Diagnostic Value of Rk39 Dipstick in Paediatric Visceral Leishmaniasis in Baghdad

***Dr.Nada Kadem Al-Ta'ee, MBCHB**, *FICMS /CM*, ****Dr.Yousif Abdul-Rahim, MBCHB**, *FICMS /CM*
****Dr.Mohammed Asaad Al-*bayaty* MBCHB, *FICMS /CM***

Abstract

Back ground: Visceral leishmaniasis is endemic in the middle and south of Iraq, it involves mostly infants. The disease is observed mainly among rural areas that are far from equipped medical centers. Therefore, there is a need for anon- invasive, cost- effective, reliable, easily available and fast method of diagnosis of this dngerous disease.

***Objective*:** The aim was to compare the validity and predictive values of the recombinant K39 antigen (rK39) test with that of the indirect fluorescent antibody test (IFAT) test (the usual laboratory method) in the detection of visceral leishmaniasis.

Methods: **A** Cross-sectional study was done in AL-Mansour Pediatric Hospital (in AL-Rusafa), and Central Pediatric Hospital (in AL-Karkh) in Baghdad for 6 months duration (from the 1st of December 2004 to 30th of May 2005). Children less than five years who suspected to be infected with visceral leishmaniasis were selected and investigated by IFAT test and rK39 dipstick strip.

***Results*:** The validity of rK39 test when evaluated with the standard methods IFAT test showed a sensitivity of 90.5%, specificity of 90.7%, predictive value positive of 77.9%, predictive value negative of 96.4% and 90.7% accuracy.

***Conclusions*:** The rK39 antigen strip test is valid in the diagnosis of suspected children with visceral leishmaniasis as the test is rapid, sensitive, and specific.

***Keywords*:** visceral leishmaniasis, rK39 dipstick,

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Introduction

The term leishmaniasis refers collectively to various clinical syndromes caused by obligate intracellular protozoan parasite of the genus *Leishmania*, which affect mostly the reticuloendothelial system [1, 2].

Infection is acquired through the bite of an infected female sand fly of the genus *Phlebotomus* (Old World) or *Lutzomyia* (New World), and has wide range of symptoms in human being, depending on the species of *leishmania* and the host immunity [2, 3].

Visceral leishmaniasis (Kala-azar, Dum-Dum fever) is the sever systemic form of the disease; mucosal (Espundia) and cutaneous leishmaniasis (Baghdad, Alibo, Jarash or Delhi boils) are the other forms **.** These syndromes are widespread geographically and often represent zoonotic infection with variable transmission to man. It is a vector- borne disease, with rodents and canids as common reservoirs, and human being is usually an incidental host [4, 5].

Visceral Leishmaniasis (VL) is an important public health problem, has a worldwide distribution and fatal outcome if left undiagnosed and/or untreated [6].

The World Health Organization (WHO) reported 500.000 cases of VL occurring every year, which spread in 88 countries [7]. About 367 million people at risk of leishmaniasis in different parts of Africa, Asia, Europe, North America, and South America, and about 59,000 people die from it annually [8].

VL is endemic disease in Iraq since 1954. According to the data of Kala-azar section in the Communicable Diseases Control Center (CDC) in Baghdad, the main focus is the middle and the southern governorates of Iraq. The numbers of cases reported in Iraq during the years 2000-2005 were 17515; 95% of them were occurred in children less than 5 years, while they were 18409 cases during the period of 1976–1995 [9]. The incidence of VL has been reported to be high during winter and spring and as the number of cases started to increase in December and continue till March. During the last few years, there was an increase in VL cases in some of the middle and the southern governorates of Iraq (Wasit, ThiQar, Maysan, Basrah and Al-Muthana) [9, 10, 11].

The diagnosis of kala-azar is classically made by demonstration of *Leishmania* parasites in various tissues. Of the procedures currently used, lymph node aspiration is easy to perform but has a sensitivity of only 58%. Bone marrow aspiration is more sensitive (70%) but is resented by patients. Splenic aspiration has excellent sensitivity (96%); however, it carries some risk and should preferably be done in a hospital [11, 12]. Much progress has been made in the development of less invasive tests to assist in the diagnosis of VL, the indirect florescent antibody (IFAT) test was the best one with high sensitivity and specificity, used since 1978 for diagnosis (13).Clearly there is a need for simple serological tests that can be used under field

conditions; in the year 2004, the newly developed specific visceral leishmaniasis antigen strips recombinant K39 (rK39) was introduced for use in newly developed specific visceral leishmaniasis antigen strip recombinant K 39 (rK39) was introduced for use in Iraq [14]. It is important to evaluate the performance and the validity of K39 in the diagnosis of visceral Leishmaniasis among our patients, so the aim of this study evaluate the sensitivity, specificity, predictive value positive, predictive value negative and accuracy of rK39 test in comparison with standard diagnostic method, IFAT test among children less than 5 year in Baghdad.

Methods

This is a cross-sectional descriptive study conducted during the period from 1st of December 2004 to 30th of May 2005 (seasons of the disease in Iraq). The study sample was from the children less than five years attending to AL-Mansour Peadiatric Hospital (in AL-Rusafa), and Central Pediatric Hospital (in AL-Karkh) in Baghdad. Two hundred and eighty blood samples were taken from children suspected to have VL. The presumptive diagnosis was made by pediatrician on the basis of the classic clinical presentation of prolonged fever (more than 10 days), pallor, splenomegaly, hepatomegaly or hepatosplenomegaly [1,2,3,5]. All the suspected children were tested serologically by Rk39 and IFAT.

-Laboratory tests:

1- rK39 test (Kalazar Detect® Rapid Test):

Blood samples were collected in plain tube without anticoagulant. Sera were separated and brought at room temperature prior to testing. The tests were carried out by using the commercially available diagnostic kit (Kalazar Detect®). The test strip was placed into a test tube, so that the end of the strip is facing downward as indicated by the arrows on the strip. The result was read in 10 minutes. The test is positive when a control line and test line appear in the test area. A positive result indicates that the Kala-azar Detect ® dipstick detected antibodies to members of *L. donovani complex*. A faint line is considered as a positive result. As a guide for interpretation, the red color in the test region will vary depending on the concentration of present anti-Leishmanial antibodies. The test line for "weakly positive" sera samples may show results between a weak positive red line to a faintly red, almost white background. The test is negative when only the control line appears. A negative result indicates that detect dipstick did not detect antibodies to members of *L.donovani complex*.[14]

2- Indirect fluorescent antibody test (IFAT):

All samples were tested at Baghdad Teaching Clinical Laboratory. The frozen antigen-coated slides were washed in phosphate buffered saline (PBS) and allowed to dry at room temperature. The sera were inactivated for 30 minutes in a water bath at 56°C. The test sera were diluted from 1/10 to 1/80, Positive and negative control sera were tested at dilutions of 1/80 and 1/160. 30 µl of diluted serum samples were distributed on to each slide circle and incubated for 30 minutes at 37°C. The serum samples were removed by vigorous washing in PBS, followed by immersion of the slides in PBS for 10 minutes, the slides allowed to dry. 30 µl of diluted fluorescein isothiocyanate (FITC)-conjugated anti-immunoglobulin were distributed on to each slide circle and incubated for 30 minutes at 37°C. The slide was read under a fluorescent microscope.[15]

-Statistical analysis: The results of the study were analyzed statistically by using the following procedures [103].

1- Descriptive statistics**:** Statistical tables including observed frequencies with their percentages (Cross tabulations).

2- Evaluating the validity of the rK39 tests when compared with the standard tests (IFAT). Data were analyzed using the following procedure:-The sensitivity and specificity are two measures of the validity of a screening test. The SENSITIVITY is defined as the probability of testing positive if the disease is truly present and is calculated by: Sensitivity= (True positive by the test)/ (True positive +false negative) x100%

The SPECIFICITY is defined as the probability of screening negative if the disease is truly absent and is calculated by: Specificity = (True negative by the test) / (True negative +false positive) x100%.

The predictive positive value (PV+) or the yield of the test is the probability that a person actually has the disease given that he or she tests positive and is calculated by: PV+= (True positive by the test)/ (Total positive by the test) x100%.

The PREDICTIVE VALUE NEGATIVE (PV-) is the probability that an individual is truly disease-free given a negative screening test and is calculated by: (PV+) = (True negative by the test)/ (Total negative by the test) x100%.

The Accuracy = (True positive + True negative) /Total number 100%[16].

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Results

The results of this study revealed that 74 out of 280 children (26.4%) were infected with VL depending on IFAT as a gold standard test in diagnosis. The number of examined and infected children according to their age groups is shown in **(Table- 1)**

(Table-1)
The number of examined and infected children according to their age groups of the study sample depending on IFAT.

Age	No. of child examined	No. of child infected & diagnosed by IFAT	% of child infected
< 2month	40	0	0
2m -<1 year	35	33	44.6
1 -<2 year	50	14	18.9
2 -<3 year	65	11	14.9
3 -<4 year	45	10	13.5
4 - 5 year	45	6	8.1
Total	280	74	100

The used of rK39 dipstick in diagnosis of VL revealed 86 (30.7%) infections among the study sample as shown in table two.

(Table-2)
The number of examined and infected children according to their age groups of the study sample depending on rK39 dipstick.

Age	**No. of child examined**	**No. of child infected and diagnosed by Rk39**	**% of child infected**
< 2month	40	0	0
2m -<1 year	35	37	43
1 -<2 year	50	16	18.6
2 -<3 year	65	15	17.4
3 -<4 year	45	11	12.8
4 - 5 year	45	7	8.2
Total	280	86	100

(Table -3)
The validity of rK39 test in comparison with IFAT as a gold standard test.

Tests		**IFAT test results (Gold standard)**		**Total**
		Positive	**Negative**	
rK39 test results	**Positive**	67 **(True positive)**	19 **(false positive)**	86 **Test positive**
	Negative	7 **(false negative)**	187 **(True negative)**	194 **Test negative**
Total		74 **(Infected children)**	206 **(Non infected children)**	280 **Study sample**

➡ **Sensitivity** = (67/74) X 100% = **90.5%**
➡ **Specificity** = (187/206) X100% = **90.7%**
➡ **PV^+** = (67/86) X100% = **77.9%**
➡ **PV^-**= (187/194) X100% = **96.4%**
➡ **Accuracy** = (67+187/280) X100% = **90.7%**

The validity of rK39 test when evaluated with the standard methods IFAT test showed a sensitivity of 90.5%, specificity of 90.7%, predictive value positive of 77.9%, predictive value negative of 96.4% and the accuracy of rK39 test with good percent of 90.7%) which mean that it is a good screening and diagnostic test for Kala-azar in our hands.

Discussion

Visceral leishmaniasis is a life threatening disease if not diagnosed and treated early [6]. The disease has known to be endemic in Iraq for the last 50 years. Early diagnosis and prompt treatment of the disease could prevent its high mortality. Thus, a simple method of diagnosis is essential [15]. The clinical diagnosis of leishmaniasis is difficult due to the variable symptomatology. The definite diagnosis often depends on parasite isolation by in vitro culture or by detection of parasite particles from lymph node or bone marrow biopsy specimens. However, these techniques are invasive, time-consuming, and expensive. One of the major drawbacks of parasitological diagnosis is the expertise required from both the physician to perform the procedures and the laboratory technician to stain and read the slides accurately. This expertise is very difficult to obtain in practice outside reference tertiary hospitals or specialized treatment or research centers [1, 2, 3, 5].

Research has focused on the development of cheap, simple and reliable serological tests for kala- azar, which could replace parasitological diagnosis in the field. Thus, the detection of specific anti-*Leishmania* antibodies in patient's sera remains an important diagnostic tool. Among the different tests available, most widely used are immunofluorescent-antibody tests (IFAT), direct agglutination tests, enzyme-linked immunosorbent assays (ELISA) These tests are mostly based on purified, water-soluble antigen fractions of promastigote or amastigote stages. In most countries around the Mediterranean basin, IFAT is considered the gold standard for serological diagnosis of leishmaniasis, and it is generally recognized as the most sensitive and specific test [18]. However, IFAT is expensive, time consuming, and not suitable for field diagnosis [17].

A recent development has been the identification of the K39 *Leishmania* antigen, a member of the kinesin family of proteins, containing a repetitive, and immunodominant epitope of a kinesin-related protein that is highly conserved among viscerotropic *Leishmania* species. Detection of IgG antibodies to this antigen has been found [9] to be extremely sensitive and specific in the diagnosis of VL various studies of human patients with visceral leishmaniasis and of dogs with canine leishmaniasis have demonstrated that rK39 are sensitive (93 to 100%) and specific (94 to 100%) [15, 16].

The technique used (rK39) dipstick had several major advantages compared with IFAT in the field setting, the simplicity and ease of use and handle,

individually packed, less cost (4$ comparing to 10$ for each IFAT test), and rapidity of the rK39 dipstick are especially important in a setting such as rural areas in Iraq, where IFAT can be performed by only a few if no laboratories, and travel to a referral center was difficult [19]. Additionally, (rK39) dipstick may be stored at ambient temperature (up to 30°C) and is suitable for field use [18].

The present study was made on infants, who are more commonly affected by visceral leishmaniasis in Iraq, and only those with suggested signs and symptoms were included in order to increase the accuracy of the test. Our study showed that IFAT was 90.5% sensitive, 90.7% specific in suspected children. These findings is in agreement with Iqbal *et al.* (from Kuwait) [19]. But in different from other areas. The highest sensitivities (100%) occurred in patients from India and Nepal [, 21, 22, 23].

Patients in Venezuela had significantly lower percentages of true positive test results (88%), and the rK39 antigen strip test was least (70%-80%) sensitive in patients from Sudan and other African countries. The specificity also had varied from the highest (100%) in India and Nepal to lowest (90%) in Sudan [19,.23,24].. These differences in results might be related to the different strains in different countries and the striking similarities between our results and those of Iqbal et al. [20] may be explained by the causative agent of visceral leishmaniasis.

In conclusion, the present study confirms the benefit of the rK39 antigen strip test in the diagnosis of patients with VL in suspected children as the test is rapid, sensitive, and specific. The rK39 strip test would be highly desirable as diagnostic tool, because it can be done in the field and remote areas and would allow control interventions to be implemented in situ. More studies are needed to confirm its accuracy in non symptomatic children in order to estimate its benefit as a screening test as well.

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***Community Medicine Department**
***Al- Kindy College of Medicine**
***Head of the Community Medicine Department**
***Al- Kindy College of Medicine**
E-mail: *Naddat2006@yahoo.com*

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