BRUCELLOSIS AND DENGUE FEVER – A CO-INFECTION OR CROSS REACTIVITY?

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Brucellosis and Dengue fever can present with acute febrile illness with other nonspecific symptoms and share common haematological and biochemical abnormalities making their clinical differentiation a diagnostic challenge. We present two cases admitted with acute febrile illness and other nonspecific symptoms. In both patients diagnosis of brucellosis was confirmed by positive blood culture and or positive serology by tube agglutination test method, in both patients Dengue virus IgM and or IgG was also positive. This may represent co-infection or cross-reactivity between serological tests used for the diagnosis of brucellosis and dengue fever. To the best of our knowledge this has not been previously reported. Both these cases are presented here to share our experience with others.

Brucellosis is a multi-system zoonotic disease and is endemic in many parts of the world including Saudi Arabia[1]. Dengue fever virus infection is also found in large areas of tropics and subtropics[2]. It is being increasingly reported in parts of western and southern regions of Saudi Arabia[3]. Both diseases can present with similar nonspecific symptoms with or without haematological abnormalities like pancytopaenia or thrombocytopaenia which makes their differentiation a diagnostic dilemma[4,5]. Diagnosis of both diseases usually depends on serology because of its widespread availability and ease of use, in addition to other definitive diagnostic techniques such as blood culture and use of polymerase chain reaction (PCR).

We describe two cases admitted with acute fever and nonspecific symptoms with simultaneous positive brucella blood culture or positive serology by tube agglutination test method and positive Pan Bio Dengue Duo IgM and IgG capture ELISA used for diagnosis of dengue fever. We will discuss possible causes and present a review of literature.

CASE REPORTS

Patient 1: An eighteen year African male living in Saudi Arabia for the last two years was admitted with history of high grade fever, rigors, generalized aches and pains and sweating of five days duration. He denied any history of ingestion of dairy products or contact with animals. Past medical and family history was unremarkable. On examination, he was febrile with a temperature of 40°C. He had no skin or mucosal lesion or palpable lymph nodes. Abdominal examination revealed a palpable spleen of two centimeters below the left costal margins. Rest of the examination was unremarkable. Investigation showed a white blood count (WBC) of 2.5 x 10^9/L. with a normal differential count. Hemoglobin was 9.3 gram/ deciliter (gm/dl) with a haematocrit of 26.3. His platelets were 30 x 10^9/L. Erythrocyte Sedimentation Rate (ESR) was 70 mm in first hour. Prothrombin time (PT) and partial thromboplastin time (PTT) were normal. Blood film was negative for malarial parasite. Serum urea, creatinine, random blood glucose and electrolytes were normal. His total bilirubin was 37 micromole / liter. Serum alanine aminotransferase (ALT) was 216 units/ liter (U/L) and aspartate aminotransferase (AST) was 333 U/L. Alkaline phoshphatase (ALP), serum protein and albumin were normal. Lactate dehydrogenase (LDH) was 409 U/L (Table 1). Chest X-ray and transthoracic echocardiogram were normal. Abdominal ultrasound examination revealed moderate splenomegaly. Brucella tube agglutination test serology requested on the day of admission was negative. Repeated brucella titer with dilution was strongly positive with a titer of 1: 1280 for brucella abortus and 1: 640 for brucella mellittenesis. Dengue virus serology requested on third day of admission showed a positive dengue IgM capture ELISA and a negative IgG capture ELISA. Repeated dengue IgM and IgG capture ELISA 25 and 30 days after admission were negative. Serology for Hepatitis B Surface Antigen (HBsAg), antibody to hepatitis C virus (Anti HCV), IgM Hepatitis A virus (HAV), IgM Hepatitis E Virus (HEV) and antibodies to Human Immunodeficiency virus

(HIV) was all negative. Antinuclear antibodies, Rheumatoid factor, Monospot test and Direct Coomb's test were also found to be negative. One week after admission blood culture was reported as positive for brucella melitensis. Patient was started on oral doxycycline and intramuscular streptomycin. He started improving and became afebrile ten days after admission. Patient was discharged for home and advised to continue antibiotics for a period of six weeks.

Table 1: *Main haematological and biochemical Data in two patients with Brucellosis and Dengue Fever.*

	Case 1	Case 2
Haemoglobin gm/dl	9.3	13.3
WBC $\times 10^9$/L	2.5	2.82
Platelets $\times 10^9$/L	30	143
ESR mm in first hour	70	23
Creatinine micromoles/L	86	74
Bilirubin micromoles/L	37	12
AST U/L	333	103
ALT U/L	216	101
Alkaline Phosphatase U/L (N = 50-136	86	62
Albumin gm/L	34	38
CK U/L	250	154
LDH U/L	409	170

WBC = White Blood Cells; ESR = Erythrocyte redimentation rate. AST = Aspartate Aminotransferase; ALT = Alkaline Aminotransferase; ALP = Alkaline Phosphatase. CK = Creatinine Kinase; LDH = Lactate dehydrogenase.

Patient 2: A nineteen years old Saudi female was admitted with one week history of sudden onset of high-grade fever associated with chills. She also complained of severe frontal headache and generalized body aches. There was no history of raw milk ingestion, contact with animals or recent travel. On examination, she was febrile with temperature of 38°C; her pulse was 90 / minute and regular and blood pressure was 130 / 75mm of Hg. She did not have any skin rash or palpable lymph nodes. There were no signs of meningeal irritation. Systemic examination was unremarkable.

Investigation revealed a white blood count of 2.82 x 10^9/L. Haemoglobin was 13.3gm/dl and platelets were 143 x 10^9/L ESR was 23 mm in first hour. Urea, creatinine, electrolytes and blood glucose were normal. Her AST was 103 U/L and ALT was 101 U/L. Gamma glutamyl transferase (GGT) and alkaline phosphetase were normal (Table 1). Blood culture and urine culture were negative. Chest X ray, abdominal and pelvic ultrasound were reported as normal. Serology for HbsAg, anti HCV, IgM HAV, IgM HEV and monospot were negative. Serology for brucella on 2nd day of admission revealed a positive titer of 1:160 for both brucella abortus and brucella mellitensis.

Repeated serology for brucella on 7th day of admission showed that titer for brucella abortus and melitensis had risen to 1: 320. Dengue virus serology requested on 2nd day of admission was positive for both IgM and IgG by capture ELISA. She was treated with oral Rifampicin and Co-trimaxazole. She started improving quickly and became afebrile and asymptomatic after seven days. She was discharged for home and was advised to continue antibiotic for six weeks. During her follow up visit in out patient clinic she was well and asymptomatic.

DISCUSSION

The Brucellae are a group of gram negative coccobacillary organisms, of which four species are significant pathogens in humans. These include brucella melitensis, brucella abortus, brucella suis and brucella canis. The disease is transmitted from animals to humans by ingestion of infected animal products, close contact with an infected animal or inhalation of aerosol[6]. The disease remains a significant health problem in Mediterranean region including some parts of Saudi Arabia, Africa, Western Asia, Central and South America[1]. Brucellosis is a multi-system disease with a broad spectrum of clinical manifestations.

Dengue Fever is a mosquito-borne viral illness. It is caused by one of the four serotypes of dengue virus, belonging to the family Flaviviridae and predominantly transmitted by Aedes Egypti and a few other members of Aedes species[5]. The disease is now endemic in more than hundred countries in Africa, Mediterranean region, South America and South East Asia[2]. Dengue virus causes a broad spectrum illness ranging from mild undifferentiated fever to classical dengue fever, as well as dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS).

When brucella infection presents acutely, it shares common manifestations with dengue fever in endemic areas that makes their differentiation a difficult diagnostic challenge. Both diseases can present with non-specific febrile illness with myalgia, arthralgia, headaches and haematological manifestations including thrombocytopaenia or pancytopenia. The definitive diagnosis of brucellosis will depend on the isolation of organisms from

blood or tissue samples. The percentage of cases with positive culture varies from 15 to 70 percent[7]. Serology with the use of serum agglutination test remains the most widely used diagnostic tool for diagnosis of brucellosis. A titer above 1:160 is considered positive for diagnosis of disease in appropriate clinical settings[8]. Cross-reactivity of serum agglutination test with Escherichia Coli 0116 and 0117, Yersinia Enterocolitica, Vibrio Cholera, Salmonella Urbana, Fracisella tularensis, malaria, infectious mononucleosis and tuberculosis has been described[9-11]. A specific polymerase chain reaction (PCR) for brucella species is not available worldwide but shows promise for future use[12]. In our hospital we use tube agglutination test method (Shield Diagnostic, UK) for serological diagnosis of brucellosis. A titer of > 1 : 80 is considered as positive test and suggest diagnosis of recent brucella infection. The diagnosis of dengue fever also commonly depends on serology. Primary dengue infection is characterized by the presence of a significant or rising IgM titer 3-5 days after infection and these generally persist for 1-3 months after infection. In secondary dengue infection IgG levels increase rapidly to higher levels than observed in primary or past dengue infection and remain at these levels for 30-40 days and majority are also accompanied by elevation of IgM[13]. The combined Pan Bio Duo dengue IgM and IgG capture ELISA test is a commonly used accurate and rapid test for the detection of antibodies to dengue viruses in samples of human serum. The overall sensitivity of dengue IgM and IgG capture ELISA test is 90-100% in diagnosing primary and secondary dengue infection and specificity for other flaviviruses is above 75% and non-flavivirus is about 100%[14]. Although serological cross reactivity between different dengue serotypes, yellow fever virus, Japanese Encephalitis, and Murry Valley encephalitis is common with the use of other assays, it is uncommon in Pan Bio Duo dengue IgM and IgG capture ELISA assay[15]. Moreover, patient with non-dengue infection did not show elevation of IgM antibodies. In ministry of health of the Kingdom of Saudi Arabia central laboratory we used PANBIO Dengue Duo IgM Capture and IgG Capture ELISA. A dengue IgM capture > 11 papino units and dengue IgG >22 papino units are considered as a positive test.

To our knowledge co-infections with brucellosis and dengue fever or cross-reactivity of Brucella serum agglutination test with PanBio Dengue Duo IgM and IgG capture ELISA used for serological diagnosis of dengue fever has not been reported previously. We conducted a Medline (Pub-med) search in English literature from 1966 through November 2005 using the combination “Brucellosis and Dengue fever”, “Dengue serology and cross reactivity”, “Brucella serology and cross reactivity” or “dengue and brucella serology”. We also reviewed the manufacturer's literature for Brucella and Dengue fever tests used for diagnosis in our patients for any evidence of cross-reactivity. During this search, we did not find any documented case of cross-reactivity between the two diseases or co-infection of brucellosis and dengue fever. Both of our patients had the diagnosis of Brucella confirmed by blood culture and/ or serology with-simultaneous positive dengue IgM and/or IgG capture ELISA serology at the time of hospitalization. In our first patient, the positive dengue IgM capture and negative IgG capture ELISA done on 9th day from the illness suggest primary dengue infection (co-infection). However cross reactivity with other flavivirus group could be another explanation for positive dengue IgM capture ELISA results. This seems unlikely explanation because of the fact that other group of flavivirus infections is not endemic in Western region of Saudi Arabia and cross-reactivity does not produce positive dengue IgM capture ELISA results. However the cross-reactivity will be a problem if dengue IgG capture ELISA is positive and dengue IgM capture ELISA is negative[25]. The repeated dengue IgM and IgG capture ELISA test within one month from onset of symptoms was negative. This may suggest that initial positive IgM capture ELISA test may be a false positive result which became negative after treatment for brucellosis or really represent co-infection. Our second patient was a young Saudi female who also presented with an acute non-specific febrile illness. Although blood culture was negative for brucella, the diagnosis of acute brucellosis seemed quite definite due to the rising titer of antibodies for both brucella abortus and melitensis. She also had a positive IgM and IgG capture ELISA for dengue virus. This may suggest secondary dengue infection (co-infection), as clinical features and other laboratory parameters are indistinguishable for both diseases. However cross-reactivity can not be ruled out as the definitive diagnostic test in the form of virus isolation from blood or other body tissues was not available.

It was **Concluded** that Simultaneous positive Pan Bio Dengue Duo IgM and / or IgG capture ELISA for diagnosis of dengue fever and the tube agglutination test used for serological diagnosis of brucella species (Shield Diagnostics) could represent co-infection or cross reactivity that needs cautious interpretation of results. In such circumstances the use of definite diagnostic tests such as isolation of bacterium or virus from blood or other tissues samples or the use of PCR for causative organisms should be considered for appropriate management.

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