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RESEARCH ARTICLE

VISCERAL LEISHMANIASIS: HAEMATOLOGIC AND HAEMOSTATIC CHANGES
AMONG SUDANESE PATIENTS

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ABSTRACT

Visceral leishmaniasis (VL) is a protozoal parasitic disease caused by *Leishmania donovani* complex, and transmitted to man by the bite of infected female sandfly. The parasite infects the macrophages in the liver, spleen and bone marrow producing many haematological abnormalities. VL is a major public health problem in Sudan. The aim of this study was to determine the haematologic and haemostatic changes among VL patients in Sudan. One hundred subjects were enrolled: 50 patients with established diagnosis of visceral leishmaniasis who were admitted to Tropical Disease Hospital, Sudan; and 50 age and sex matched healthy individuals as controls. Complete blood count was performed by automated cell counter (Sysmex KX-21N); D-Dimer was measured using i-CHROMA™ system (Boditech – Korea); APTT, PT and TT were measured using coagulometer (Sysmex CA 50). Patients included 30 male and 20 female, their median age was 10 year, with minimum age of 1 and maximum of 51 years. All patients were anaemic, 70% (35/50) were leucopenic, 62% (29/50) were thrombocytopenic and 44% (22/50) were pancytopenic. APTT, PT and TT were prolonged among patients when compared to controls (p value = 0.000, 0.001 and 0.000 respectively). Mean D-Dimer level was significantly higher among the cases when compared with the controls (p value 0.000). In conclusion, our study shows that the haematologic and haemostatic features of Sudanese VL are, generally, similar to the picture seen in other areas worldwide.

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INTRODUCTION

Visceral leishmaniasis (VL), also known as kala azar, is a protozoal parasitic disease caused by *Leishmania donovani* complex, and transmitted to man by the bite of infected female sandfly (Nail and Imam, 2013). It results from the infection of phagocytes within the reticuloendothelial system from the initial site of cutaneous infection. The proliferation of parasites in macrophages in the liver, spleen and bone marrow of patients with VL gives rise to progressive hepatosplenomegaly and bone marrow suppression and disturbs the function of these organs producing many haematological abnormalities (Varma and Naseem, 2010). Unless treated, patients develop pancytopenia and immunosuppression and are prone to super-infections with other microbes (WHO, 2010). The incubation period of the disease varies from 10 days to one year. The infection may be asymptomatic or may lead to fully blown kala-azar. Initially, there is low grade recurrent fever and malaise, followed by progressive wasting, anaemia and hepatosplenomegaly and if untreated, proves fatal within 2-3 years. In some patients, the disease takes a more acute course. The cause of death is often a secondary infection (Malla and Mahajan, 2006).

Leishmania infections are worldwide in distribution; it is endemic in more than 60 countries (Murray, 2002). The World Health Organization estimates the annual global rate for VL prevalence at 2.5 million and incidence at 0.5 million cases, and it causes 60-70 thousand deaths every year (WHO, 2010). VL occurs mainly (90% of cases) in Bangladesh, Brazil, Ethiopia, India, Nepal, South Sudan, and Sudan (Desjeux, 2004). It has been known to exist in Sudan since the beginning of 19th century (Zijlstra and El-Hassan, 2001). The disease is endemic in several regions over a wide area, including the eastern and the former southern part of the country (the new South Sudan Republic) with small foci in the Nuba Mountains and the state of Darfur, in western Sudan, and in Kapoita at the former southern Sudan/Kenyan border (Zijlstra and El-Hassan, 2001). This study aimed to determine the haematologic and haemostatic changes among VL patients in Sudan.

MATERIALS AND METHODS

One hundred subjects were enrolled in this cross-sectional study: 50 patients with established diagnosis of visceral leishmaniasis (diagnosis based on the identification of LD-bodies in the bone marrow) who were admitted to Tropical Disease Hospital, Sudan; and 50 age and sex matched healthy individuals as controls. None of the patients were receiving

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anticoagulant therapy at the time of the study. Patients with other known causes of haemostatic changes such as pregnancy, smoking, diabetes, malignancy and hypertension were excluded; known anaemic patients or patients with any other source of infection were also excluded. Informed consent was obtained from each subject before enrollment in the study. Five ml of venous blood was collected from each subject: 2.5 ml in 3.8% trisodium citrate (9:1 vol/vol), kept on ice until centrifugation at 2500g for 30 minutes at 4°C, plasma samples were immediately frozen and stored at - 80°C for subsequent coagulation analysis; and 2.5 ml in EDTA for blood count. Laboratory analysis was performed at the Department of Haematology, Faculty of Medical Laboratory Sciences, Alneelain University.

D-Dimer was measured using i-CHROMA™ system (Boditech – Korea). The test uses the sandwich immunodetection method. D-Dimer is bound with an antibody in buffer and the antigen-antibody complexes are captured by antibodies that have been immobilized on the test strip as sample mixture migrates through nitrocellulose matrix. Signal intensity of fluorescence on detection antibody reflects the amount of the antigen captured and is processed by i-CHROMA™ Reader to show D-Dimer concentration in the specimen. The working range of i-CHROMA™ D-Dimer test is 50 – 10,000 ng/ml. APTT, PT and TT were measured using coagulometer (Sysmex CA 50) which rely on scattered light detection method. Complete blood count was performed by automated cell counter (Sysmex KX-21N). Statistical analysis was performed using statistical package for social science (SPSS) software. Evaluation of patient's data was performed using the t-test. Results with p value < 0.05 were considered statistically significant.

RESULTS

Patients included 30 male and 20 female, there median age was 10 year, with minimum age of 1 and maximum of 51 years. All patients were tested for the blood count, APTT, PT, TT and D-Dimer level. Table 1 showed results of the blood count, APTT, PT and TT. All patients were anaemic with maximum haemoglobin level of 107 g/l, 70% (35/50) were leucopenic with TWBC lower than 4.0 X10⁹/L, 62% (29/50) were thrombocytopenic with platelets count lower than 150 X10⁹/L and 44% (22/50) were pancytopenic.

Table 1. Haematological values, APTT, PT, TT and D-Dimer level between VL patients and controls

VL patients	Control	P value	
Hb mean±SD (g/l)	76.5±16.8	122.7±28.0	0.000
RBC mean±SD(X1012/L)	3.2±0.7	4.4±0.6	0.000
PCV mean±SD (%)	23.2±5.2	36.2±7.9	0.000
TWBC mean±SD (X109/L)	3.6±2.0	5.6±1.4	0.000
Platelets mean±SD (X109/L)	147.5±89.0	269.2±90.8	0.000
D-Dimer mean±SD (ng/ml)	753.8±262.8	236.3±83.9	0.000
APTT mean±SD (seconds)	61.7±44.0	31.1±28.4	0.000
PT mean±SD (seconds)	35.3±45.9	12.5±1.1	0.001
TT mean±SD (seconds)	17.7±6.6	12.6±0.9	0.000

APTT, PT and TT were prolonged among patients when compared to controls (p value = 0.000, 0.001 and 0.000 respectively). Mean D-Dimer level for patients was 753.8±262.8 ng/ml, the levels were elevated in 86% of patients

(43/50), and were always normal among the control group with a mean level of 236.3±83.9 ng/ml. Mean D-Dimer level was significantly higher among the cases when compared with the controls (p value 0.000).

DISCUSSION

Sudan is one of the five countries in the World that constitute 90% of VL cases (Desjeux, 2004). The disease contributes significantly to the propagation of poverty, because treatment is expensive and hence either unaffordable or it imposes a substantial economic burden (Ngure *et al.*, 2008). In this study we determined the haematological and haemostatic changes among Sudanese patients with VL. The study included 50 Sudanese patients with VL, their complete blood count, APTT, PT, TT and D-dimer levels were measured and compared with 50 age and sex matched normal subjects as control. All patients were anaemic with maximum haemoglobin level of 107 g/l, our finding is in agreement with previous Studies (Marwaha *et al.*, 1991; Al-Jurrayan *et al.*, 1995; Singh *et al.*, 1999; Patrela *et al.*, 2010). Red cell survival and ferrokinetic studies have suggested that haemolysis is the major cause of anemia in VL (Woodroff *et al.*, 1072; Pippard *et al.*, 1986), but nutritional factors might be common one. The majority of our patients are leucopenic, this finding is in agreement with previous Studies (Marwaha *et al.*, 1991; Al-Jurrayan *et al.*, 1995; Singh *et al.*, 1999; Patrela *et al.*, 2010). The main cause for leucopenia has been attributed to hypersplenism (Varma and Naseem, 2010). Thrombocytopenia is observed in the majority of patients, similar finding was reported in previous Studies (Marwaha *et al.*, 1991; Singh *et al.*, 1999; Patrela *et al.*, 2010). Splenic sequestration is possibly the main contributory factor (Varma and Naseem, 2010). We observed a significant increase in the mean of APTT, PT, TT and D-dimer level among VL patients, when compared with the controls. Al-Jurrayan *et al.* (Al-Jurrayan *et al.*, 1995) recorded coagulation abnormalities in 10 (11%) of the 94 VL patients in their study, in the form of prolonged PT and APTT, with 4 (36%) of these having disseminated intravascular coagulation. Alteration of the liver function among VL patients is possibly the main contributory factor. Liver dysfunction may be caused directly by protozoa itself or indirectly to the effect related to the immune response of the parasites (Varma and Naseem, 2010).

Conclusion

In conclusion, we evaluated the haematologic and haemostatic feature among VL patients in Sudan by determining blood cell count, APTT, PT, TT and D-Dimer level. Our study shows that the haematologic and haemostatic features of Sudanese VL are, generally, similar to the picture seen in other areas worldwide.

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Authors contributions

O. A. Abd Elsieed and M.H.A. Abdalla conceived the idea of the study, collected and analyzed samples and data and wrote the manuscript.

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