Brucellosis-Induced Pancytopenia in Children: A Prospective Study

Mona Afify[1], Salha H Al-Zahrani[1] , and Mohamad A El-Koumi[2]
[1]Department of Biology, Science College for Girls, King AbdulAziz University.
[2]Department of Pediatrics, Al-Khafji Joint Operation Hospital[*], Kingdom of Saudi Arabia.
Corresponding author :drmonaafify@hotmail.com

ABSTRACT: Brucellosis, a zoonotic infection, constitutes a major health and economic problem in many parts of the world, including countries of the Mediterranean Basin, The Middle East and The Arabian Gulf. Hematological complications in brucellosis are common and can be multi-factorial due to the pathogen's tropism for central (bone marrow) and peripheral (spleen) organs of the reticuloendothelial system (RES). 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. The study was carried out in Al-Khafji region. All children suffering from fever of more than 5 days without clinically evident cause and associated with symptoms suggestive of brucellosis were screened by a rapid slide serum agglutination test for presence of brucellosis. Sixty patients who had tube agglutination titre $\geq$ 1: 160 or had positive blood and/or bone marrow (BM) culture for brucellosis, were enrolled in the study. At enrollment, the following investigations were performed: CBC, blood culture and BM, aspiration was carried out in all patients with pancytopenia, to exclude malignancy. Obtained results revealed that: out of 60 children with brucellosis, 50 (83%) ingested raw animal milk and 45% had a positive family history of brucellosis. The commonly presenting symptoms and signs included; excessive sweating (68%), bone aches (62%), chills (55%), arthritis (32%), and hepatosplenomegaly. The most commonly detected hematological manifestations included; anemia (in 43%), leukopenia (in 38%) and leukocytosis (in 20%). Meanwhile, pancytopenia was detected in 11 patients (18%). Positive blood culture for brucella was seen in 38% (23 patients). *melitensis* from 21 patients was cultured *in vitro*.. Out of 9 BM cultures, 3 were positive for *B. melitensis* and 6 cultures were negative. Out of 11 patients with pancytopenia, 9 patients (82%) presented with bone aches and weakness, 7(64%) presented with sweating and chills, 6(55%) of patients had petechiea and purpura, 5 (46%) had splenomegaly and 5 (46%) had hepatomegaly. The majority of patients with brucella-induced pancytopenia had agglutination titres of > 1: 320 and all of them had positive blood culture for *B. melitensis*. In conclusion in a patient with fever, arthalgia, chills and hematological abnormalities such as anemia, leukopenia, thrombocytopenia or pancytopenia, brucellosis should be kept in mind, especially in geographical areas where the disease is still endemic, as in our region.[Mona Afify, SalhaHAl-Zahrani,and Mohamad A El-Koumi. **Brucellosis-Induced Pancytopenia in Children:AProspective Study** *J AmSci*2012;8(10):112-116].(ISSN:1545-1003). http://www.jofamericanscience.org. 17

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1.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, *B. melitensis* being the most invasive and produces the most severe disease and *B. abortus* is the least invasive **(Al-Eissam, 1999)**. In Saudi Arabia, human infection with *B. melitensis* is commonly encountered (80%-100%), and infection with *B. abortus* is less frequent, but infection with other species has not been reported **(Bilal et al., 1991).**

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 shepherds, farmers and veterinarians), and through inhalation of infectious aerosols (e.g., by workers in abattoirs and microbiology laboratories) **(Young, 2000).**

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 **(Sari et al., 2008).** 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 **(Dilek et al., 2008).** The disease also produces a variety of nonspecific hematological abnormalities. The BM and the spleen are commonly involved, and such involvement may result in a hypoplastic pattern on the peripheral blood smear **(Sari et al., 2008).**

Hematological complications in brucellosis are common and can be multifactorial due to the pathogen's tropism for central (BM) and peripheral (spleen) organs of the RES. Changes in the hematological parameters are observed in most patients, but pancytopenia is rare **(Lambros et al., 2010).**Hemo-phagocytosis, hypersplenism or granulomatous changes in the BM may be responsible for pancytopenia occurring during brucellosis. Additionally, BM involvement due to simultaneous presentation of malignant diseases with brucellosis rarely lead to pancytopenia **(Eser et al., 2006).**

Incidence of pancytopenia is 3-21% among adult patients affected by brucellosis **(Omidi et al., 2009).** Meanwhile, although the presentation of acute brucellosis with mesenteric lymphadenitis and pancytopenia is rare, it must be considered in patients such in endemic areas **(Dag et al., 2011).** On other hand, although brucellosis is a rare cause of pancytopenia, it should be considered in differential diagnosis with pancytopenia of children **(Sen et al., 2010).**

2.PATIENTS AND METHODS

This study was carried out at Al-Khafji Joint Operation Hospital during years 2010 through 2011. All children suffering from fever of more than 5 days, without clinically evident cause of fever and associated with symptoms suggestive of brucellosis such as weight loss, weakness, anorexia and arthalgia 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*. In case of a positive result, tube agglutination test was performed. Titre of 1/20 up to 1/360 was done for each serum to avoid prozone effects. Titre above 1/160 and rising antibody titre was considered to be positive. From positive cases of slide agglutination test, ten ml of blood samples and/or bone marrow aspirates were taken 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 indicated 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 aerobic in 5% CO_2 atmosphere and anaerobically. Gram stain, oxidase, catalase, urease and other biochemical reactions were performed for identification of brucella organisms.

All children with positive tube agglutination test or positive blood or BM cultures were enrolled in the study. All the enrollment, 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. After that a through clinical examination was performed.

At enrollment, beside the laboratory diagnosis of brucellosis, the following investigations were performed complete blood count (CBC), erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP). As required, coagulation parameters such as prothrombin time (PT), activated partial thromboplastin time (APTT) and plasma fibrinogen level were measured. Fibrinogen assay was performed by using kit (Diagnostica Stago, France). All investigations were considered abnormal according to the established reference values in childhood **(Pesce, 2011)**.

Pancytopnia was considered if white blood cells, platelets count and hemoglobin values are low according to age **(Pesce, 2011)**. In cases with bicytopenia, severe cytopenia and pancytopenia, BM aspiration/biopsy was also performed. When results are abnormal or when needed, the CBC was repeated **(Dilek et al., 2008).**

Data entry and statistical analysis were performed by application of the SPSS (version 15) statistical package. P-values <0.05 were considered significant.

3.RESULTS

One hundred thirty-three patients were screened for brucellosis and 84 were positive by rapid slide test. Non of screened children with titre < 1: 160 had positive blood or BM culture for brucellosis. Sixty children, diagnosed as brucellosis by titre $\geq$ 1:160 of tube agglutination method, were enrolled. Their ages ranged from 5 to 16 years (mean±SD: 7.6±1.8), 43 (71.7%) of them were males. Fifty patients (83.3%) ingested raw milk and 27 (45%) had a positive family history of brucellosis. Excessive sweating was present in 41 patients (68.3%), bone aches in 37 patients (61.7%), and chills were present in 33 patients (55%). Nineteen patients (32%), 11 patients (18%), 9 patients (15%) and 4 patients (7%) were found to have arthritis, hepatomegaly, spleno-megaly and hepatosplenomegaly, respectively (**Table 1**).

Table (2) summarizes hematological manifestations, cultures and agglutination titres among 60 children suffering brucellosis. Twenty-six patients (43.3%) had anemia, 23 (38.3%) had leukopenia, 12 (20%) had leukocytosis and 11 patients (18.3%) had pancytopenia. Among 23 patients (38.3%) with positive blood culture, *B. melitensis* was isolated from 21 (35%) and *B. abortus* from 2 (3.3%). BM culture was carried out for 9 patients (15%), 3 of them (5%) was positive for *B. melitensis.* Thirty-eight patients (63.3%) had an

agglutination titre of 1/160-1/320, and 22 (36.7%) had an agglutination titres of 1/320-1/640 or more.

Out of 60 patients with brucellosis, 11 patients (18.3%), 7 males and 4 females, with age ranging from 5 to 13 years, had pancytopenia at diagnosis. Among these 11 patients, 9 patients (81.8%) had bone aches and weakness, 6 patients (55%) had petechiae, pupura and/or bleeding and 5 patients (45.5%) had hepatomegaly and/or splenomegaly. The mean±SD of hemoglobin, WBCs counts and platelet counts accounted for 5.9±2.8, 3.12±1.3 and 32.7±4.7, respectively. The majority of patients with pancytopenia (72.7%) had an agglutination titre of 1/320-1/640 more. Blood culture was positive for *B. melitensis* in all patients with pancytopenia (**Table 3**).

4.DISCUSSION

Brucellosis is primarily an infectious disease of domestic animals that is transmissible to humans. The source of infection is likely to fresh unpasteurized milk or milk products, or by direct contact with infected animal tissues **(Soker et al., 2001).**

The World Health Organization estimates the number of new cases of brucellosis at more than 500,000 per year in the world. Although brucellosis has been controlled in many developed countries, it remains an important health problem in developing countries, particularly in Mediterranean region, Middle East and West Asian countries **(Afscharpaiman and Mamishi, 2008).** Hematological complications such as anemia and leukopenia are more frequently seen in acute brucellosis cases. However, acute brucellosis should also be considered in the differential diagnosis in the presence of other hematological abnormalities such as severe thrombocytopenia, pancytopenia, acute hemolytic anemia, and disseminated intravascular coagulation **(Dilek et al., 2008).**

In this study, out of 133 patients with fever of more than 5 days, 60 children were diagnosed as acute brucellosis by titre of $\geq$ 1:160 of tube agglutination method. The majority of patients (83%) ingested raw animal milk and 45% of them had a positive family history of brucellosis. **Al-Eissa, (1999)** reported that in Saudi population, brucellosis presents in both sexes and in 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 normadic heritage and dietary habits of the people.

Patients with brucellosis usually present with fever, chills, malaise, weight loss, joint involvement hepatosplenomegaly and lymphadenopathy **(Dilek et al., 2008).** In this study, the main symptoms at presentation in 60 children with brucellosis were excessive sweating (in 68%), bone aches (in 62%) and chills (in 55%). The main signs in these patients were arthritis (in 32%), and hepatosplenomegaly. Nearly similar results were obtained by other studies **(Eser et al., 2006; Al-Anzi & Asma, 2007 and Dilek et al., 2008; Koura & Mohammed, 2009),** both in children and adult patients.

In this study, hematological laboratory investigations detected in 60 children with brucellosis included anemia (in 43%), leukopenia (in 38%), leukocytosis (in 20%) and pancytopenia (in 18%). In South-Western Saudi Arabia, **Benjamin and Annobil, (1992)** reported leucopenia (in 38%), anemia (in 64%), and thrombocytopenia (in 28%). 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% **(Al-Eissa & Al-Nasser, 1993 and Issa & Jamal, 1999 ;Koura & Mohammed, 2009).** Furthermore, **Koura and Mohammed (2009)** detected pancytopenia in 10% of children suffering brucellosis. The relative frequency of pancytopenia with brucellosis varies from 3% to 21% in the previous studies, being highly relative in adults but low relative in children **(Aysha & Shayib, 1986; Al-Eissa & Assuhaimi, 1993 and Yildirmak et al., 2003).**

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 the associated septicemia **(Dilek, 2008).** Although anemia in brucellosis is expected to be due to BM involvement, numerous other pathogenetic mechanisms can be (and have been) implicated. **Lambros et al. (2010)** reported that brucellosis induced an autoimmune process, culminating in autoimmune hemolysis.

In this study, blood culture was positive for brucellosis in 23 children (21 for *B. melitensis* and 2 for B.abortus). BM culture was done for 9 children, 3 of them was positive and isolated *B. melitensis*. The majority of children with brucellosis (63%) had serum agglutination titres of 1/160-1/320.

In this study, the most common symptoms and signs, in 11 children with pancytopenia, included bone aches and weakness (in 82%), sweating and chills (in 64%), petechiea and purpura (in 55%), hepatomegaly and splenomegaly (in 46%). The majority of children with pancytopenia (73%) have an agglutination titres of 1:320-1:640 or more. Furthermore, all cases with pancytopenia had positive blood culture. Nearly similar results were obtained by other studies **(Bilal et al., 1991; and Al-Eissa & Al-Nasser, 1993;Sari et al., 2008; Dilek et al., 2008; Omidi et al., 2009; Koura & Mohammed, 2009).**

Recommendations :

1) Surveillance, testing and massive immunization of animals in areas of Saudi Arabia with high infection rate.

2) An organized national brucellosis control program to eradicate the disease.

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

Characteristic(s)	n	%
Age (years):		
Mean ± SD	7.6±1.8	
Range	5-16	
Males	43	71.7
Duration of fever (days), X±SD	9.6±5.3	
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	19	31.7
Hepatomegaly	11	18.3
Splenomegaly	9	15
Hepatosplenomegaly	4	6.7
Lymphadenopathy	4	6.7
Petechiae and purpura	3	5

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

	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

* positive for B.melitensis

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

Finding	n=11	%=18-31
Symptoms, no (%)		
Bone aches & weakness	9	81.8
Sweating & chills	7	63.6
Painful & swollen joints	5	45.5
Signs, no (%)		
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 x 10^9/ml	3.12±1.3	
Platelets x 10^9 /ml	32.7±4.7	
Agglutaination titres, n(%):		
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.

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