



## Sero-prevalence of visceral leishmaniasis by using two diagnostic kits (rKE16 and rK39)

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### Abstract

The dipstick test was evaluated for sero-diagnosis of visceral leishmaniasis. We compared two types of dipstick (rKE16, rK39) tests. The sensitivity of both tests were determined using sera from fifty-two children suspected of having visceral leishmaniasis and thirty healthy children as a control group collected from Central Teaching Hospital of Pediatric in Baghdad. Fifty (96.15%) cases were confirmed to have infection by rKE16 dipstick test while, forty-six (88.46%) cases were positive by rK39 dipstick test. Non of the sample taken from healthy control showed reactivity in any of these tests. The study indicated that rKE16 test had better sensitivity than rK39 in the diagnosis of VL(100%), (92 %) respectively.

**Keywords:** visceral leishmaniasis , diagnosis VL, dipstick.

### التقييم المصلي للشمانيا الاحشائية باستخدام نوعين من عدد التشخيص (rKE16 and rK39)

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### الخلاصة:

اختبار الـ dipstick يستخدم لتقييم الشمانيا الاحشائية مصليا. في هذه الدراسة تم المقارنة بين نوعين من اختبار الـ dipstick (rKE16, rK39) فيلس تحسس كلا الاختبارين اجري باستخدام عينات مصول مأخوذة من 50 طفلا مشكوك باصابتهم بالشمانيا الاحشائية و 30 طفلا اصحاء كمجموعة سيطرة جمعت من مستشفى الطفل المركزي في بغداد. 50 (100%) اظهرت ايجابية للتحسس بواسطة الـ rKE16 بينما 46 (92%) اظهرت ايجابيه للتحسس بواسطة الـ rK39. لم تظهر اي عينة من مجموعة السيطرة ايجابية للتحسس بكلا الاختبارين. اظهرت الدراسة ان الـ rKE16 افضل تحسسا من الـ rK39 في تشخيص الشمانيا الاحشائية عينة 50 (100%) عينة 46 (92%) على التوالي.

### Introduction

Leishmaniasis is a vector-transmitted protozoan disease distributed throughout the world's tropical and subtropical regions. Leishmaniasis is caused by 20 different species which are pathogenic to human, and belong to the genus *Leishmania*, an intracellular parasite transmitted by the bite of the phlebotomine sandfly [1, 2].

Visceral leishmaniasis is a chronic and vector-borne potentially fatal parasitic disease caused by *Leishmania (L.) donovani* / *L. infantum* / *L. chagasi* complex which is associated with fever, malaise, anaemia, anaruxia, fatigue, enlargement of the liver, spleen and lymph nodes, hypergammaglobulinemia and to the progressive suppression of cellular immunity [3]. According to

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WHO, 2010 [4] million cases are estimated to cause over 50,000 deaths per year . More than 90% of the visceral leishmaniasis (VL) occurs in Bangladesh, Brazil, India, Nepal, and Sudan [5]. A range of serodiagnostic assays have been developed and widely used to detect presence of anti-leishmanial antibody, many of these tests are found to be cross-reactive with *Trypanosomes* and *Mycobacterium*, as a consequence recent applications of recombinant antigen based tests with higher sensitivity to detect leishmanial infection are much in practice [6,7]. Crystal KA is an immunochromatographic test for the qualitative determination of anti-leishmanial antibodies, using a patented recombinant antigen (rKE16) is highly specific for VL caused by parasite members of *L. donovani* complex of old world [8]. Therefore, this study aimed to evaluate the sensitivity of two diagnostic dipstick tests (rKE 16 and rK39) for visceral leishmaniasis in children group suspected of having the disease.

### **Materials and Methods**

#### **Blood sample collection:**

Five ml of venous blood were collected from each child . The blood was placed in vacuum tube and left to stand for one hour at room temperature for clot formation. For serum collection, the tube was centrifuged for 10 minutes at 3000 rpm , then dispensed into a sterile eppendorf tubes and stored at -20 until used.

#### **Crystal –KA (rKE16)**

Crystal KA is an *in vitro* qualitative screening test to diagnose Kala azar using human serum or plasma.

#### **Assay procedure**

- 1- reagents and samples were cooled to 25C° for use.
- 2- Four drops of reaction buffer were dispensed into the test tube
- 3- (20 µl) of serum sample was added by plastic bulb dropper (20 µl) and mixed properly by agitation
- 4- Dipstick was brought from blister pack and labeled with patients identification code
- 5- Dipstick was placed vertically into the tube containing diluted sample, until the sample liquid reached the arrow mark
- 6- The dipstick was removed from the sample after 15 minute, and the result was seen

#### **Interpretation of results**

##### **Negative result**

Presence of only one pinkish red band at control C region and no band in test region T indicates that the sample is non-reactive for leishmaniasis.

##### **positive result**

Presence of two pinkish red bands, one at C and one at T region indicate that the sample is reactive for leishmaniasis.

##### **Invalid result**

Absence of pinkish red band at control C region with presence or absence of band at T region indicate invalid results.

It can be due to deterioration of sample/reagent, if sample is not or presence of particulate matter in sample. In such case the test using new dipstick and fresh sample.

#### **rK39 dipstick**

##### **Assay procedure**

- 1- The Kala-azar dipstick strip was removed from the pouch or vial
- 2- One drop of serum was placed on the absorbent pad on the strip bottom.
- 3- The test strip was placed into a test tube so that the end of the strip is faced downward.
- 4- The mixture was allowed to migrate into the strip by capillary tube, then 2-3 drops of the buffer solution were added provided with the test kit to the pad
- 5- The results were seen in 10 minutes.

#### **Interpretation of Results**

##### **Positive result**

The test is positive when a control line and test line appear in the test area as shown. A positive result indicates that the dipstick detected antibodies to members of *L. donovani* complex. A faint line is considered a positive result. As a guide for interpretation, the red colour in the test region will vary depending on the concentration of the available 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. ("Weakly positive" samples are those with low affinity or low titer antibodies against the recombinant test antigen.).

#### Negative result

The test is negative when only the control line appears. A negative result indicates that the *Leishmania* dipstick did not detect antibodies to members of *L.donovani* complex. No test line is present.

#### Invalid result

No lines appear at either the control or test line areas The test is also invalid if no control line appears, but a test line is seen. It is recommended to retest using a new dipstick and fresh serum.

#### Results and Discussion

This study included fifty-two children suspected of having visceral leishmaniasis and thirty healthy children as a control group. Which tested by using two immunochromatographic dipstick assays to detect antibodies to *leishmania donovani* complex. The results showed that fifty (96.15%) samples were confirmed to have infection by rKE16 dipstick test while forty-six (88.46%) samples were positive by rK39 dipstick test. Non of the samples taken from healthy control showed reactivity in any of these tests. Table-1. All 52 studied children proved having visceral leishmaniasis, their ages ranged between 5 months to 2 years and the infection rate was significantly higher  $p<0.0$  in age more than one year (61.53%), 21 of them were males and 31 females, the percentage of females was significantly higher  $p<0.01$  than males (59.61%), (40.38%) respectively. The duration of illness varied between 15 days to 4 months with significant  $p<0.01$  majority of children being ill for more than one months (61.46%). Table-2.

**Table 1-** Sero-prevalence of visceral leishmaniasis in surveyed children collected from Central Teaching Hospital of Pediatric in Baghdad

	No. of cases	rKK16		rK39	
		+Ve No.(%)	-Ve No.(%)	+Ve No.(%)	-Ve No.(%)
Suspected cases	50	50 (100%)	0 (0%)	46 (92%)	4 (8%)
Control group	30	0 (0%)	30 (100%)	0 (0%)	30 (100%)

**Table 2-** The percentage distribution of VL in studied children according to different clinical parameters

		No. of cases(%)	Total No.(%)	Chi-square value
Age	Less than one year	20(40)	50 (100)	9.514 **
	More than one year	30 (60)		
Sex	Male	19 (38)	50 (100)	7.277 **
	Female	31(62)		
Duration of illness	Less than one month	18 (36)	50 (100)	9.672 **
	More than one month	32(64)		

\*\* (P<0.01).

Current study indicates that rKE16 test had better sensitivity than rK39 in the diagnosis of VL (100%) ,(92 %) respectively.

Inspite of , rK39 has been shown to be specific for antibodies arising during VL caused by members of the *L. donovani* complex, and had highly sensitive and predictive values for onset of acute disease and evokes high antibody titres in VL patients [10]. But previous results from Sudan and other countries agreed with present results that revealed this antigen showed decreased sensitivity and specificity, in Sudan the rK39 ELISA test is reported to miss 7 per cent parasitologically proven cases. In strip test format, the sensitivity is further compromised to only 67 percent in Sudan, 71.4 percent in Southern Europe and 60 to 90 percent in Brazil [11, 12].

A recombinant antigen rKE16 was cloned and characterized from an Indian isolate of *L. donovani* strain [13]. Sivakuma *et al.* 2006<sup>13</sup> reported that the antigen (rKE16) is found to be 100 percent

sensitive and specific. In fact it has better sensitivity than rK39 which showed 98 percent sensitivity for the diagnosis of Indian kala-azar and PKDL. It also showed 100 percent concordance with rK39 in sera of leishmaniasis patients from China, Pakistan, and Turkey, these results were in agreement with the current results that showed 100% sensitivity by this kit.

This diagnostic kit (rKE16) has several major advantage compared with others testes in the field setting, the simplicity and ease of use, less cost, and rapidity of rKE16 dipstick are especially important in a setting such as rural areas in Iraq [14]. While, the only disadvantage of the rKE16 test is inability to differentiate between recent and old infection [15]. The (rKE16) antigen has now been commercialized and has a tremendous potential for the serological diagnosis of VL worldwide [13].

This study was disagreed with the previous studies that showed most infection was in children group less than 2 years [16]. While, Al.Saqur *et al.* (2006) [17] found that the age has adverse effect with infection in children less than two years whether in Baghdad or Wasit. In the former it was 43.3% in less than one year and then decrease to 6% in 4-5 years, while in Wasit the results was slightly different. Five cases 2.5% were detected in infant less than one month, but the highest incidence recorded in 1- <2 years of age group 37.9%.

The increase of infection among 1- < 2 years age group may be due to the movement and activity of children leading to possibility to contact with vector sand fly that spread in the environment beside their immune system is not well developed [18].

In the current study the distribution of VL according to the gender showed significant different percentage of females and males which were (62%) and (38%) respectively this results disagreed with previous results of Al.Saqur *et al.* (2006) [17] who showed the distribution of VL according to gender in Baghdad was nearly similar in males and females which was 52.2% and 47.8% respectively. While in Wasit, it was higher in males than females which was 60.6% and 39.4% respectively. On the other hand the result of the current study was similar with Akbarpour *et al.* (2012) [19] which revealed those males infected with VL were less than female cases in percent (0.88:1). There is no real explanation for the increase of one gender on another; it may due to the highest exposure of male children for the vector than female especially when the type of clothes of female children were more covering of the body than male so the protection from the insect bite was more also [20]. But AL-Alousi *et al.* (1980) [21] disagreed with above and found equal percentage of infection in both males and females.

From this study we conclude that dipstick (rKE16) more sensitive than (rK39) in diagnosis of visceral leishmaniasis.

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