The validity of Kala-azar rapid detection test in the laboratory diagnosis of visceral leishmaniasis in Baquba

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Abstract

Background: visceral leishmaniasis is the most severe form of leishmaniasis. The disease caused by protozoan parasites of the *Leishmania* genus. It is the second-largest parasitic killer in the world, being responsible for an estimated 500,000 cases each year worldwide.

Objectives: To explore the validity of rapid detection test for the laboratory diagnosis of visceral leishmaniasis against the conventional blood smear technique.

Patients, materials and methods: The present study was carried out at Al-Batoul Teaching Hospital during the period from January to July 2009. A total of 75 patients with VL were included. 28 (37.3%) of them were female and 47(62.7%) were male. The age range was 1 month- 8 years. Additionally, 30 apparently healthy individuals were enrolled as control groups. 13(43.3%) of them were female and 17(56.7%) were male. Venous blood sample were collected from each subject; sera were separated and kept frozen till use. All sera were tested for anti-rK39 IgG antibodies using the InBios Kala-azar detect rapid test (Seattle, WA) according to the manufacturer's protocol.

Results: The results showed that out of 75 patients, 10 (13.3%) and 9 (12%) were positive for kala-azar by microscopical blood film examination and kala-azar rapid screening test respectively. The distribution of positive cases according to the gender revaled hat the infection rate among males was higher than in females, and the most affected age was 1-4 years.

Conclusion: The validity of kala-azar rapid setection test was comparable to that of microscxopical examination for detection of kala-azar cases among clinically suspected patients.

Keywords: Visceral leithmaniasis, Kala azar, Black feve

Introduction

Black fever, visceral leishmaniasis (VL), also known as kala-azar, or Dumdum fever. It is a potentially fatal disease caused by an intracellular protozoan parasite of the *Leishmania donovani* complex that is transmitted by phlebotomine sandflies [1]. VL is highly prevalent in developing countries with poor socioeconomic conditions and low standard of health services [2] . In Each year there are about 500 000 new cases and more than 50 000 deaths worldwide [3,4].

For successful control of the disease, efficient and reliable diagnosis of VL is essential. Demonstration of the causative parasites in aspirates from lymph nodes, bone marrow, and the spleen is the most specific diagnosis [5]. High anti-leishmania antibody levels were observed prior to detection of parasite specific T cell responses[6] and several serologic tests, such as the enzyme-linked immunosorbent assay (ELISA)[7,8],the direct agglutination test (DAT),[9,10] and the indirect immunofluorescent antibody test (IFAT) [11] have provided variable diagnosis. Among these techniques, the direct agglutination test (DAT) has been well accepted as a routine serologic test due to its simplicity and high sensitivity and specificity [12,13]. Several studies using the InBios rapid strip test for the diagnosis of VL have found it as highly sensitive and specific [14,15]. The present study is an attempt to explore the validity of "Kalazar Detect Rapid Test" kit which is a rapid immunochromatographic strip assay that used routinely in local laboratory in Iraq for qualitative detection antibody to recombinant antigen specific for visceral leishmaniasis.

Patients, Materials and Methods:

The present study was carried out at Al-Batoul Teaching Hospital during the period from January to July 2009. A total of 75 patients with clinical picture suggestive of VL were included. 28 (37.3%) of them were female and 47(62.7%) were male. The age range was 1 month- 8 years and the mean age 2.1±1.4 years. Additionally, 30 apparently healthy individuals were enrolled as control groups. 13(43.3%) of them were female and 17(56.7%) were male. The age range was 6 months-6 years with mean age 2.2 ± 1.4 years. Peripheral blood film was done for each subject, and stained with leithman stain and examined by two

experts for detection of *L. donovani* parasite. About 3-5 milliliters of venous blood were collected from each subject; sera were separated and kept frozen till use.

All sera were tested for anti-rK39 IgG antibodies using the InBios Kala-azar detect rapid test (Seattle, WA) according to the manufacturer's protocol. The DiaSys Leishmania one step test is a qualitative, membrane based immunoassay for the detection of antibody to viseral Leishmania in serum or plasma. The membrane is pre-coated with recombinant viseral Leishmania antigen on the test line region and anti-protein A antibody on the control line region. During testing, the serum sample reacts with the dye conjugate which has been pre-coated in the test device. The mixture then migrates upward on the membrane chromatographically by capillary action to react with recombinant viseral Leishmania antigen on the membrane and generate a red line. Presence of this red line indicates a positive result, while its absence indicates a negative result. A red line at the control line region is always appearing. The presence of red line serves as verification for sufficient sample volume and proper flow and as a control for the reagents.

Results:

The microscopical examinations of blood films of the patients revealed that 10 out of 75 (13.3%) were positive for leishmania parasite. Whereas, all the blood films of the healthy control were negative, table (1).

Table (1): Positivity rate by blood film examination among study groups.

Study groups	Blood film examination		Total (%)
	Positive (%)	Negative (%)	
Patient group	10 (13.3)	65 (68.7)	75 (100)
Healthy control	0 (0)	30 (100)	30 (100)

The results of the rapid kala-azar serological test showed that 5 (6.7%) of the patients sera were positive and 4 (5.3%) gave weak positive results. Therefore, the sensitivity of the test was 90%. Beside that none of the control sera were positive, So the specificity was 100%, table (2).

Table (2): Positivity rate by Kala-azar rapid detection test among study groups.

Study groups	Kala-azar rapid detection test			Total (%)
	Positive (%)	Weak positive (%)	Negative (%)	
Patient group	5 (6.7)	4(5.3)	66(88)	75 (100)
Healthy control	0 (0)	0 (0)	30 (100)	30 (100)

Regarding the results of the Kala-azar rapid detection test according to gender of study groups, it was found that the infection rate was slightly higher in male compared to that in female (60% *vs* 40%), table (3).

Table (3): Results of Kala-azar rapid detection test according to the patient's gender.

Gender	Patient group		
	Positive (%)	Weak positive (%)	Negative (%)
Female	2 (40)	0 (0)	40 (6o.6)
Male	3 (60)	4 (100)	26 (39.4)
Total	5 (100)	4 (100)	66 (100)

The distribution of positive cases according to the age of patients revealed that the majority of positive cases were within the age group 1-4 years, table (4).

Table (4) Results of Kala-azar rapid detection test according to the age of patient

Age group	No.	Positive cases		Weak positive cases	
		No.	%	No.	%
< 1 year	14	1	7.1	0	0
1-4 years	54	3	5.5	3	5.5
5-8 years	7	1	14.2	1	14.2
Total	75	5	6.7	4	5.3

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Discussion

Undoubtedly, the importance of the present study comes through the fact that leishmaniasis is distributed worldwide and 13 million people are estimated to be infected, with about 1.8 million new cases each year. Approximately 50% of these patients are children [16, 17]. Moreover, leishmaniasis represents a major public health problem in the Eastern Mediterranean Region and foci of zoonotic cutaneous leishmaniasis, caused by Leishmania major, occur in Afghanistan, Egypt, Iran, Iraq, Jordan, Libya, Morocco, Palestine, Pakistan, Saudi Arabia, Sudan, Syria, Tunisia and Yemen [18].

In the present study, the sensitivity of kala-azar rapid test was 90% and the specificity was 100%. These results are in agreement with previous reports which documented a sensitivity ranged between 89%- 92%, while the specificity ranged between 59%-100% as compared with splenic aspirate and direct agglutination tests [19,20,21] . Moreover, the sensitivity and specificity of kala-azar rapid test was comparable to that of direct agglutination test even among HIV positive patients [22]. Additionally, the kala-azar rapid test was less expensive than DAT, and has the advantages of ease of use and obtaining results within minutes. Therefore, it can be concluded that the validity of kala-azar rapid test is good for the detection of visceral leishmaniasis among clinically suspected patients as expressed by its high sensitivity and specificity.

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