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Original paper

Hematological features of visceral leishmaniosis (kala-azar) of infected children in Baghdad, Iraq

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ABSTRACT. *Leishmania donovani* complex are the exclusive causative agents of systemic and fatal visceral leishmaniosis. In Iraq however, the case numbers of this fatal disease continue to be sustained. The present study aimed to characterize the prevalence and the hematological features of Iraqi children hospitalized cases with fatal visceral leishmaniosis. Hospitalized children considered positive for fatal visceral leishmaniosis depending on bone marrow aspiration and indirect florescent antibody test IFAT titer 1/34 and up. Prevalence of fatal visceral leishmaniosis in 115 children at Baghdad city revealed male were higher infected (54%) than female (46%). The 1–3 years age was the most affected (53.3%). The most prevalence (74.9%) in cold season in compare with lowest prevalence (26.0%) at hot season. Hematological features examination of infected children revealed hypochromic and microcytic anemia with leukopenia and neutropenia and lymphocytosis.

Keywords: visceral leishmaniosis, anemia, hematological, prevalence

Introduction

Leishmaniosis is a vector-borne disease caused by many species of the *Leishmania* genus. These species cause cutaneous leishmaniosis (CL), visceral leishmaniosis (VL), mucocutaneous leishmaniosis (MCL), and post-kala-azar dermal leishmaniosis (PKDL). Visceral leishmaniosis is deadly. Parasites invade internal organs and have the potential to attack the central nervous system by crossing the blood – brain barrier in a parasitic systemic disease [1]. VL among species are responsible for the disease in Middle East, East Africa, and the Indian subcontinent, while *L. infantum* species are incriminated in Europe, North Africa and South and Central America [2].

In 2002, the central health laboratories in Baghdad detected 14,502 cases infected with kala-azar using an indirect fluorescent antibody test (IFAT) from sixteen provinces in Iraq [3]. It seems that Wasit Province had the highest prevalence, and then it follows by Diala, Babylon and Baghdad. The annual number of reported cases with visceral leishmaniosis in Iraq is over 1,000 and according to the data obtained from the section of the endemic disease institute, the number of cases reported during the years 1971–1984 was 12,038 and about 90% of them were from Baghdad and central governorates. There has been an uptick in southern regions in recent years [4]. Leishmaniosis is a complex disease caused by protozoan intracellular parasites, belonging to the genus *Leishmania* (Kinetoplastida, Trypanosomatidae). The infection transmitted through female Phlebotominae sand flies of the genus *Lutzomyia* in New World (America) further to *Phlebotomus* in the Old World (Asia, Africa and Europe) [5]. The parasites causing leishmaniosis may live and multiply in humans, domestic or stray dogs, and rodents. Among the 20 or so *Leishmania* spp. described as pathogenic for humans, those of the *Leishmania donovani* complex are the exclusive causative agents of systemic and fatal visceral leishmaniosis, the *L. donovani* complex could be treated as a single entity [6]. Incidence of leishmaniosis has been shown to be geographic patterns and disease hotspots [7]. Iraq is

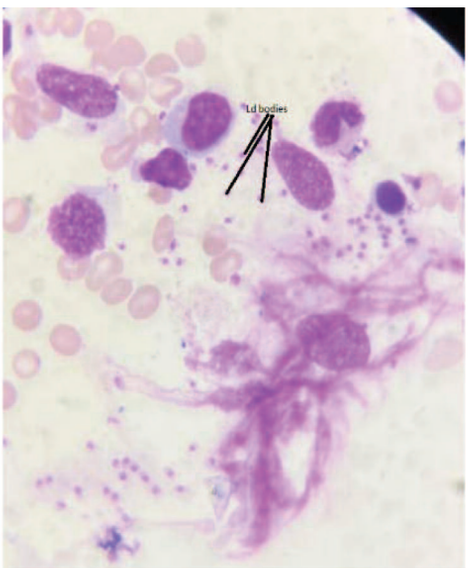

Figure 1. Bone marrow biopsy showing the presence of Ld bodies (amastigote) from children with VL Leishman stain (100×)

considered as the endemic area of leishmaniosis where both forms of the disease, cutaneous (Baghdad boil) and visceral (kala-azar) found. Leishmaniosis is widespread in Iraqi governorates; the first cases were recorded in Mosul and Baghdad cities [8]. In Iraq however, the case numbers of this fatal disease continue to be sustained.

The aim of the present study was to characterize the prevalence and the hematological changes of Iraqi children hospitalized cases with VL.

Materials and Methods

Population of the study

The prevalence of VL was studied in three Baghdad hospitals and included 115 children, aged 4 months to 5 years being admitted as VL patients. The diagnosis depends upon IFAT test and bone marrow aspirate. The clinical information including

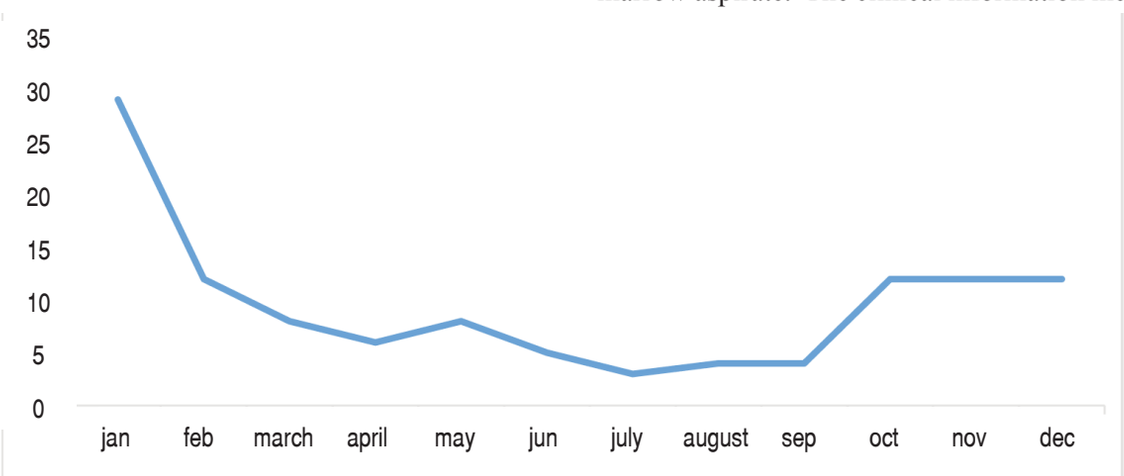

Figure 2. Prevalence of VL in Iraqi children through the year Chi-square value 56.87 at P<0.0001

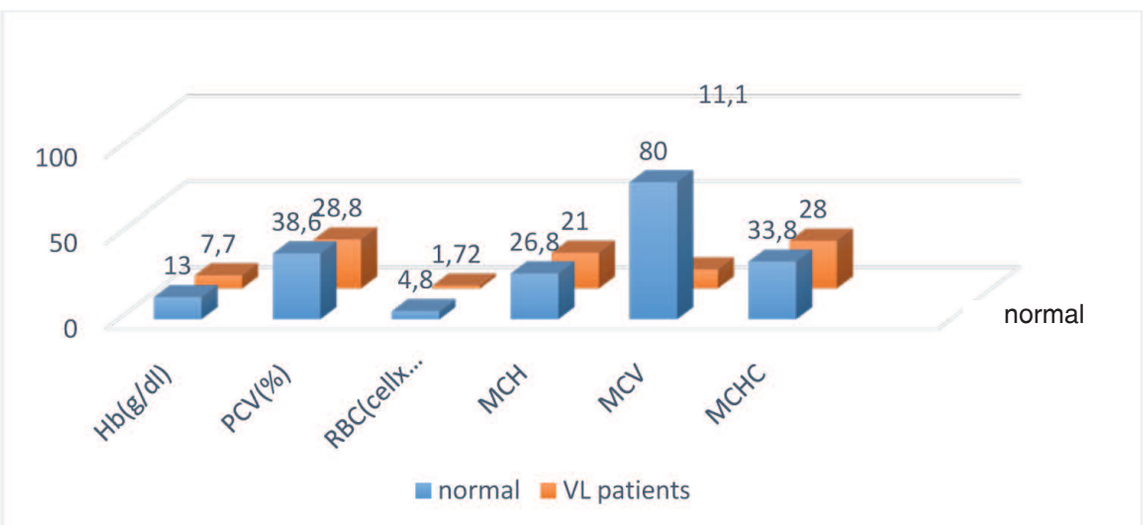

Figure 3. Erythrocytes indices tests for visceral leishmaniosis patients and control groups

Table 1. Hematological features of VL patients

Hematological parameter	Minimum	Maximum	Mean	SD
Hb (g/dl)	3.3	9.7	7.7	1.57
PCV (%)	19	36	28.8	5.4
RBCs (cell×10^3/µl)	1	3.2	1.72	1.05
MCH(pg)	14	28	38.1	64.69
MCV(fl)	1.3	41	11.1	19.62
MCHC(g/dl)	18	43.8	28	8.82
Total WBCs (cell×10^3/µl)	0.56	12.8	5.51	9.8
Neutrophil	17	57	42.2	9.4
Eosinophil	0	2	0.73	6.63
Monocytes	1	8	3.66	2.03
Lymphocytes	41	76	53.36	8.92

age, sex, and symptoms. Symptoms of persistence the infection such as anemia, hepatosplenomegaly, with black skin. Hematological features were evaluated in anticoagulant fresh blood sample of 40 patients. Bone marrow aspirate was stained by Leishman stain.

Table 2. Hematological picture of the VL patients distributed according to gender

	Group	N	Mean	SD	SE Mean	P-value
Hb	Female	20	7.9450	1.08893	.24349	0.70
	Male	20	8.1450	2.05874	.46035	
PCV	Female	20	26.4000	3.33088	.74481	0.31
	Male	20	28.2450	7.36782	1.64749	
RBC	Female	20	1.9400	1.10330	.24670	0.48
	Male	20	1.7050	.97575	.21818	
MCH	Female	20	19.2450	8.03102	1.79579	0.85
	Male	20	18.8000	7.01750	1.56916	
MCV	Female	20	5.1950	2.30365	.51511	0.83
	Male	20	5.3550	2.56832	.57429	
MCHC	Female	20	28.3095	5.82091	1.30159	0.81
	Male	20	27.7320	9.13847	2.04342	
WBC	Female	20	3.2650	1.48617	.33232	0.51
	Male	20	3.5900	1.59733	.35717	
NET	Female	20	41.2500	9.52490	2.12983	0.93
	Male	20	41.4500	4.89334	1.09418	
EOS	Female	20	.5500	.68633	.15347	0.80
	Male	20	.6000	.59824	.13377	
MONO	Female	20	4.2000	2.09259	.46792	0.40
	Male	20	3.7000	1.65752	.37063	
LYMP	Female	20	54.0000	9.55868	2.13739	0.93
	Male	20	54.2000	5.08455	1.13694	

Table 3. Hematological picture of the VL patients distributed according to age

	Age (years)	No.	Means±SE	LSD
Hb (g/dl)	≤1	5	6.16±1.08B	1.36
	1–3	25	8.62±0.22A	
	3–5	10	7.53±0.46A	
PCV (%)	≤1	5	27.38±4.6A	5.49
	1–3	25	28.24±0.97A	
	3–5	10	25.0±1.44A	
RBCs (cell×10^6/µl)	≤1	5	1.22 ±0.19B	0.98
	1–3	25	1.78±0.22AB	
	3–5	10 2.	22±0.28A	
MCH (pg)	≤1	5	24.1±3.7A	6.69
	1–3	25	19.94±1.4AB	
	3–5	10	14.2±1.5B	
MCV (fL)	≤1	5	4.78±1.2A	2.29
	1–3	25	5.77±0.4A	
	3–5	10	4.28±0.78A	
MCHC (g/l)	≤1	5	19.64±4.0B	6.66
	1–3	25	30.08±1.17A	
	3–5	10	27.0±2.6A	
WBCs (cell×10^3/µl)	≤1	5	2.96±0.69A	1.46
	1–3	25	3.73±0.28A	
	3–5	10	2.89±0.54A	
Neutrophils %	≤1	5	39.2±1.77A	7.35
	1–3	25	41.72±1.24A	
	3–5	10	41.5±3.59A	
Eosinophils %	≤1	5	1.0 ±0.3A	0.6
	1–3	25	0.4±0.13A	
	3–5	10	0.6±0.16A	
Monocytes %	≤1	5	3.4±0.6A	1.82
	1–3	25	4.24±0.35A	
	3–5	10	3.5±0.73A	
Lymphocytes %	≤1	5	56.4±1.9 A	7.4
	1–3	25	53.52±1.24A	
	3–5	10	54.4±3.63A	

Explanation: different capital letters denoted significant differences ($P<0.05$) among age

Data analysis

Data were analyzed using SPSS statistical analysis program version-9. Data presented as means SE. Chi-square value 56.87 at $P<0.0001$ considered for the prevalence of the disease.

Results

Bone marrow aspiration of the VL infected children

Examination of stained bone marrow aspiration for 10 infected children revealed the presence of the Ld-bodies of the *Leishmania* amastigote in all examined samples (Fig. 1).

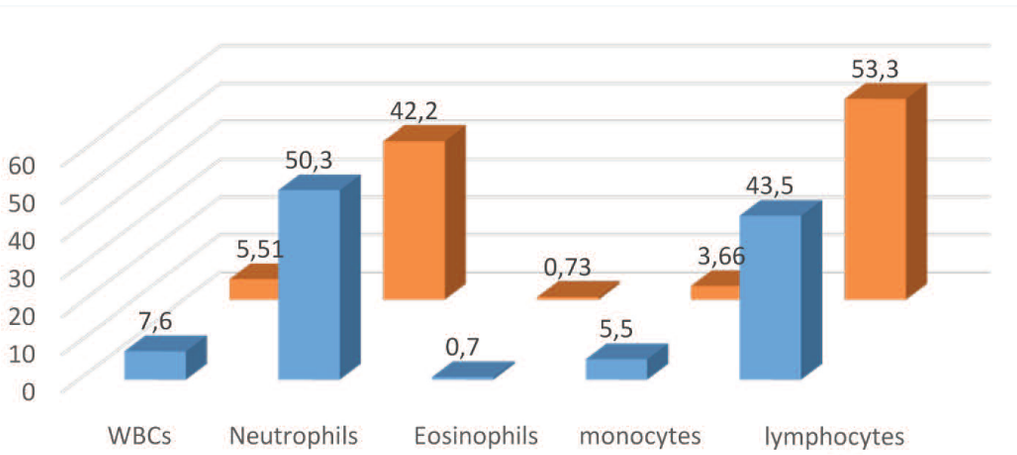

Figure 4. White blood cells tests for visceral leishmaniosis patients and control groups

Prevalence countenance of the VL patients

Diagnosis of VL in the children considered positive when results of IFAT in arrange between 1/16–1/128. From 115 cases studied, male were higher infected (62.54%) that female (53.46%). The patients included in the study distributed in to 3 groups according to age: less than one year, 1–3 years, and 3–5 years old. The majority of the cases (53.3%) were between 1–3 years and 33.3% were 3–5 years. The less percentage was 13.3% among children less than one year. With the aim of analyzing the effect of climatic conditions on the incidence rate of infection, the cases number admitted for each month were recorded (Fig. 2). The highest incidence (74%) were found in months that characterized by relatively cold climate in which the temperature is between 1–25°C, and lowest (26%) in hot climate with 30–50°C (Fig. 3). The most prevalence of VL in Iraqi children was found in January (25%) when compare with the lowest prevalence (1.7%) in July.

Hematological picture of the VL patients

Results of hematological features related to anemia such as Hb, PCV, and RBCs were reduced in VL patients when compare with normal values, indicating a hypochromic and microcytic type of anemia (Tab. 1, Fig. 3). In general, all patients with different age and sex suffered from marked leukopenia (5.51) and neutropenia (42.2) as shown in figure 4. Furthermore, patients almost had lymphocytosis (53.3), and decrease in monocytes (3.66) but there were no changes in eosinophils. According to sex, statistical analysis of hematological features showed that there were no significant differences between males and females with VL (Tab. 2). Nevertheless, results revealed that there are statistical differences between the different ages, especially in RBCs, Hb, MCV, MCH, and MCHC for VL infected children (Tab. 3).

Discussion

The demonstration of the amastigote in the bone marrow aspiration films considered the golden diagnostic test for VL [9], furthermore the IFAT detect the circulating anti-*Leishmania* antibodies [10]. These antibodies are produced by plasma cells against the surface antigens of the parasites. The results of the current study showed that there were statistically significant differences in prevalence of VL monthly and seasonal distribution. The highest number of cases was reported in the cold season, this is in agreement with another report [11]. These observations corresponds to the active season of the sand flies, the vector of VL is during hot season. Considering the incubation period between the infected sand fly bite and the appearance of the symptoms is about 3–6 months [12]. In another hand, incubation period is patient age and immune status in addition to species of parasite. Furthermore, high level of humidity in cold season of Iraq, which provide good environment for parasite spread [13]. However, the present study revealed that the most affected age between 1–3 years, which agree with previous results, found that 25% of suspected patients were positive for *Leishmania* DNA at age of 1–5 years [3]. In regarding to hematological features, the anemia consider the most common feature of the VL [14–16]. The cause of anemia seen in the present patients is multifactorial: sequestration and

destruction of red blood cells (RBC) in enlarged spleen, and reduced in red blood cells number [17]. Hemophagocytosis due to immune mechanism and alterations in RBC membrane permeability have been implicated and high ESR were frequently found hematological pictures in visceral leishmaniosis [18]. Dietary factors appear to be most important factors [19] in bad prognosis of anemia. Reduced plasma iron level of host results in the infected macrophages with parasite restricts iron and heme availability to the parasite in a process termed nutritional immunity [20]. This may limit the marrow response to hemolysis. In Mediterranean population a very rapid onset of anemia with hemolysis is commonly observed. Results of laboratory tests related to hematological changes for the majority of patients revealed anemia, leukopenia, neutropenia, and lymphocytosis correlated with peripheral edema [11,21]. Anemia, indicators and other hematological parameters such as leukopenia may also play important role for diagnosis of the clinical cases [22]. This neutropenia may be due to destroyed premature white blood cell (especially neutrophils) by the parasite [23]. 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 [24], Iran [25], and Ethiopia [17].

In conclusion, the VL in Iraqi children was most common at age of 1–3 years and the hematological features were anemia of hypochromic and microcytic in addition to leukopenia and neutropenia.

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