Hematological alteration among visceral leishmaniasis patients attending in Western Tigrai, Ethiopia, 2018/2019: A cross-sectional study

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Research

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Abstract

Background: Hematological abnormalities are common in visceral leishmaniasis patients, which is one of the main public health problems worldwide. The most common hematological abnormalities are anemia, leucopenia and thrombocytopenia. Therefore, this study aimed to assess the hematological alteration among visceral leishmaniasis patients attending Western Tigrai, Ethiopia, 2019. Method: Hospital based comparative cross sectional study was conducted from November 2018 to March 2019 in Western Tigrai, Ethiopia. A total of 100 Visceral Leishmaniasis patients and 100 control groups were included in this study. Blood was collected and analyzed by mindray BC-5800 hematology analyzer. Data was entered and analyzed using Statistical package for social science version 23. Student independent t-test was used for data analysis. P value <0.05 was considered as statistically significant at 95% confidence level. Result: From the total 100 visceral leishmaniasis patients the following abnormalities were reported: 96(96%) anemia, 95(95%) leucopenia, 92(92%) neutropenia, 73(73%) Lymphopenia, 45(45%) eosinopenia and 97(97%) had thrombocytopenia. Red blood cell, hemoglobin, hematocrit, red cell indices and platelet were significantly lower ($p<0.05$) in visceral leishmaniasis patients compared with the control groups. Similarly the total White blood cell, neutrophil, lymphocyte, eosinophil and basophil count were significantly lower ($p<0.05$) in visceral leishmaniasis patients compared with the control groups. Conclusion: The major changes in peripheral blood of patient with visceral leishmaniasis are reduced number of red blood cells, reduction in leukocytes and decreased platelet count. Visceral leishmaniasis patients presented with anemia, leucopenia and thrombocytopenia. So this finding indicates that visceral leishmaniasis causes alterations of hematological parameters. Key words: Amastigote, Anemia, Hematological Parameters, Promastigote, Visceral leishmaniasis, Ethiopia .

Background

Leishmaniasis is groups of parasitic diseases caused by the genus Leishmania. Clinically, it is categorized as; visceral, cutaneous mucocutaneous, diffuse cutaneous and post kalaazar dermal leishmaniasis [1]. Visceral leishmaniasis is one of the fatal human diseases with about an incidence of 0.2 to 0.4 million cases worldwide, causing 20,000-40,000 deaths annually [2]. It is mainly distributed in East Africa, South Asia, South America, and Mediterranean Region, with an estimated 50,000 to 90,000 new cases annually. More than 90% of VL cases were reported in Brazil, Ethiopia, India, Kenya, Somalia, South Sudan, and Sudan [3].

VL causes diverse hematological abnormalities like hepatosplenomegaly, anemia, leucopenia and thrombocytopenia. Normocytic normochromic anemia is common major feature of VL with Hb level of 7-10g/ dl. VL causes anemia due to numerous factors; Sequestration and destruction of red blood cells (RBC) in enlarged spleen, opportunistic infection, chronic disease**,** certain nutritional deficiencies, immune mechanism and alterations in RBC membrane permeability have been identified [1].

As reports indicates that thrombocytopenia and leucopenia is also causes the hematological changes in VL patients [1].The leucopenia in VL patients is mainly due to decrease in neutrophilic leukocytes. This is

believed to be due to hypersplenism. Eosinophil number is decreased significantly, while the percentages of monocytes are increased. In the other hand, absolute number of lymphocytes is slightly decreased but, there is controversial issues among different authors about the absolute number of lymphocytes in VL [4].

Several studies have reported pancytopenia in the late stages of VL [5-7]. There is variation in the frequency of pancytopenia reported by several researchers [5-8]. The cause of pancytopenia is assumed to be due to sequestration of blood cells in the spleen [4]. Even though several studies have been pointed out hematological alteration of visceral leishmaniasis, there is paucity of data in Ethiopia; particularly in the study area. So, this study aimed to assess the hematological alteration of among VL patients western Tigrai, Ethiopia.

Materials And Methods

Study design, area, and period

Hospital based comparative cross-sectional study was conducted to assess hematological alteration among visceral leishmaniasis patients attending Kahsay Abera and Mearg Hospitals, Western Tigrai, Northern Ethiopia , 2019. Western zone of Tigrai is the biggest in terms of geographic and territorial possessions that stretches along the border of Sudan in the North West, the Amhara Adminstative region in the south and the Eritrea border in the north where people live in clusters over a wide range of areas. It is subdivided into three districts; from north to south they are Kafta Humera, Wolqayt and Tsegede. It is one of the agriculture surplus areas in Ethiopia known for producing sorghum and exportable products like sesame which makes it a net contributor to the national economy. Based on the 2007 Census conducted by the Central Statistical Agency of Ethiopia, this zone has a total population of 356,598, of whom 71,823 or 20.14% are urban inhabitants.

Kahsay Abera Hospital is the district Hospitals found in VL endemic region in north Ethiopia with close to 210 beds and an estimated 742,000 catchment population including the migrant population from Sudan, and Eritrea. Mearg hospital is the district hospital found in VL endemic area in Tsegede Woreda, Western Tigrai which have an estimated 299,594 catchment population including migrant population and have 134 beds at emergency, medical, surgical, gynecology, and pediatrics wards.

Study participants

Two study groups were involved: Case and control groups. Case groups include all VL patients confirmed at Kahsay Abera and Mearg Hospital laboratories during the study period. VL patients were diagnosed by experienced physician and VL patients who have history of any other chronic disease (kidney disease, liver disease, cancer, HIV/AIDS, diabetic mellitus, hypertension tuberculosis, and malaria) were excluded. Patients under treatment of anti VL were also excluded from the study.

Control group includes all apparently healthy patient attendants of Kahsay Abera and Mearg Hospitals, who were match with cases in age and sex without having VL. Control groups were examined and screened by experienced physician and individuals who have history of any chronic disease (kidney disease, liver disease, cancer, HIV/AIDS, diabetic millitus, hypertension tuberculosis, and malaria) were also excluded from the study.

Sample collection and laboratory analysis

About 3 mL venous blood sample was collected on vacutainer tube containing EDTA after cleaning with 70% ethanol. As soon as the sample was collected and labeled, it was transported to the hematology working area to be analyzed. Blood analysis was done for the hematological parameters using mindray BC-5800 hematology analyzer and peripheral blood morphology was examined.

Data Quality Assurance

To assure the quality of data in the study, data collectors and supervisors was trained and a regular supervision and follow-up was made by supervisors and principal investigator. Three levels of commercially prepared hematology cell controls (Normal, Low and High) were done to assure the analytical part. Analysis was performed by following standard operating procedure (SOP) after running and passing of these levels of controls. The peripheral morphology smear was re-examined by trained and experienced laboratory personnel.

Ethical considerations

Before starting the study, ethical clearance was obtained from the ethical review committee of the department of Medical Laboratory Sciences of Addis Ababa University. Further permission was also obtained from Tigrai Regional Health Bureau and from Administrators of selected hospitals. Furthermore, after explaining the importance of the study, an informed written consent was obtained from study participants.The confidentiality of the information collected was maintained by using code numbers for participants.

Dissemination of the result

A finding of this study was presented to the scientific community in the Addis Ababa University, department of Medical laboratory science, College of Health Sciences. The result was disseminated to the study health facilities, weredas and zonal health administrations, Tigrai regional health bureau. Finally it will be submitted to peer reviewed local and international journals for publication.

Result

Socio demographic Characteristics of study participants

The study included 200 study participants that comprise 100 VL patients with mean age 28±9.6 years and 100 controls with mean age 27.6 ± 4.7 years. Ninety one (91%) was males in case group and 90(90%) were males in control group. Forty nine percent of the VL cases and 55% of the control groups were single. Most of the VL cases were primary school which accounts and 56% of the controls were diploma and above. About 51% of VL cases and 56% of healthy controls were primary school. The majority of the VL cases and control groups were from rural area (Table 1).

Clinical features of visceral leishmaniasis patients

In this study the main clinical signs and symptoms presented at the initial evaluation were: fever (100%), splenomegaly (100%), general weakness (85%), skin mucosal pallor (72%), bleeding (67%), weight loss (65%), anorexia (52%) and hepatomegaly (36%) (Table-2).

Hematological abnormalities among VL patients

In this study the most common hematological abnormalities were thrombocytopenia, anemia leucopenia, neutropenia and pancytopenia (Table 3).

Frequency of hematological abnormalities of VL patients

The red blood cell morphological characteristics of VL showed that normocytic normochromic , microcytic hypochromic, dimorphic cells and macrocytic normochromic RBC cells were the most common red blood cell morphological findings which were present in 60(60%), 35 (35%), 4(4%) and 2 (2%) respectively (Figures -1).

Anemia severity

The severity of anemia depends on the hemoglobin level where Hgb<8 g/dl have termed as sever for both sex, Hgb =8-9.9g/dl (moderate) for females and Hgb =8-10.9g/dl for male (moderate) and Hgb =10-11.9g/dl for females (mild) and 11-12.9 (mild). Based on this classification, from the 96 anemic patients, majority of them 52 (54.17%) shows severe anemia. Mild anemia was seen in only 4 patients (4.17%) (Figure -2).

Hematological parameters tests of Visceral Leishmaniasis patients and healthy control groups

Based on the analysis, the absolute mean number of RBC, level of hemoglobin, the percentage of hematocrit, the RBC indices and the absolute platlate count were significantly lower in VL cases compared with the control groups. Similarly the absolute count WBC (10^9/l), neutrophil count, lymphocyte count and eosinophil count were significantly lower in VL cases compared with the control groups, but RDW-CV, lymphocyte percentage, monocyte percentage and monocyte count was significantly higher in VL cases compared with the control (Table-4).

Discussion

Visceral leishmaniasis is a potentially deadly human disease with an estimated occurrence of 0.2 to 0.4 million cases worldwide, causing 20,000-40,000 deaths annually [2]. In the current study, the main clinical symptoms and signs of VL patients were splenomegaly ,hepatomegaly ,fever ,weight loss ,jaundice ,skin mucosal pallor ,diarrhea ,anorexia ,abdominal pain ,general weakness and bleeding , similar to studies conducted Nepal [9] ,India [10],Yemen [7],Pakistan [11],Kuman [8],Iran [12, 13].

The present study showed that anemia, leucopenia, neutropenia, lymphopenia, thrombocytopenia, and pancytopenia were the most common hematological problem present in VL patients. These findings are in line with studies reported from Yemen [14], Nepal [9], India [15], Iran [12, 13], Sudan [16, 17], Gonder, Ethiopia [18].

In present study significantly decreased mean Hgb, RBC, HCT and RBC indices values was reported in VL patients compared to control groups, similar to report in Sudan [17]. The 96% anemia observed in VL patients in this study, is consistent with studies done in Iran (97.1%) [12] ,Yemen (100%) [14] India (100%) [7], Sudan (100%) [16],and Gondar, Ethiopia (94.4%) [18]. However, the prevalence of anemia in this study was higher than other studies done in Nepal (90%) [9] and Iran (87.3%) [13]. The cause of anemia in these VL patients may be multifactorial: sequestration and destruction of red blood cells (RBC) in enlarged spleen, immune mechanism and alterations in RBC membrane permeability, plasma volume expansion. Moreover, hypersplenism, nutritional deficiencies of iron, folate and vitamin B12 may also have some additional role. Other suggested causes include increased sensitivity to complement, inhibition of erythrocyte enzymes, production of hemolysin by the parasites and presence of cold agglutinins [1, 4].

Most of the RBC morphology of VL in this study showed that normocytic normochromic cells followed by microcytic hypochromic cells similar to studies conducted in Nepal [9],India [1, 4].The finding of this study is in contrast with previous studies conducted in India [10], Yemen [19] and Kumaon [8] , which showed that microcytic hypochromic cells was the predominant blood cell morphology followed by normocytic normochromic cells.

Total white blood cell count and neutrophil count of VL patients significantly decreased compared to controls , similar with the study conducted in Sudan [17].The prevalence of leucopenia in this study was 95% which is similar with a study done in Gonder, Ethiopia (95.4%) [18]. The prevalence of leucopenia in this study was higher than studies done in Yemen (87%) [14], Nepal (67.5%) [9], India (83.3%) [7], Iran (67.6%) [13].The cause of leucopenia is may be due to delayed presentation to hospital which was attributing to hypersplenism causing leucopenia. Neutropenia was the most common abnormality seen in 92% in this study, which was similar with a study done in Gonder (90.1%) [18], but which was higher than studies in Yemen (73.5%) [20] and Sudan (88%) [16].This increases of neutropenia may be due to destroyed premature white blood cell (especially Neutrophils) by the parasite [4].

Similarly the prevalence of Lymphopenia in this study was 73%, which was higher than in studies done in Yemen (53.2%) [14] and Gonder ,Ethiopia (37.9%) [18].The prevalence of Lymphopenia in this study was lower than study done in Sudan (94%) [16]. Also eosinopenia was observed in VL patients in this study.

The result of this study is in line with pervious study conducted in India [4, 10], Yemen [14], and Sudan [16].The suggested mechanism for development of this leucopenia is due to hypersplenism [1].

Platlate count of VL patients in this study is significantly decreased compared to control groups. The result of this study is consistent with a study conducted in Sudan [17]. The prevalence of thrombocytopenia in this study was 97%, which is consistent with studies done in Yemen (94%) [14], Sudan (100%) [16] and Gonder ,Ethiopia (90.1%) [18]. However this study showed slightly higher prevalence than studies done Nepal (72.5%) [9], India (83.3%) [15], Kumaon (85%) [8], Iran (91.2 %) [12]. Splenic sequestration and immune mediated mechanisms are mainly thought to be responsible for development of thrombocytopenia [4]. Pancytopenia is the most common hematological abnormality seen in 89% VL patients in this study, similar to a studies conducted in India [7, 15], Pakistan [21], Yemen [19], Iran [13]. The reason for pancytopenia could be due to long duration of symptoms and splenomegaly before presentation leading to increased peripheral destruction of blood cells [1].

Conclusion

To conclude, the major changes in peripheral blood of patient with visceral leishmaniasis were reduced number of red blood cells, reduction in leukocytes and decreased platelet count. VL patients presented with splenomegaly, fever, bleeding, anemia, leucopenia and thrombocytopenia. This finding indicates that visceral leishmaniasis causes alterations of hematological parameters.

Limitation of the study

Limited published studies are available making it difficult for comparison of this study with other findings. Also being a cross-sectional study by design, it cannot observe prospectively and thus cannot associate causal relationships between the factors under study.

List Of Abbreviations

HCT-Hematocrit, Hgb-Hemoglobin, LD-Leishmania donovani, MCH-Mean Cell Hemoglobin ,MCHC-Mean Cell Hemoglobin Concentration, MCV-Mean Cell Volume ,MPV-Mean Platelet Volume, RBC-Red Blood Cell, RDW-Red Blood Cell Distribution Width , SOP-Standard Operating Procedure, SPSS-Statistical Package for Social Sciences, VL-Visceral leishmaniasis ,WBC-White Blood Cells ,WHO-World Health Organization.

Declarations

Ethics approval and consent to participate

Ethical clearance was obtained from the ethical review committee of the department of Medical Laboratory Sciences of Addis Ababa University. Further permission was also obtained from Tigrai Regional Health Bureau and from Administrators of selected hospitals. Furthermore, after explaining the importance of the study, an informed written consent was obtained from study participants.

Consent for publication

Not applicable.

Availability of data and materials

The data sets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Not-applicable

Authors' contributions

GG participated in the conception and design of the study, data collection, analysis and interpretations of the findings, and preparing the manuscript. BT, GG, and GB carried out reviewing, editing, and organizing the papers. FM, GT, and HH participated in the data entry and analysis with their constructive comments. GA and AG participated in the laboratory analysis of Hematology tests. AT, BB and HT revised, editing, reviewed the whole document starting from the proposal up to manuscript writing. All authors read and approved the final manuscript.

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Tables

Table 1: **Socio-demographic characteristics of study participants in Kahsay Abera and Mearg hospitals**

Socio demography	VL cases (n=100)	Control groups (n=100)	P- value
Gender			
Male n (%)	91(91%)	90(90%)	0.809
Female n (%)	9(9%)	10(10%)	
Age in year n (%)			
15-20	28	4	
21-25	20	31	
26-30	23	42	
31-35	9	15	
>35	20	8	
Mean ±SD	27.98±9.634	27.64±4.758	0.752
Marital status			
Single n (%)	49	55	
Married n (%)	37	42	
Divorced n (%)	13	3	
Widowed n (%)	1	0	
Educational status			
Illiterate	29	24	
Primary	51	56	
Secondary	20	16	
Diploma and above	0	4	
Occupation			
Student	15	23	
Farmer	24	15	
Daily labour	60	56	
Merchant	1	4	
Government employee	0	2	
Residence			
Urban	6	9	
Rural	94	91	

Table 2: **The frequency of clinical features of visceral leishmaniasis patients in Kahsay Abera and Mearg hospitals**

Sign and symptom	No (%)
Splenomegaly	100(100%)
Hepatomegaly	36(36%)
Fever	100(100%)
Weight loss	65(65%)
Jaundice	21(21%)
Skin mucosal pallor	72(72%)
Diarrhea	15(15%)
Anorexia	52(52%)
Abdominal pain	17(17%)
General weakness	85(85%)
Bleeding	67(67%)

Table 3: **Frequency of hematological abnormalities of visceral leishmaniasis patients in Kahsay Abera and Mearg hospitals**

Hematological abnormalities	N (%)
Anemia	96
leucopenia	95
neutropenia	92
Lymphopenia	73
Eosinopenia	45
Thrombocytopenia	97
Pancytopenia	89

Table 4: **Hematological parameter tests for visceral leishmaniasis patients and control groups in in Kahsay Abera and Mearg hospitals**

Parameters	VL cases(n=100) (mean±SD)	Controls (n=100) (mean±SD)	Test of significance (95% confidence interval)	
			T	p-value
RBC ($\times 10^{12}$/L)	3.14± 0.89	4.76 ±0.49	-15.86	0.001
HGB (g/dl)	8 ±2.21	14.4± 1.5	-24.08	0.001
HCT (%)	24.8 ±6.6	41.95± 3.16	-23.4	0.001
MCV (fl)	79.8 ±6.9	85.76 ±4.85	-7.07	0.001
MCH (pg)	25.5±3.13	29.87 ±1.9	-11.8	0.001
MCHC (g/dl)	32.0 ±2.34	34.79 ±1.27	-10.1	0.001
RDW CV (%)	17.6±3.53	13.8± 2.94	8.3	0.001
WBC ($\times 10^9$/L)	2.1 ±1.0	6.7± 1.7	-22.6	0.001
Lympho (%)	42.7± 13.4	30.6± 10.7	7.0	0.001
NUETR (%)	47.4± 13.6	60.3 ±13.9	-6.6	0.001
Eosino (%)	1.5± 1.2	2.9± 1.8	-7.5	0.001
Baso (%)	1.5± 1.4	1.31± 1.22	0.825	0.411
Lympho ($\times 10^9$/l)	0.88 ±0.5	1.9± 0.7	-12.4	0.001
Neutrophil ($\times 10^9$/l)	1.0 ± 0.61	4.1 ±1.5	-19.0	0.001
Eosino ($\times 10^9$/l)	0.032±0.032	0.2±0.14	-11.8	0.001
Platelet ($\times 10^9$/L)	70.8±36.8	230± 59.4	-21.8	0.001
MPV (fl)	9.4 ±1.3	9.7 ±1.0	-1.7	0.092
PDW	14.6±4.2	12.5±2.2	2.14	0.001

Figures

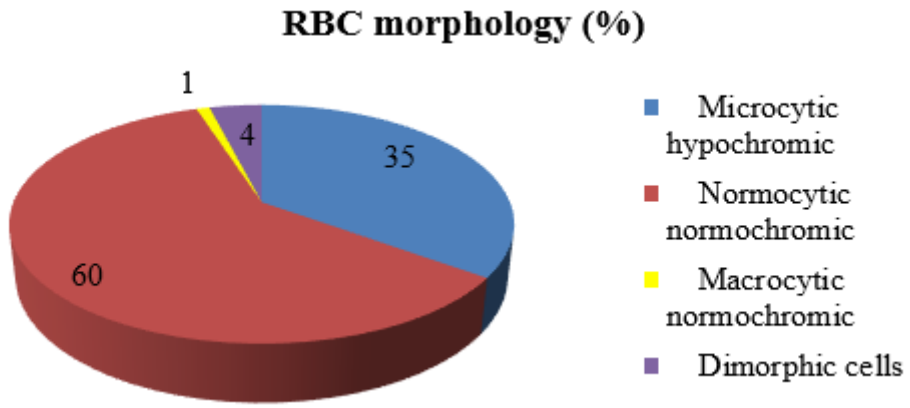

Figure 1

Morphological classification of anemia among visceral leishmaniasis patients in Kahsay Abera and Mearg hospitals

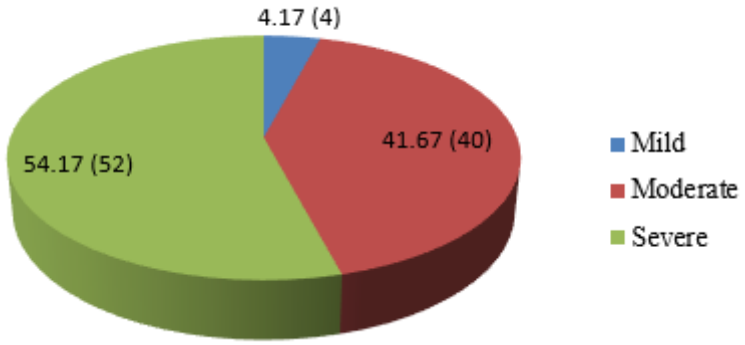

Figure 2

Severity of anemia among visceral eishmaniasis patient in Kahsay Abera and Mearg hospitals