune thrombocytopenia responded well to IVIG and recovered within an average of three to seven days [11,13,14]. This time was also consistent with the hematological recovery time of our patients. Thus, IVIG treatment may be useful in emergency cases or in life-threatening conditions secondary to

thrombocytopenia in patients with brucellosis and *Brucella*-associated HLH [11,13,14]

The findings of brucellosis, including pancytopenia, fever, and splenomegaly were also among the main clinical symptoms in HLH. The mechanisms of hemophagocytosis in non-viral infections may be related to overproduction of activating cytokines, such as TNF-α and γ-interferon, which contribute to macrophage activation, or could be the result of a poorly regulated or inappropriate T-helper lymphocyte response to intracellular pathogens [15].

Hyperinflammation caused by excessive cytokine levels as occurs in HLH should be treated with either corticosteroids or immunosuppressive agents. The aim of therapy is to suppress cytokine release and control cell proliferation using immunosuppressive or immunomodulatory agents and cytotoxic drugs [3]. Corticosteroids are cytotoxic for lymphocytes and inhibit the expression of cytokines. IVIG, etoposide, cyclosporin A, and antithymocyte globulin are the other treatment choices, either in combination with corticosteroids, or alone [3,15]. Corticosteroids, etoposide, and cyclosporine A either in combination or alone are used according to HLH-2004 protocol [3]. Successful use of IVIG alone or in combination with steroid or etoposide in these patients has been reported [16,17]. Moreover, in the presence of sHLH, one of the mainstays of the treatment strategies is to treat the underlying disease and conditions. As mentioned in another report, cyclosporin A, steroids, or IVIG alone or in combination may be used in low-risk HLH patients for prompt management of cytokine-induced symptoms as well as treatment of the underlying disease [18]. We used IVIG in our patients in addition to the specific antimicrobial therapy.

Conclusions

In patients who have been diagnosed with brucellosis and who have prolonged fever, hepatosplenomegaly, and cytopenias, sHLH should be considered. In cases of laboratory findings of high serum ferritin and triglycerides, and low serum fibrinogen levels and pancytopenia, bone marrow aspiration should be assessed for diagnosing sHLH.

References

1. Buzgan T, Karahocagil MK, Irmak H, Baran AI, Karsen H, Evirgen O, Akdeniz H (2010) Clinical manifestations and complications in 1028 cases of brucellosis: a retrospective evaluation and review of the literature. Int J Infect Dis 14: 469-478.
2. Caylak SD, Sozen H,Gonen I, Sahin C (2014) A brucellosis Case Presenting with Vesicular and Maculopapular Rash and Febrile Neutropenia. J Microbiol Infect Dis 4: 39-41
3. Henter JI, Horne A, Aricó M, Egeler RM, Filipovich AH, Imashuku S, Ladisch S, McClain K, Webb D, Winiarski J, Janka G (2007) HLH-2004: Diagnostic and Therapeutic Guidelines for Hemophagocytic Lymphohistiocytosis. Pediatr Blood Cancer 48: 124-131.
4. Larroche C, Mouthon L (2004) Pathogenesis of hemophagocytic syndrome. Autoimmun Rev 3: 69-75.
5. Karakukcu M, Patiroglu T, Ozdemir MA, Gunes T, Gumus H, Karakukcu C (2004) Pancytopenia, a rare hematologic manifestation of brucellosis in children. J Pediatr Hematol Oncol 26: 803-806.
6. Akbayram S, Dogan M, Akgun C, Peker E, Parlak M, Caksen H, Oner AF (2011) An analysis of children with brucellosis associated with pancytopenia. Pediatr Hematol Oncol 28: 203-208.
7. Erduran E, Makuloglu M, Mutlu M (2010) A rare hematological manifestation of brucellosis: reactive hemophagocytic syndrome. J Microbiol Immunol Infect 43: 159-162.
8. Al-Eissa YA, Assuhaimi SA, al-Fawaz IM, Higgy KE, al-Nasser MN, al-Mobaireek KF (1993) Pancytopenia in children with brucellosis: clinical manifestations and bone marrow findings. Acta Haematol 89: 132-136.
9. Aysha HM, Shayib AM (1986) Pancytopenia and other haematological findings in brucellosis. Scand J Haematol 36: 335-338.
10. Akdeniz H, Irmak H, Seckinli T, Buzgan T, Demiröz AP (1998) Hematologic changes in brucellosis cases in Turkey. Acta Med Okayama 52: 63-65.
11. Citak EC, Citak FE, Tanyeri B, Arman D (2010) Hematologic manifestations of brucellosis in children: 5 years experience of an anatolian center. J Pediatr Hematol Oncol 32: 137-140.
12. David A, Iaria C, Giordano S, Iaria M, Cascio A (2012) Secondary hemophagocytic lymphohistiocytosis: forget me not! Transpl Infect Dis 14: 121-123.
13. Sevinc A, Buyukberber N, Camci C, Buyukberber S, Karsligil N (2005) Thrombocytopenia in brucellosis: case report and literature review. J Natl Med Assoc 97: 290-293.
14. Tsirka A, Markesinis I, Getsi V, Chaloulou S (2002) Severe thrombocytopenic purpura due to brucellosis. Scand J Infect Dis 34: 535-536.
15. Filipovich A, McClain K, Grom A (2010) Histiocytic disorders: recent insights into pathophysiology and practical guidelines. Biol Blood Marrow Transplant 16: 82-89.
16. Nomura S, Koshikawa K, Hamamoto K, Okubo S, Yasunaga K (1992) Steroid and gamma globulin therapy against virus associated hemophagocytic syndrome. Rinsho Ketsueki 33: 1242-1247.
17. Chen RL, Lin KH, Lin DT, Su IJ, Huang LM, Lee PI, Hseih KH, Lin KS, Lee CY (1995) Immunomodulation treatment for childhood virus associated hemophagocytic lymphohistiocytosis. Br J Haematol 89: 282-290.

18. Shinsaku I, Tomoko T, Akira M (2001) Recent developments in the management of hemophagocytic lymphohistiocytosis. Expert Opin Pharmacother 2: 1437-1448.

Corresponding author
Yöntem Yaman
Department of Pediatric Hematology
Sütçü Imam University Medical School
Kahramanmaraş, Turkey
Phone: +90 5064070607
Fax: +90 344 280 1037
Email: yontemyaman@gmail.com

Conflict of interests: No conflict of interests is declared.