Original Article

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A Prospective Study of Brucellosis in Children: Relative Frequency of Pancytopenia

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Abstract

Objective: Hematological complications of brucellosis are common. Pancytopenia, although mainly reported in adults, has also been described in children with brucellosis. This investigation was conducted to estimate the relative frequency of pancytopenia in children with brucellosis.

Methods: The current study was conducted in Al-Khafji Joint Operations Hospital, Saudi Arabia. Sixty patients with brucellosis were enrolled in the study. Complete blood count (CBC) and blood culture were performed for all cases. Bone marrow (BM) aspiration was considered only in those with pancytopenia.

Findings: Out of 60 children with brucellosis, 50 (83%) ingested raw animal milk and 27 (45%) had a positive family history of brucellosis. The common presenting symptoms and signs included: excessive sweating (68%), bone aches (62%), chills (55%), arthritis (32%), hepatomegaly (18%) and splenomegaly (15%). The main hematological manifestations included: anemia (43%), leukopenia (38%) and leukocytosis (20%). Pancytopenia was detected in 11 (18%) patients. Blood culture for Brucella was positive in 38% (23 patients). *B. melitensis* from 21 patients was cultured in vitro. Out of 9 BM aspirate cultures, 3 were positive for *B. melitensis*. Out of 11 patients with pancytopenia, 9 (82%) patients had bone aches and weakness, 7 (64%) patients sweating and chills, 6 (55%) patients petechiae and purpura.

Conclusion: The current study concludes that although pancytopenia is an uncommon complication of brucellosis in children, it does occur. Therefore, brucellosis should be considered in the differential diagnosis of pancytopenia in children, particularly in endemic areas such as Saudi Arabia.

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Key Words: Endemic Brucellosis; Fever of Unknown Origin; Pancytopenia

Introduction

Brucellosis, a primarily contagious disease of domestic animals, is caused by small, fastidious gram-negative coccobacilli of the genus Brucella. There are four important species pathogenic to humans: *B. melitensis*, found primarily in goats, sheep and camels; *B. abortus* in cows; *B. suis* in pigs; and *B. canis* in dogs. The Brucella species differ in degree of virulence and invasiveness with *B. melitensis* being the most invasive and most severe disease while *B. abortus* is the least invasive[1]. In Saudi Arabia, human infection with *B. melitensis* is common (80-100%) while infection with *B. abortus* is less frequent. Infection with other species has not been reported[2].

Humans are commonly infected through ingestion of raw milk, cheese or meat, or through direct contact with infected animals, products of conception or animal discharges (e.g., among

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shepherds, farmers and veterinarians), and through inhalation of infectious aerosols (e.g., by workers in abattoirs and microbiology laboratories)[3]. Human brucellosis can be an acute or a chronic febrile illness and presents with a variety of manifestations after an incubation period, which can vary from 1 to 6 weeks or several months. Brucellosis may be difficult to distinguish clinically from a number of other infections such as typhoid fever, tuberculosis, infective endocarditis, and acute rheumatic fever[4]. The symptoms of acute illness are fever, chills, headache, muscle and joint pains, malaise, nausea, night sweats and loss of appetite persisting 3 to 6 weeks. Brucellosis shows multisystem involvement[5]. The disease also produces a variety of nonspecific hematological abnormalities.

The BM and spleen are commonly involved, and such involvement may result in a hypoplastic pattern on a peripheral blood smear[6]. Hematological complications of brucellosis are common and can be multifactorial due to the pathogen's tropism for central organs (e.g., BM) and peripheral organs (e.g., spleen) of the reticuloendothelial system (RES). Changes in the hematological parameters are observed in most patients, but pancytopenia is rare[6]. Hemophagocytosis, hypersplenism or granulomatous changes in the BM may be responsible for pancytopenia in brucellosis. Additionally, BM involvement due to simultaneous presentation of malignant diseases with brucellosis rarely leads to pancytopenia[7].

Incidence of pancytopenia is 2-14% among adult patients affected by brucellosis[8]. Although the presentation of acute brucellosis with mesenteric lymphadenitis and pancytopenia is rare, it must be considered in patients in endemic areas[9]. The aim of this study was to estimate the relative frequency of pancytopenia in Saudi children with brucellosis.

Subjects and Methods

This study was conducted at Al-Khafji Joint Operations Hospital, Saudi Arabia, from August 2011 to October 2012. All children suffering from fever for more than 5 days, without clinically evident cause for fever, with symptoms suggestive of brucellosis such as weight loss, weakness, anorexia and polyarthalgia were screened for brucellosis by a rapid slide serum agglutination test using plasmatic stained febrile antigens reagent code number FA/018 for *B. abortus* and FA/020 for *B. melitensis*. If a positive result was obtained, tube agglutination test was performed.

Titer of 1/20 up to 1/360 was done for each serum to avoid prozone effects. Titer of 1/160 or more and rising antibody titers were considered to be positive. Of the positive cases, by slide agglutination test, 10 ml blood samples and/or bone marrow aspirates were obtained under complete aseptic procedures, inoculated and mixed on Hemoline Performance Diphasique, BioMerieux blood culture system and Oxoid signal blood culture system code BC0100. The medium was designed to create pressure in the sealed bottle when organisms were growing. A positive result is signaled when the blood/broth mixture rises above the green locking sleeve of the growth indicator device. Positive growth was subcultured on blood, chocolate and MacConkey's agar media, both aerobically in 5% CO_2 atmosphere and anaerobically. Gram stain, oxidase, catalase, urease and other biochemical reactions were performed for identification of Brucella species.

All children with positive tube agglutination test or positive blood or BM cultures were enrolled in the current study. Baseline data were collected including demographic data, documented family history of brucellosis, ingestion of raw milk, cheese or meat or contact with infected animals or their products, and history of hematological disorders. Thorough clinical examinations were performed.

Laboratory workup included completed blood count (CBC), erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP). When indicated, coagulation profiles, including prothrombin time (PT), activated partial thromboplastin time (APTT) and plasma fibrinogen level, were assayed. Pancytopenia was considered if age-corrected white blood cell count, platelet count and hemoglobin were low[10]. In cases with bicytopenia and pancytopenia, BM aspiration/biopsy was also considered. CBC repeat was performed in cases with cytopenia[5]. Data entry and statistical analysis were performed by

Table 1: Demographic and clinical characteristics of 60 children diagnosed as having brucellosis, presented as number (n) and percentage (%)

Parameter	Characteristic(s)	n (%)
Males		43 (71.7)
History of raw milk ingestion		50 (83.3)
Family history of brucellosis		27 (45)
Symptoms	Excessive sweating	41 (68.3)
	Bone ache	37 (61.7)
	Chills	33 (55)
	Painful or swollen joints	22 (36.7)
	Weakness	21 (35)
Signs	Arthritis and/or arthralgia	19 (31.7)
	Hepatomegaly	11 (18.3)
	Splenomegaly	9 (15)
	Hepatosplenomegaly	4 (6.7)
	Lymphadenopathy	4 (6.7)
	Petechiae and purpura	3 (5)

application of the Statistical Package for the Social Sciences (SPSS; IBM, Inc., NY, USA). *P* values <0.05 were considered statistically significant.

Findings

One hundred thirty-three patients were screened for brucellosis. Of these, 84 were positive by rapid slide test. None of the screened children with titer <1:160 had positive blood or BM culture for brucellosis. Sixty children, diagnosed as brucellosis whose titer ≥1:160 by tube agglutination method, were enrolled in this prospective study. Age of the enrolled children ranged between 5-16 years (Mean±SD: 7.6±1.8), of which 43 (71.7%) were males. Table 1 shows other demographic and clinical symptoms children with brucellosis. Mean duration of fever in patients was 9.6 (±5.3) days.

Table 2 summarizes the hematological manifestations, cultures, and agglutination titers among the 60 children suffering brucellosis. Among the 23 (38.3%) patients with positive blood culture, *B. melitensis* was isolated in 21 (35%) cases and *B. abortus* in only 2 (3.3%) cases. BM culture was conducted for 9 (15%) patients. Of these patients, 3 (5%) were positive for *B. melitensis*.

Out of all patients with brucellosis, 11 patients (18.3%) had pancytopenia at diagnosis of which 6 (55%) patients had petechiae, pupura and/or

Table 2: Hematological manifestations, cultures and agglutination titres among 60 study children, presented as number (n) and percentage (%).

	Presentation	n	%
Hematological manifestations	Anemia†	26	43.3
	Leucopenia††	23	38.3
	Leucocytosis	12	20.0
	Pancytopenia‡	11	18.3
	Lymphocytosis‡	5	8.3
Cultures :	Positive blood culture	23	38.3
	B.melitensis	21	35
	B.abortus	2	3.3
	Positive bone marrow culture*	3	5
	Negative bone marrow culture	6	10
Agglutination titres	1/160-1/320	38	63.3
	1/320-1/640 & more	22	36.7

† Defined as hemoglobin level and or red blood cells is below reference value for age and sex[22,23].
†† Defined as total leucocytic count is below reference value for age and sex[22,23].
‡ Defined as total leucocytic count/lymphocytic count is above reference value for age and sex[22,23].
* Positive for B. *melitensis*

Table 3: Clinical and laboratory findings of 11 children suffering pancytopenia

	Finding	n=11	%
Symptoms	Bone aches & weakness	9	81.8
	Sweating & chills	7	63.6
	Painful & swollen joints	5	45.5
Signs	Petechiae & purpura	6	54.5
	Hepatomegaly	5	45.5
	Splenomegaly	5	45.5
	Hepatosplenomegaly	3	27.3
	Arthritis	3	27.3
	Generalized lymphadenopathy	3	27.3
CBC, X±SD	Hemoglobin (g/dl)	5.9(2.8)	
	WBC $x10^9$/ml	3.12(1.3)	
	Platelets $x10^9$ /ml	32.7(4.7)	
Agglutination titres	1:160–1:320	3	27.3
	1:320–1:640 & more	8	72.7
	Positive blood culture*, n(%)	11	100

* positive for *B. melitensis*

bleeding. The majority of patients with pancytopenia (72.7%) had an agglutination titer of 1/320-1/640 or more. Interestingly, blood culture was positive for *B. melitensis* in all patients with pancytopenia (Table 3).

Discussion

Brucellosis is primarily an infectious disease of domestic animals that is transmissible to humans. The source of infection is likely to be fresh unpasteurized milk or milk products consumption or via direct contact with infected animal tissues[11]. Although brucellosis has been controlled in many developed countries, it remains an important health problem in developing countries, particularly in the Mediterranean region, Middle East and West Asian countries[12]. Hematological complications such as anemia and leukopenia are more frequently seen in acute brucellosis cases. However, other hematological abnormalities such as severe thrombocytopenia, pancytopenia, acute hemolytic anemia, and disseminated intravascular coagulation are not infrequent[5]. In the current study, out of 133 patients with fever lasting more than 5 days, 60 children were diagnosed as having acute brucellosis based on tube agglutination test method. The majority (83%) of patients declared raw animal milk/raw dairy products consumption while 45% had a positive family history of brucellosis. Al-Eissa reported that brucellosis in Saudi population presents in both genders and all ages and that the main form of acquiring disease is through ingestion of raw milk and milk products obtained mainly from infected goats or camels, a traditional custom fostered by the nomadic heritage and dietary habits of the people[1]. Patients with brucellosis usually present with fever, chills, malaise, weight loss, joint involvement, hepatosplenomegaly and lymphadenopathy[5]. In the current study, the main symptoms at presentation in 60 children with brucellosis were excessive sweating (68%), bone aches (62%) and chills (55%). The main signs in these patients were arthritis/arthralgia (32%), hepatomegaly (18%) and splenomegaly (15%). Hematological dyscrasias in study children with brucellosis included anemia (43%), leukopenia (38%), leukocytosis (20%) and pancytopenia (18%). These findings were in accordance with other reports conducted in both pediatric and adult patients with proven brucellosis[7,13,14].

Similarly, in South-Western Saudi Arabia, Benjamin and Annobil have reported an incidence of leucopenia in 38%, anemia in 64%, and thrombocytopenia in 28% of brucellosis candidates[15]. Many other studies of hematological changes during the active course of brucellosis showed that leukopenia occurred in 33% of patients, anemia in 44%, thrombocytopenia in 5% and pancytopenia in 14%[16,17]. Furthermore, Mantur BG et al detected pancytopenia in 10% of children suffering

brucellosis[14]. The relative frequency of pancytopenia with brucellosis varies from 2% to 14% in previous studies, being relatively higher in adults than in children[18-20]. The possible mechanisms suggested for pancytopenia include hypersplenism, granuloma formation in the BM, phagocytosis of formed elements by reticuloendothelial cells or BM depression due to associated septicemia[5]. Although anemia in brucellosis is expected to be due to BM involvement, numerous other pathogenetic mechanisms can be (and have been) implicated. Bourantas et al reported that brucellosis induced an autoimmune process, culminating in autoimmune hemolysis[6]. In this study, blood culture was positive for brucellosis in 23 children (21 for *B. melitensis* and 2 for *B. abortus*). BM culture was performed in 9 children.

Of these patients, 3 were positive for *B. melitensis*. The majority (63%) of children with brucellosis had serum agglutination titers of 1/160-1/320. In this study, the most common symptoms and signs in 11 children with pancytopenia included bone aches and weakness (82%), sweating and chills (64%), petechiae and purpura (55%), hepatomegaly and splenomegaly (46%). The majority (73%) of children with pancytopenia had agglutination titers of 1:320-1:640 or more. Furthermore, all cases with pancytopenia had positive blood culture, a finding which was nearly consistent with that obtained by other investigators[2,4,5,8,14,20,21].

Conclusion

In conclusion, despite pancytopenia being an Infrequent sequel of brucellosis in most of the literature, it was frequently seen in the current study. Thus, brucellosis should always be considered in the differential diagnosis of pancytopenia, particularly in endemic areas such as Saudi Arabia. Surveillance, testing and massive immunization of animals in endemic areas as well as an organized national brucellosis control program are prerequisites to eradicate the disease and its complications.

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Authors' Contribution

M. Koumi: Concept/ Design, Acquisition of Data, Data Analysis and Interpretation, Drafting of the Manuscript and Critical Revision of the Manuscript.
M. Afiffy: Data Analysis and Interpretation, Drafting of the Manuscript and Critical Revision of the Manuscript
S. Al Zahrani: Data Analysis and Interpretation, Drafting of the Manuscript and Critical Revision of the Manuscript
All authors approved final version of the paper.

Conflict of Interest: None

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