

Serological Detection of Visceral Leishmaniasis in Infected Children in Al-Kut City

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ABSTRACT

The research included a study of visceral leishmaniasis (VL) from an epidemiological and hematological perspective. 230 serum samples were collected from children suspected of being infected with VL visiting al-Kut hospitals. The samples were tested using two immunoassay tests; CTK Biotech test and Rapid InBios test. The infection rate was 8.25%. The ELISA infection rate was 7.38%. The results of the study showed that there were significant differences between the incidence of VL in children and their ages (Table 2), as the highest rate of infection was (10.76%) in the age group (1-5) years, and the lowest rate of infection was (3.63%) in the age group (5-9) a year. When distributing infections among age groups, it was found that infection appears clearly in children, as the highest infection rate was recorded in the age group (1-5) years at a rate of (10.76%). The results of the laboratory examination confirmed the presence of a decrease in the number of packed cells in people with VL when compared with the size of the packed cells in healthy people. The current study also shows results (43.75%) of those infected with the disease had a hemoglobin level of (6.1 gm/dl-8), while (5.55%) of those infected had a hemoglobin level of (gm/dl > 12.1). The study also showed that the white blood cell counts of (37.5%) of the infected people ranged between (Cell/cm 2000-4000), while the white blood cell counts of (5.55%) of the infected people was (Cell/cm < 12000). The results of the current study confirmed the presence of a decrease in the total rate of WBCs for children with VL compared to the control group. In conclusion, our findings indicate that VL impact significantly on the values of hematology; however, furthermore studies are needed to estimate the effect of disease on different biochemical markers.

1. Introduction

Leishmaniasis is a parasitic disease that is prevalent in many regions of the world. It prevails on four continents and is considered by the World Health Organization (WHO) to be one of the six largest parasitic diseases affecting humans (WHO, 2010). There are multiple types of this disease, each of which has different clinical manifestations (Al-Hayali, 2021) the cutaneous type, the diffuse cutaneous type, the mucocutaneous type, and finally the visceral type (Rahi *et al.*, 2021). VL (visceral leishmaniasis) is one of the endemic parasites shared between humans and animals that is transmitted by sand flies and affects residents of tropical and subtropical regions, infecting approximately (12 million) people annually in (88) countries (Khaleel, 2021). The disease is transmitted from animals to humans through an infected insect called the sandfly. Dogs, cats, and rodents may play the role of reservoir hosts in the life cycle (Al-Bayati *et al.*, 2021). The parasite exists in two different phases during its life cycle: the amastigote phase, which is found inside the cells of the reticuloendothelial system of the spinal host, and the promastigote phase, which is found in the intestines of the vector insect. VL, also known as black fever or kala-azar, is the most dangerous, as the parasite invades the internal viscera, including the liver, spleen, and bone marrow. Symptoms of the disease include fever, enlargement of the liver and spleen, and anemia. The disease may lead to death if not treated (Saleem *et al.*, 2021). Iraq is one of the most common countries with parasitic diseases. The main habitats where it spreads and the study very few in Iraq Wasit-Kut Governorate in particular on the size and spread of VL are disproportionate to the seriousness of this disease, especially after taking into account the increase in cases of infection recorded in children in recent years, so this study sought to explain the relationship between immunity and the correct diagnosis used for this disease, and to indicate the extent of infections according to gender and age (Rahi *et al.*, 2021).

2. Methodology

Study site

The study was conducted in the Wasit Governorate of the city of Kut. Information and variables were collected about the children included in the study through a special form. (230) Blood samples were collected from children suspected of being infected with the disease from patients in Kut hospitals. Blood samples were collected through venous puncture using a specific sterile syringe and then placed in tubes containing the anticoagulant EDTA to conduct blood tests.

Serological tests

The immunosorbent strip test (prepared by Bios International Inc. USA and CTK Biotech, Inc.USA) and ELISA were used. The immune strip test is one of the rapid qualitative tests for detecting immune antibodies specific to the visceral leishmania parasite. In sera, the principle of the examination depends on the antigen present in the test line area on the strip with the antibodies present in the serum, as the immune complexes formed adsorb with the reagent dye placed on the test line and this indicates a positive result of the test.

Epidemiological examination

Using an information form that includes a set of information such as (gender, age, and review date) to determine the spread of the disease and its distribution among children.

Blood tests

This value was determined using glass capillary tubes with open ends containing a layer of heparin or any anticoagulant filled with blood. One of the two nozzles of the capillary tube is closed and placed in a hematocrit centrifugation device for 2-3 minutes, after which three layers are observed, representing the volume of compacted red and white blood cells and the volume of plasma. The percentages of these components are read using a hematocrit reader uses Saleem, Al-Samarai (2018)

This method relied on diluting the blood with a solution of potassium ferricyanide (Drabkin's solution), which oxidized the hemoglobin to methemoglobin, which eventually turned into methemoglobin cyanide, and its optical density was read using a spectrometer at a wavelength of 540 nm.

They used a blood cell counter, and a Hematocytometer, which consists of a glass slide known as a *Neubautrs* chamber slide. They used a special pipette for counting white blood cells, which contains two grades of 0.5 and 11.0. Also, contains a white glass ball to distinguish it from the special pipette for counting red blood cells last this study used the *Chi-Square* test (Gharban et al., 2023).

Statistical analysis

The GraphPad Prism Software was served to identify significant differences between values of G1 and G2 groups at $P < 0.05$ (*), (Gharban, 2022). Values were represented as Mean \pm Standard Errors (M \pm SE).

3. Results and Discussion

After collecting the samples required from children suspected of being infected by visitors to the Kut City Center hospitals, two diagnostic methods were used, namely the in Bios Rapid test the CTK Biotech test, and the ELISA test methods (Table 1).

Table 1 shows the No. and (+,-) tested samples and % of cases of VL, according to the used test type

Type test	No. samples	Positive		Negative	
		No.	%	No.	%
In Bios Rapi Test	230	19	8.25	211	91.73
CTK Biotech Test	230	19	8.25	211	91.73
ELISA	230	17	7.38	213	92.6

The Dipstick immunoassay test is one of the important tests that has been used in the field and can be considered a rapid examination to detect acute and early infection in suspected cases. The test relies on the use of the K39 r antigen, where the interaction occurs between the antibodies present in the serum of the suspected patient and the antigen fixed on the strip (Singh et al., 1995; Madjeed et al., 2022). The sensitivity and specificity of this test differed in regions of the world, as it showed a sensitivity and specificity of (98%) in India, (97%) in Iran, and (100%) Hussein et al., 2019 in Nepal (Bern et al., 2000), while it showed limited or little sensitivity and specificity in other regions (Khaleel 2021): sensitivity reached (71.4%) in southern Europe, (73%) in South America, and (67%) in Sudan (Zijlstra et al., 2001; Kadhim et al., 2020).

The current study showed that the sensitivity and specificity of the ELISA test compared with Dipstick reached 93% and 98% for each of them, respectively. Our results were similar to Taher (2006) and Rahiet *et al.*, 2021, as the sensitivity and specificity reached (97.99) %, respectively. Likewise, the results of the current study were similar to Al-Hayali (2021), which reached a sensitivity of 91.76% and a specificity of 96.15%. Our results differed from Sinha and Sehgal (1994) with Al-Bayati *et al.* 2021 who indicated that the sensitivity of ELISA reached (75%).

Study of epidemiological and blood tests

The results of the study showed that there were significant differences between the incidence of VL in children and their ages (Table 2), as the highest rate of infection was (10.76%) in the age group (1-5) years, and the lowest rate of infection was (3.63%) in the age group (5-9) a year.

Table 2 Number of positive and % of VL, according to age groups using a dipstick and ELISA test

Ages	No. of sample	No. of + by Dipstick test	%	No. of + by ELISA test	%
≥ 1	30	2	6.6	2	6.6
1-5	130	14	10.76	14	10.76
5-9	55	2	3.63	1	1.81
9-12	15	1	6.6	-	-
Total	230	19		17	

When distributing infections among age groups, it was found that infection appears clearly in children, as the highest infection rate was recorded in the age group (1-5) years at a rate of (10.76%). These results are consistent with Sukker, 1985; Tarish, 2009; Gani *et al.*, 2010; Al-Hayali 2021. The current study showed that infection appears clearly in children between the ages of one and nine. These results are consistent with Al-Hayali 2021. Leishmaniasis in Iraq is classified as Mediterranean kalasar, which affects children under five years of age. The reason for the high incidence of infection among children is the incomplete growth and development of the children's immune system, as well as the absence of the immune factor Euglobulin present in the serum of adults, which works to destroy the parasite and which is missing when Children Al-Bayati *et al.* 2021

The results of the study also showed that the size of the packed cells in 75% of the infected people was between (24 - 26), while the size of the packed cells in 5.55% of the infected people was between (27 -29), (Table 3).

Table 3 Measurement of the size of packed cells in children with VL

Average PCV	No. of positive		Control group	The normal range
	No.	%		
%			No.	%
16-19	1	5.55	30	36-43
20-23	2	12.5		
24-26	13	75		
27-29	1	5.55		
Total	17	7.38		

The results of the laboratory examination confirmed the presence of a decrease in the number of packed cells in people with VL when compared with the size of the packed cells in healthy people (Figure 1). The study was consistent with (Madjeed *et al.*, 2022) due to a decrease in the number of red blood cells and a decrease in the level of hemoglobin (Kumer *et al.* (1992) Shati *et al.*, 2022, as well as a result of the accumulation of red blood cells, which often increases the chances of being attacked and eaten by phagocytic cells in the tissues of the reticuloendothelial system Madjeed *et al.*, 2022

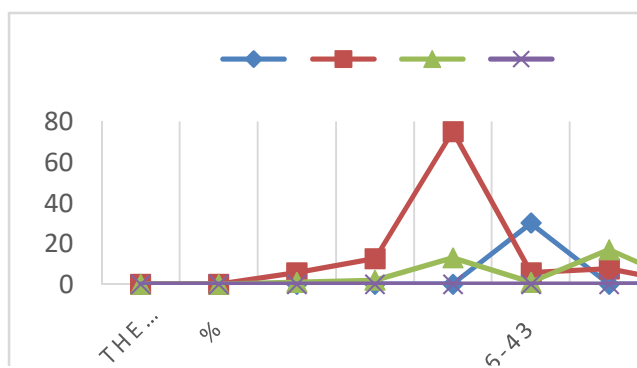


Figure 2 Measurement of the size of packed cells in children with VL

The current study also shows results (43.75%) of those infected with the disease had a hemoglobin level of (6.1 gm/dl-8), while (5.55%) of those infected had a hemoglobin level of (gm/dl > 12.1), (Table 4).

Table 4 Hemoglobin rate (Hb gm/dl) for children with VL

Hemoglobin concentration rate Hb(gm/dl)	Sample +		Control group	
	No.	%	No.	Normal Hemoglobin concentration rate Hb(gm/dl)
4-6	2	12.5	30	12.3-13-6
6.1-8	8	43.75		
8.1-10	5	27.7		
10.1-12	1	5.5		
12.1>	1	5.5		
Total	17	7.38		

Laboratory examination to measure Hb in those infected with VL showed a decrease in the level of Hb at all affected ages compared to the control group, and thus our results are consistent with some of researchers; Behaman *et al.*, 2000; Madjeed *et al.*, 2022)

According to many researchers, the cause of low blood hemoglobin is the decomposition of red blood cells in the spleen due to their fragility, which increases as the infected by VL progresses due to increased permeability of the cell wall to calcium ions, or a decrease in the formation of red blood cells in the bone marrow as a result of the leishmaniasis, In addition to the loss of appetite in children during infection, it reduces the amount of iron entering the body, which leads to a decrease in the amount of hemoglobin during infection with VL(Lewis et al, 2001; Hussein *et al.*, 2019).

The study also showed that the white blood cell counts of (37.5%) of the infected people ranged between (Cell/cm 2000-4000), while the white blood cell counts of (5.55%) of the infected people was (Cell/cm < 12000) (Table 5).

Table 5 Total WBC count for children with VL

Rang of WBC (Cell/cm)	Sample +		Control group	
	No.	%	No.	Normal Rang of WBC(Cell/cm)
4000-2000	7	38.5	30	7500-9000
6000-4100	5	31.2		
8000-6100	2	11.1		
10000-8100	1	5.5		
12000-10100	1	5.5		
12100>	1	5.5		
Total	17	7.38		

The results of the current study confirmed the presence of a decrease in the total rate of WBCs for children with VL compared to the control group, and this is agreed with (Tarish, 2006; Al-Hayali, 2021). The reason for the decrease is due to the accumulation of blood cells due to the presence of accumulated parasites in the blood-forming organs, which are the liver and

spleen. And bone marrow, and then its deficiency in the bloodstream, or as a result of a disruption in the functions of these organs as a result of the injury, which leads to a decrease in the number of blood cells.

The study showed an increase in interleukin-4 levels in serum samples from patients infected with VL (8.6 ± 110), while the levels in the control group were (5.38 ± 3.2), (Table 6).

Table 6 Shows the rate of interleukin-4 for children with VL compared to the control group

Group	Total No. of samples examined	Interleukin section rate Mean \pm S.D
Samples +	17	8.6 ± 110
Control group	30	3.2 ± 5.38

Th1 helper T cells secrete cytokines that are extremely important in resisting infection, including interferon-gamma (IFN- γ), interleukin-2 (IL-2), and tumor necrosis factor TNF- α . For example, IFN- γ activates phagocytic cells to accelerate the formation of iNOS2, which is the catalytic enzyme. To form nitric oxide kills non-flagellated intracellular amastigotes. As for type 2 helper T cells, they secrete a variety of cellular motors, namely interleukin-4 (IL-4), interleukin-5 (IL-5), and interleukin-10 (IL-10). These act in an anti-type 1 T cell way, by inhibiting their activity. Phagocytic cells thus help the parasites inside the cells to grow and develop the disease (Amit et al., 2004; Madjeed et al., 2022)

(Ahmed et al., 2003; Aljeboree et al., 2022) confirmed that the recovery or progression of VL is linked to the drivers produced by Th1 cells (γ IFN- γ) and the drivers produced by Th2 cells (IL-4, IL-10), respectively.

As for Th2 cells, they secrete IL-4, IL-5, IL-6, and IL-10, and they differentiate B lymphocytes and stimulate them to produce immune globulins, and thus these cells are more effective in humoral immunity (Chapel and Haeney, 1993; Roitt, 1997; Hyde, 2000; Al-Hayali 2021). Cytokinetics of Th2 cells (IL-4, IL-5, and IL-10) plays the largest role in chronic infections (Farah et al., 2001; Aljeboree et al., 2022).

Interleukin 4 stimulates the proliferation of B lymphocytes, T lymphocytes, and mast cells, as well as stimulates activated B cells to produce both IgG and IgE (Heinrich & Bagby, 2000).

The level of interleukin IV, IL-4, is constant and is important in regulating inflammatory and allergic responses. It stimulates the production of IgE from B cells through its combination with its recipients in target cells. IL-4 regulates the differentiation of T cells into Th2 helper T cells and B cells into producing IgE (Zheng and Rudensky, 2007; Saleem and Al-Samarai, 2018).

4. Conclusion

Our findings indicate that VL impact significantly on the values of hematology; however, furthermore studies are needed to estimate the effect of disease on different biochemical markers.

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