Original Article

A comparative study of effectiveness of diagnostic tests (rK39 strip, IFAT and bone marrow stained smear) for visceral leishmaniasis.

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Summary:

Background: Iraq is an endemic area for visceral leishmaniasis (VL) (kala-azar), which is caused by the intracellular parasite Leishmania donovani. A high sensitive and specific laboratory diagnostic approach is needed because of the high fatal evolution of the disease without treatment and the serious toxicity of most widely used first-line drug, sodium stibogluconate. The aim of this study was to evaluate the efficiency of recombinant kinesin 39 immunochromatographic (rK39 IC) strip test, indirect immunofuorescent antibody test (IFAT) and bone marrow stained smear in diagnosis of acute VL cases.

Patients and Methods: A total of 394 clinically suspected cases of kala-azar (218 males and 176 females, whose ages ranged from 1 month to 15 years), with 36 healthy children and 41 children infected with bacterial or parasitic infection other than VL were investigated for kala-azar by two serological tests (rK39 strip test and IFAT) with microscopic examination of stained bone marrow smear.

Results: One hundred and thirty-five out of 394 clinically suspected cases were diagnosed as kala-azar patients. The sensitivity and specificity were 88.7% and 100% respectively for rK39 strip test and 95.2% and 87.8% respectively for IFAT, while the sensitivity of bone marrow smear was 79.2%.

Conclusion: Recombinant kinesin 39 IC strip test have good sensitivity and specificity in predicting acute VL cases.

Key Words: visceral leishmaniasis, kala-azar, rK39 strip, IFAT.

Introduction:

Leishmaniasis is a vector borne disease caused by obligate intra-cellular protozoa of the genus Leishmania, and capable of causing spectrum of clinical syndromes affecting millions of people in endemic areas of the tropics and subtropics (1).

As reported in several studies, most VL patients present with prolong fever, enlarge spleen and weight loss. This clinical picture is shared by several other endemic diseases such as malaria, disseminated tuberculosis or typhoid fever, which are also commonly seen in the same focus (2). Laboratory testing is therefore necessary to confirm the diagnosis of VL. A highly sensitive and specific diagnostic approach is needed, because of the high fatal evolution of the disease without specific treatment and the serious toxicity of most widely used first-line drug, sodium stibogluconate (3). Beside that, in Iraq, among many reasons, the incomplete studies of the epidemiological aspects were due to lack the usage of diagnostic tests that could be used not only in the detection of entire cases of VL, but also as an epidemiological screening test, such tests should be easy to perform not only in reference laboratories but also in district one and in study areas.

The aim of this study is to evaluate the efficiency (sensitivity and specificity) of rapid immunochromatographic (IC) technique [strip test with recombinant Kinesin 39 (rK39) antigen] and indirect immunofluorescent antibody test (IFAT) in diagnosis of Iraqi children with VL.

In most countries, diagnosis of VL relies on microscopic examination of lymph nodes, bone marrow or spleen aspirates (4). Bone marrow aspiration is safe procedure but its sensitivity for diagnosis of kala-azar is only 7086%- (5). Although the microscopical method is considered the golden standard for the diagnosis and it is relatively simple and cheap, but one of the major drawbacks of parasitological diagnosis is expertise required from both the physician to perform the procedure and the laboratory technician to stain and read the slide accurately (4).

Several sensitive and specific immunodiagnostic methods have been developed. They are useful in identifying specific cases and can be used for community surveillance (6).

The Kinesin related protein encoding gene has been recently

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discovered in Leishmania chagasi that contains a repetitive 117-bp sequence encoding 39 amino acid residues (K39) conserved at the c-terminal in all examined VL-causing isolates (6). A promising ready-to-use immunochromatographic strip test based on rK39 antigen has been developed as a rapid test for use in difficult field conditions (3). Several studies from the Indian subcontinent reported the test to be 100% sensitive (7, 8, 9). However, when evaluated in Sudan, the sensitivity of the test was only 67% (10). In contrast to variations in sensitivities from region to region, the rK39 antigen strip test has (in general) uniformly high specificity (92% to 100%) (8, 7, 11, 12, 13, 14).

Indirect immunofluorescent antibody test is a group specific test using the whole promastigote as a source of antigen. Some workers considered IFAT as the most sensitive and best suited for follow up as it allowed a good discrimination between the acute and remission phase of VL (15). In general, IFAT is simple and easy test with a sensitivity of 87% to 100% and a specificity of 86 % to 100% (16, 12, 13, 14,), however, it requires more expensive and specialized equipment, and it is not suitable for large scale examination of sera (11).

Patients, Materials and Methods:

The study was carried out during the period from April 2007 to December 2008.

•**Study Groups:** Two study groups were involved:

A- Clinically suspected VL group: A total of 394 clinically suspected patients of kala-azar (218 males and 176 females), whose ages ranged from 1 month to 15 years, with a mean age of (3.7) years and who had examined and defined as suspected cases of kala-azar by specialized pediatricions, were included in this study. These cases were from:

Al-Zahraa Maternity and Paediatries Hospital in Al-Najaf province.

Kerbala Children Hospital in Kerbala province.

Al-Elwyia Children Hospital in Baghdad province.

B- Control Groups:

1- Healthy control group:

A total of 36 age and gender-matched children from different health centers and hospitals in Al-Najaf province were involved in this study. They were apparently healthy by specialist physical examination, with no history of kala-azar.

2- Infected control group with diseases other than VL:

The study involved 41 age and gender-matched children with proven bacterial and parasitic infections other than VL.

•**Diagnostic Methods: 1- Bone Marrow Smears (Microscopical Examination)**

According to WHO blood safety and clinical technology by which seventy-two bone marrow samples were aspirated from clinically suspected VL children by the physicians in the ward and stained by Leishman stain, then examined under oil immersion 100x objective lens for the presence of intracellular amastigotes or free amastigotes from ruptured cells (17).

2- Serological Examination: Recombinant K39 Immunochromatographic Test (IC)

Three hundred and seventy-three suspected cases of VL, 20 healthy controls and 41 cases from infected control group were involved in this test, which is a rapid test for detection of specific antibodies to VL in human serum (3).Recombinant kinesin 39 IC strip test for VL is a rapid, qualitative, membrane based immunoassay for the detection of specific antibodies to a recombinant antigen (rk39) in human serum.

Indirect Immunofluorescent Antibody Test (IFAT)

One hundred and sixty-two suspected cases of VL, 20 healthy controls and 41 cases from infected control group were involved in this test.This test is designed for the in vitro determination of specific human antibodies in serum or plasma, which attach to the antigen of Leishmania donovani promastigotes. The attached antibodies are stained with fluorescein-labelled anti-human antibodies and visualized by using fluorescence microscopy (12).

Results:

Among 373 of the entire 394 clinically suspected VL cases, rK39 IC strip test picked up 126 (33.8%) suspected cases with positive result; while among 162 clinically suspected VL cases, IFAT picked up 61 (37.7%) suspected cases with positive result (Table 1).

Table (1): Evaluation of serodiagnostic tests (IFAT & rK 39IC) in diagnosis of clinically suspected VL cases and healthy controls from infections other than VL.

Test		Suspected VL cases		Other infections (cross- reactivity)	
		No. (162)	%	No. (41)	%
IFAT	Positive	61	37.7	5	12.2
	Negative	101	62.3	36	87.8
		No. (373)	%	No. (41)	%
rK39 IC	Positive	126	33.8	0	0
	Negative	247	66.2	41	100

Sensitivity of rK39 IFAT and IC in Diagnosis of VL: Among 62 parasitologically proven VL cases, 59 (95.2%) cases were positive by IFAT using whole promastigotes of L. donovani as antigen (Figure 1) which represents the sensitivity of IFAT in diagnosis of VL (Table 1), while 55 (88.7%) cases were positive by rK39IC strip test (Figure 2) which represents the sensitivity of rK39 IC strip test in diagnosis of VL (Table 1).

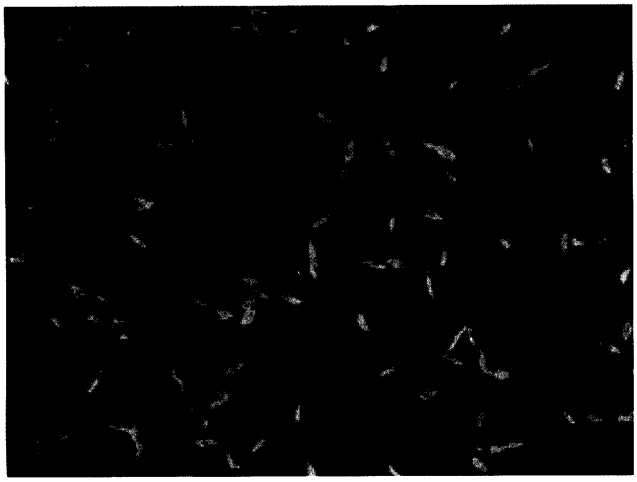
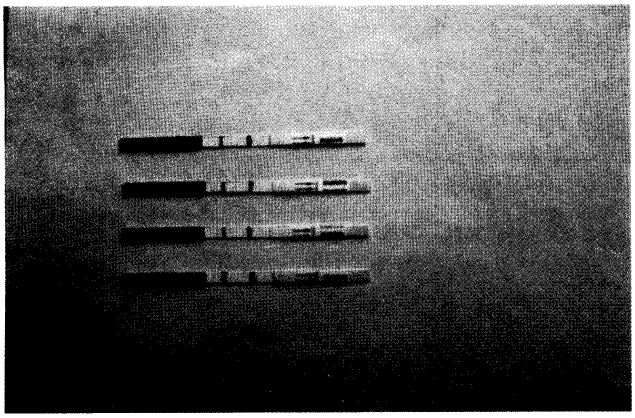
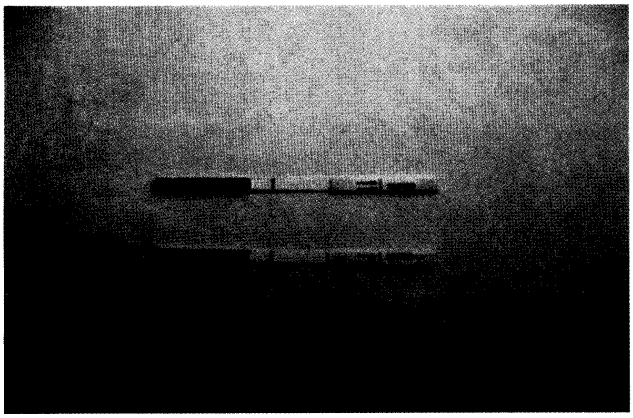


Figure1:Immunofluorescenceof L. donovani promastigotes by IFAT (dark green staining identified positively labeled promastigotes, (magnification power-40X).



(A)



(B)

Figure 2: rK39 IC strip with positive (A) and negative (B) results for VL.

Table (2): A comparison between IFAT and rK39 IC strip test in diagnosis of parasitologically proven VL cases and healthy controls.

TEST		CONFIRMED VL PATIENTS (NO.:62)		HEALTHY CONTROLS (NO.:36)	
		No.	%	No.	%
IFAT	Positive	59	95.2	0	0
	Negative	3	4.8	36	100
rK39 IC	Positive	55	88.7	0	0
	Negative	7	11.3	36	100

Specificity of IFAT and rK39 IC in Diagnosis of VL

Regarding the cross reactivity of VL antigens with sera from 41 patients with infections other than VL (tuberculosis, acute amoebic dysentery, urinary schistosomiasis, brucellosis, toxoplasmosis, malaria and typhoid fever), 5 (12.2%) sera [(2) tuberculosis, (1) brucellosis, (1) toxoplasmosis and (1) typhoid fever] showed positive IFAT for L. donovani; and 36 (87.8%) showed negative IFAT for L. donovani which represents the specificity of IFAT for diagnosis of VL. In other hand, no cross reaction was obtained by rK39 IC strip test, which represents the 100% specificity of rK39 IC strip test in diagnosis of VL (Table 1 & 3).

Table (3): Comparison of results obtained by two serological tests (IFAT and rK39 IC) with heterologous antisera against L. donovani antigens.

NON VL PATIENTS	NO. OF SERA TESTED	IFAT POSITIVE	RK39 IC POSITIVE
Tuberculosis	8	2	0
Acute amoebic dysentery	7	0	0
Urinary schistosomiasis	3	0	0
Brucellosis	5	1	0
Toxoplasmosis	7	1	0
Malaria	3	0	0
Typhoid fever	8	1	0
Total	41	5	0