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Hematological Alterations Induced by Visceral Leishmaniasis

Twasol Elsheikh Musa Altayeb[1], Nasreldeen Ali Mohammed[1], Sara Abdelghani[2], Lienda Bashier Eltayeb[3*]

[1]Department of Hematology, Faculty of Medical Laboratory Sciences, Al-Neelain University, Khartoum, Sudan
[2]Department of Parasitology, Faculty of Medical Laboratory Sciences, Al-Neelain University, Khartoum, Sudan
[3]Department of Medical Laboratory Science, College of Applied Medical Science, Prince Sattam bin Abdulaziz University, Al-Kharj, Saudi Arabia

Abstract

The aim of this study was to assess visceral leishmaniasis (VL) among infected Sudanese patients in Al–Gedaref state.

***Methods and Results*:** A case-control study was conducted among patients with VL attending Al–Gaderif Teaching Hospital. A total of 80 subjects were included in the study: 40 patients with VL (the main group [MG]) and 40 apparently healthy individuals (the control group [CG]). The complete blood count (CBC) was determined using the Sysmex KX-21 N hematological analyzer. The platelet-poor plasma was used to determine prothrombin time (PT), thrombin time (TT), and activated partial thromboplastin time (aPTT).

The age group of 12-21 years was the most frequent (40%) among VL patients. Male patients were significantly more frequent (72.5%) than females ($P<0.05$). In MG, the Hb level was 8.71±1.73 g/dL, compared to 14.25±4.11 g/dL in CG, which reflected the severity of the disease. WBCs and neutrophils decreased significantly, compared to CG, but lymphocytes increased significantly. Thrombocytopenia was observed among pediatric patients, indicating bleeding tendency as one of the VL complications. The platelet and coagulation profile of patients was also altered. PT and aPTT were prolonged significantly, compared to CG.(**International Journal of Biomedicine. 2021;11(2):151-155.**)

Key Words: visceral leishmaniasis • anemia • leukopenia • thrombocytopenia • coagulopathy

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Abbreviations

aPTT, activated partial thromboplastin time; **CBC**, complete blood count; **Hb**, hemoglobin; **Hct**, hematocrit; **MPS**, mononuclear phagocyte system; **MCV**; mean corpuscular volume; **MCH**, mean corpuscular hemoglobin; **MCHC**, mean corpuscular hemoglobin concentration; **PPP**, platelet-poor plasma; **PT**, prothrombin time; **RBC**, red blood cells; **TT**, thrombin time; **VL**, visceral leishmaniasis; **WBC**, white blood cells.

Introduction

Visceral leishmaniasis (VL), also known as Kala-Azar, is fatal if left untreated in over 95% of cases.[(1)] Al–Gedaref state in Sudan is a major endemic region for VL, with the prevalence rates varying greatly between villages, based on average rainfall and altitude. *Leishmania donovani* bodies have been identified as parasitic etiological agents causing VL, which are transmitted by the sandfly *Phlebotomus orientalis* in Eastern Sudan.[(2)] *P. orientalis* sandfly populations increase in the rainy season [(2,3)] and are concentrated in an environment with heavy abundances of Acacia seyal (locally

known as "Taleh") and Balanites aegyptica trees (locally known as "Lalob" or "Higleeg") that grow on vertisols (black cotton soil); these account for the high prevalence rate of VL in Gedaref state in Sudan.[3,4]

The L. donovani parasite persists in the spleen and bone marrow, and its expansion in these sites is associated with an increase in local hematopoietic changes.[5] Various hematologic manifestations are found in visceral forms. VL may present with splenomegaly, hepatomegaly, and fever. VL is endemic in more than 60 countries worldwide, including Southern Europe, North Africa, and the Middle East. VL is a systemic infection of the reticuloendothelial system caused by the protozoa Leishmania donovani of the genus Leishmania. The British medical doctor Ronald Ross published a paper in November 1903 commenting on the discovery of the ovoid bodies found by Leishman and Donovan in spleen pulp of patients with chronic pyrexia and splenomegaly. [6] He concluded that the ovoid bodies were not degenerated trypanosomes but a novel protozoan organism and that the clinical picture of the cases resembled that of kala-azar. [7] In a follow-up paper, Ross concluded that these ovoid bodies belonged to a new genus and proposed to name them Leishmania donovani.[8] Leishmania parasites are dimorphic organisms that live and replicate in the gut of sandflies as flagellated forms (promastigote) or as aflagellated forms (amastigotes) in mammalian cells. Amastigotes exist and proliferate in the MPS, especially the spleen, liver, and marrow; this leads to hyperplasia of the MPS with resultant disturbances in phagocytic-bearing organs, producing hematological manifestations.[2] Leishamnia spp. is endemic in tropical and sub- tropical regions, and human disease mainly occurs in parts of Africa, Asia, and the Middle East.[4,5] Poverty, war, conflicts, and migration have significantly aggravated leishmaniasis in East Africa.

Al–Gedaref state is an endemic area for VL, which affects individuals of different age groups, particularly children and young adults. Most of the patients presented to clinics with irregular bouts of fever, weight loss, enlargement of the spleen and liver, anemia, leukopenia, and thrombocytopenia. Thrombocytopenia is detected after a long duration of illness; splenic sequestration is a possible contributing factor. Liver dysfunction with jaundice, ascites, and deranged coagulation may occur in the late stages. Liver dysfunction may be caused by the protozoa itself or indirectly by an effect related to the immune response of the parasite.

The disease is more frequent among rural populations and domains. Around 95% of patients are under 5 years of age, the male-to-female ratio is 1.3:1.6. The appearance of the parasite in the human body stimulates the humeral immune system resulting in antibody production. The principal involved organs are the reticuloendothelial system; other organs such as kidneys, digestive system, and mucosa may be filled with macrophages. Bacterial infection is one of the major complications leading to death in VL patients.[9,10]

VL is characterized by a chronic course of hepatomegaly, which leads to anemia and pancytopenia. The parasite migrates to the internal organs, such as liver, spleen, and bone marrow, and if left untreated will almost always result in the death of the host. Anemia is the most common hematological manifestation of VL; the disease may be associated with leukopenia, thrombocytopenia, hemophagocytosis, and disseminated intravenous coagulation.[11-13] Normochromic normocytic anemia is a frequent and clinically significant feature of VL, and hemoglobin levels of 7–10 g/dl are commonly found. It is more severe in pediatric patients. The cause of anemia in these patients is multifactorial: sequestration and destruction of RBCs in the enlarged spleen; immune mechanisms and alterations in RBC membrane permeability have been implicated; hemolysis is the major cause of anemia; and plasma volume expansion associated with a massively enlarged spleen.

The aim of this study was to assess the VL among infected Sudanese patients in Al–Gedaref state.

Materials and Methods

A case-control study was conducted among patients with VL attending Al–Gaderif Teaching Hospital. Written informed consent was obtained from each research participant (or the participant's parent/guardian).

A total of 80 subjects were included in the study: 40 patients with VL (the main group [MG]) and 40 apparently healthy individuals (the control group [CG]). Subjects were enrolled according to the following inclusion criteria: VL patients (both sexes) under treatment. Excluded were patients newly diagnosed with VL and patients with associated diseases that may affect hemostatic profile, as well as patients taking medications affecting blood coagulation. The questionnaire was used to collect demographic and clinical data.

A total of 5ml venous blood was collected from each subject: 2.5ml in EDTA container, and 2.5ml in TSC container. PPP was used to determine PT, TT, and aPTT. The CBC was determined using the Sysmex KX-21 N hematological analyzer.

Statistical analysis was performed using the IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.). Baseline characteristics were summarized as frequencies and percentages for categorical variables and as mean±standard deviation (SD) for continuous variables. Spearman's rank correlation coefficient was calculated to measure the strength and direction of the relationship between two variables. A probability value of $P<0.05$ was considered statistically significant.

Results

Table 1 presents the age distribution among VL patients and control subjects. The age group of 12-21 years was the most frequent (40%) among VL patients. Male patients were significantly more frequent (72.5%) than females ($P<0.05$); males often work as farmers, which makes them more vulnerable to sandfly bites. Tables 2 and 3 displayed all hematological and coagulation parameters: MG was significantly different than CG. Although all patients received treatment, Hb, RBC count, and RBC indices significantly decreased, compared to control subjects. In MG, the Hb level was 8.71±1.73g/dL, compared to 14.25±4.11g/dL in CG, which reflected the severity of

the disease. WBCs and neutrophils decreased significantly, compared to CG, but lymphocytes increased significantly. Thrombocytopenia was observed among pediatric patients, indicating bleeding tendency as one of the VL complications, as reported by many previous investigators. The platelet and coagulation profile of patients was also altered. PT and APTT were prolonged significantly, compared to CG.

Table 1.

The age distribution among VL patients and control subjects

Age group, years	MG (n=40)		CG (n=40)		*P*-value
	n	%	No	%	
≤11	8	20.0	1	2.5	>0.05
12-21	16	40.0	22	55.0	
22-31	8	20.0	10	25.0	
32-41	5	12.5	4	10.0	
>41	3	7.5	3	7.5	
Total	40	100.0	40	100.0	
Male	29	72.5	20	50	<0.05
Female	11	27.5	20	50	
Total	40	100.0	40	100.0	

Table 2.

Hematological parameters in VL patients and control subjects

Parameter	MG	CG	*P*-value
Neutrophils, %	51.49±12.93	60.16±16.19	0.010
Lymphocytes, %	40.40±12.32	30.66±14.78	0.002
MCHC, g/dL	31.61±2.44	34.05±1.00	0.000
MCH, pg	25.11±3.19	28.08±1.91	0.000
MCV, fL	79.78±8.68	80.19±11.32	0.856
Hct, %	28.04±6.21	41.13±7.34	0.000
Hb, g/dL	8.71±1.73	14.25±4.11	0.000
RBC, $\times10^{12}$/L	3.49±0.81	5.06±1.29	0.000
WBC, $\times10^{9}$/L	2.97±1.21	6.36±1.89	0.000

Table 3.

Blood platelets, PT, and aPTT in VL patients and control subjects

Parameter	MG	CG	*P*-value
PT, sec	16.42±4.06	14.22±0.98	0.001
aPTT, sec	47.63±10.72	27.03±2.15	0.000
Platelets, $\times10^{9}$/L	111.0±58.15	285.2±76.38	0.000

Tables 4 and 5 show a comparison of hematological and coagulation parameters across gender and age. There was no significant correlation between genders or age groups.

Table 4.

A comparison of hematological and coagulation parameters across gender in VL patients

Parameter	Male (n=29)	Female (n=11)	*P*-value
PT, sec	16.27±3.25	16.71±5.40	>0.05
aPTT, sec	46.52±9.73	49.71±12.49	>0.05
Neutrophils, %	53.13±13.43	48.42±11.80	>0.05
Lymphocytes, %	39.18±11.66	42.68±13.40	>0.05
Platelets, $\times10^{9}$/L	116.6±58.20	100.7±58.76	>0.05
MCHC, g/dL	31.65±2.73	31.53±1.89	>0.05
MCH, pg	25.18±3.57	24.97±2.46	>0.05
MCV, fL	80.50±9.53	78.45±7.00	>0.05
Hct, %	29.38±6.70	25.55±4.38	>0.05
Hb, g/dL	8.99±1.80	8.17±1.53	>0.05
RBC, $\times10^{12}$/L	3.60±0.90	3.29±0.59	>0.05
WBC, $\times10^{9}$/L	2.99±1.90	2.94±1.77	>0.05

Table 5.

Correlations between hematological/coagulation parameters and age

Parameter	R-value	P-value
PT	0.045	0.782
aPTT	0.078	0.634
Neutrophils	0.254	0.114
Lymphocytes	-0.313*	0.044
Platelets	0.061	0.710
MCHC	-0.225	0.162
MCH	0.114	0.483
MCV	0.229	0.155
HCT	-0.127	0.433
Hb	-0.235	0.144
RBC	-0.090	0.582
WBC	0.071	0.665

Discussion

Hematological disorders in VL determine the leading role of hematologists in the diagnosis of this disease. Hematologists must ensure a high standard of suspicion for VL and include it in the differential diagnosis of patients who expressed fever, hepatosplenomegaly, anemia, leukopenia, thrombocytopenia, pancytopenia, and DIC, especially in endemic areas.

The current study noted that VL affects individuals in different age groups, but the age group of 12-21 years occurs most frequently. A study, performed in Yemen, revealed that VL predominated among five-year-old patients

(mainly 1-3 years).[14] Our results were in conflict with a study, performed in Iraq,[9] which showed that both genders were equally infected, with mean age ranging between 7 months and 12 years. An Iranian study showed that 91.5% of VL cases were in the age group ≤5 years, 57.1% of which were females.[15] The discrepancy could be attributed to different factors, such as sample size, occupation of participants, the season when samples were collected, as well as the immune status of participants.

Gamal Hamid et al.[14] concluded that children under 10 years old are the main victims of VL, as in Sudan where the risk factors for death among pediatric VL were highest for those less than 2 years old. Among adult patients, the most affected age ranged between 21 and 30 years. The significant hematological changes observed among VL patients were similar to findings reported by many investigators.[14,16-18] A study in Yemen revealed that pancytopenia and hepatosplenomegaly are the most common clinical manifestations in Yemeni children. The reason for the higher frequency of pancytopenia is probably the longer duration of symptoms and splenomegaly before presentation to the hospital and increased peripheral destruction, rather than bone marrow failure.[14]

Laboratory findings of VL patients in the current study revealed a significant decrease in Hb, RBC, and RBC indices. Similar results have been reported by many authors.[14-16,19] Multiple factors could be the leading causes of anemia among patients with VL. Reduced plasma iron level in the presence of greatly increased iron stores suggests that the reticuloendothelial hyperplasia is accompanied by abnormal iron retention by macrophages, typical of anemia of chronic diseases.[16] This may limit the marrow response to hemolysis. Hypersplenism is another primary pathogenetic mechanism of anemia, although nutritional deficiencies of iron, folate and vitamin B12 may play a further contributory role.[16,19] Malaria infection could further complicate the situation and may co-exist in the same patients since Al–Gedaref state is characterized by heavy rains, which provide suitable habitat for both sandfly and mosquito vector activities.

The WBC count and percentage of neutrophils in VL patients significantly decreased while the percentage of lymphocytes significantly increased, compared to control. Many studies showed similar results and concluded that leukopenia and neutropenia were common among pediatric patients.[11,16,17] The main cause for their development has been attributed to hypersplenism. In addition, suppression of the immune system is expected in patients with VL, as previously reported,[11] since the parasite actively secretes proteases and other factors that affect immune cells and cytokines.(9) Furthermore, the humoral and cell-mediated immune response of the host depends on the severity of VL and parasite burden.[20]

Thrombocytopenia, along with anemia, is a common clinical finding in patients with VL. Thrombocytopenia is exhibited in 40% to 65% of patients.[5] Helmi et al. in Iraq[21] and Rahim et al.[22] in Pakistan found thrombocytopenia in 80%-90% of the patients. It is postulated that the thrombocytopenia observed in the peripheral blood may have been due to hypersplenism, and partly due to poor platelet formation.[23,24]

In our study, in VL patients PT and APTT were significantly prolonged, compared to healthy control subjects. These findings were confirmed by previous studies. [12,16,25] Accordingly, the bleeding tendency existed among patients with VL in the current study. This may be due to liver dysfunction, coagulopathy, and exhaustion of plasma proteins. Previous authors concluded that bleeding is one of the main causes of death in VL patients.[2,12,,13,19]

In conclusion, VL is common in Al–Gedaref state, and it is more frequent among children. The disease affects primary hemostasis, coagulation, and fibrinolysis, and these alterations are related to the severity of clinical symptoms. Anemia and thrombocytopenia are observed in the majority of patients, and splenic sequestration is possibly the main contributing factor. A significant increase in the levels of aPTT, PT, and TT is common for VL. Liver dysfunction may be caused directly by the protozoa themselves or indirectly by an effect related to the host's immune response to the parasite.

Competing Interests

The authors declare that they have no competing interests.

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***Corresponding author**: Dr. Lienda Bashier Eltayeb. Department of Medical Laboratory Science, College of Applied Medical Science, Prince Sattam bin Abdulaziz University, Al-Kharj, Saudi Arabia. E-mail: lindarose009@hotmail.com*

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