Diagnosis of Pediatric Visceral Leishmaniasis by Immunochromatographic strip test (ICT) in Mid-Euphrates Area, Iraq

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Abstract—Visceral leishmaniasis (VL) . It is a disease caused by species of Leishmania donovani . Leishmaniasis is still one of the world's most neglected diseases, affecting largely the developing countries. VL is widely spread in different parts of Iraq, which is regarded as an endemic place, especially in the middle and south parts, the main reasons are due to adaptation of the vector sand fly in these areas.

This study was carried out during the period from January 2012 to March 2013. Five hundred and eighty seven children aged 1- 60 months were admitted to paediatric ward of Pediatric and Maternal Hospitals of Al- Najaf, Babil, Karbala, and Al-Qadisiyyah provinces with confirmed or clinically suspected visceral leishmaniasis were included; most of them had clinical manifestation of fever, hepatosplenomegaly, weight loss, Leukopenia, and anemia. The diagnosis was under the supervision of paediatrician from each hospital.

This study was evaluated Immunochromatographic strip test (ICT). ICT gave 46.84% positive results with sensitivity and specificity of 100%.

Index Terms—Visceral leishmaniasis ,Kala-azar, *Leishmania donovani, Pediatric Visceral,* Immunochromatographic strip test (ICT).

1 INTRODUCTION

Protozoa of the *Leishmania* spp. causes an obligate intramacrophage infection. The clinical syndrome is characterized by fever, weight loss, splenomegaly, lymphadenopathy and

hepatomegaly. VL (Visceral [1]Leishmaniasis) is one of the world's most neglected

iseases, largely affecting the poorest people, mainly in developing countries [1].

Leishmaniasis represents a major public health problem in the Eastern Mediterranean Region (EMR); visceral

leishmaniasis is mainly seen in 14 of the 22 countries of the region. Anthroponotic visceral leishmaniasis, caused by *L. donovani*, occurs mainly in Sudan and Somalia. Zoonotic visceral leishmaniasis, caused by *L. infantum*, occurs in most countries of the region: in Afghanistan, Iran, Iraq, Jordan, Lebanon, Saudi Arabia, Syria, Turkey, Tunisia and Yemen [2][3].

Iraq is a well-known area for endemicity with the Kala-azar disease (Jacobson, 2011), which is a long-lasting disease since 1954 [4]. Then factors such as population movement and the destruction of health and interruption of control methods, like insecticide spraying on early diagnosis and treatment of positive cases contributed to the outbreak of leishmaniasis in Iraq [5][6].

Immunochromatographic (ICT) strip test (Dipstick serologic tests): Immunochromatographic strips using K39 antigen had become popular in recent years, and became possible after the identification of the cloning and expression of k39 kinesin gene by Burns and colleagues (1993)[7], and they reported the evaluation of leishmaniasis patient antibody responses to the recombinant product, rK39 (A kinesin-related protein-encoding gene contains a repetitive 117-bp sequence encoding 39 amino acid residues (K39) conserved at the C-terminal end in all of the visceral leishmaniasis-causing isolates examined so far). rK39 contains a 39-amino acid repeat that is part of a 230-kDa protein predominant in *L. chagasi* tissue amastigotes. The sequence encodes an immunodominant protein with a repetitive epitope closely conserved between *L. chagasi* and *L. donovani*.

Detectable anti-K39 antibody was virtually absent in cutaneous and mucosal leishmaniasis patients and in individuals infected with *Trypanosoma cruzi*. The data show that rK39 may replace crude parasite antigens as a basis for serological diagnosis of visceral leishmaniasis. The cloning of the K39 antigen has resulted in the synthesis of recombinant K39 (rK39) antigen.

Using its recombinant product, an immunochromatographic based strip test has been developed in which rK39 is fixed

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on a nitrocellulose paper, and colloidal gold-protein A is used for detection. A drop of serum or blood obtained by finger prick is smeared over the tip of the strips and dipped in a small amount of buffer, with the results read within 15 minutes [8].

The rK39 strip test has been found highly sensitive and a reliable indicator of Kala-azar [8][9][10].

Maia and colleagues (2012)[11] recorded that the rK39 strip test was more sensitive and specific than the IFAT and ELISA, and they did not detect any difference in the sensitivity and specificity between strips produced by different manufacturers. Singh and colleagues study (2009)[12] showed that all the cured followed up cases showed positive result by rK39 strip test even after 180 days of completion of successful treatment. They also mentioned that, the test seems an ideal qualitative test for the diagnosis of Kala-azar. But for sero-epidemiological studies the test may be used with other parameters like ELISA.

This advancement could be made simpler by using patient's urine instead of blood so as to avoid contact with more hazardous biological material for peripheral health workers. Goswami and colleagues (2012)[13] recorded 100% sensitivity and 86.33% specificity of the K39 strip assay by using urine samples of VL patients, however urine testing had more false-positive results in comparison with blood testing. Another study mentioned that the strips cannot be used for the diagnosis of VL by using urine samples because the sensitivity of rK39 in urine was 96.4% while the specificity was low (66.7%), but with serum, sensitivity was 100% and the specificity was 100% [14]. Other researchers evaluated the presence of anti-rK-39 antibody in human saliva, and they recorded that the sensitivity in saliva was 82.5%, compared with 100% in serum. They mentioned that saliva is not suitable for diagnosis of VL because of low sensitivity [15].

ICT suffers from the same disadvantage as DAT: being positive in a significant proportion of healthy individuals in endemic regions and for long periods after cure of VL [8].

2 METHODOLOGY

This study was carried out in the laboratories of Pediatric and Maternal Hospitals of Al-Najaf, Babil, Karbala, and Al-Qadisiyyah provinces and in the Department of Biology in the Faculty of Science – University of Kufa.

2.1COLLECTION OF SAMPLES

Blood samples: Four ml of venous blood was collected from clinically suspected patients and control. Two ml was allowed to clot at room temperature then centrifuged at 3000 rpm for 5 minutes the serum was harvested into clean dry screw-capped tubes. Part of separated serum was used freshly for the Immunochromatographic strip test.

2.2 STUDY GROUPS

Patients Group: including 587 children aged 1-60 months, admitted to pediatric ward of Pediatric and Maternal Hospitals of Al-Najaf, Babil, Karbala, and Al-Qadisiyyah provinces. Most cases had clinical manifestation of fever, hepatospleenomegally, weight loss,leukopenia, and anemia. Cough and vomiting were frequently reported accompanying symptoms. Thediagnosis was under thesupervision of pediatrician from each hospital.

The Bone marrow aspiration was carried out for just 38 cases under the supervision of pediatricians at private hospitals in each province. Microscopic examination method applied and detected the Leishmaniadonovani in their Pathological Lab, and then the results of aspirated bone marrow were sent. Only 34 cases were positive of Kala-azar by the demonstration of the parasite -the amastigotes- from direct smear of bone marrow, while 4 cases were negative.

Control group: consists from 20 healthy children all were with no history of parasitic infection, and without clinical manifestation of any disease.

2.3Immunochromatographic Strip Test (ICT) or Dipstick Serologic Tests:

A-Materials

ICT Strip Test Kit: It is a product of Cortez Diagnostics Inc., CA, USA, and Catalog No. 176070-1, sufficient for 25 tests (Figure 3.1), which includes:

1. Twenty-five (25) individually pouched Test Strips, each Cortez test strip's membrane is pre-coated with a recombinant rK39.

2. One (1) vial of Chase Buffer solution.

B-Immunochromatographic Strip Test Protocol:

1. Cortez strip was removed from the foil pouch.
2. 20 µl of sera was added to the test strip in the area beneath the arrow.
3. The 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.
4. 2-3 drops (150 µl) of the Chase Buffer solution provided with this test kit were added.
5. The results were read in 10 minutes. It is significant that the background is clear before reading the test, especially when samples have low titer of anti-Leishmanial antibody, and only a weak band appears in the test region.

2.4 Statistical Analysis

Statistical analyses of all results were carried out by the help of SPSS version 17 software statistical package using chi square (P value at level of significance less than 0.05).

3 EXPERIMENTALRESULTS

The results of the ICT are presented in table1 275 (46.84%) Sera from the patients gave positive results, while 312 (53.15%) Sera from the patients and from 20 (100%) apparently healthy control, gave negative results (Figure 1). There was a significant difference (P < 0.05) between positive and negative results.

Table 1: The Results of the Immunochromatographic Strip Test.

subjects	Test results		Total
	Positive	Negative	
Healthy controls	0	20 100%	20 3.29%
Disease suspects	275 46.84%	312 53.15%*	587 96.70%
Total	275 45.30%	332 54.7 %	607 100%

*P < 0.05 significant

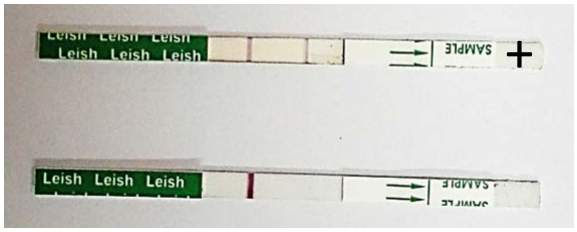

Figure 1: The Positive Result of ICT from Patient Serum and Negative From Healthy Control.

4 .DISCUSSION

In Iraq, rK39 rapid diagnostic test with ICT is now widely used for routine confirmation of VL. This test has been widely accepted in VL-endemic areas because of reported high sensitivity and specificity in published analyses [16][17][18][19][20].

In the present study ICT gave 46.84% positive results with significant difference between positive and negative results.

The results of Al Saqur *et al*. (2008)[21] revealed that the prevalence of Visceral Leishmaniasis in Wasit governorate 47.8% by using dipstick rK39.

In this study both the sensitivity and specificity of dipstick test were 100% and this similar to the results of same test used by Mandal *et al*. (2008)[22].

Different sensitivities and the specificities of the rK39 antigen strip test had been observed by many researchers on parasitologically confirmed Kala-azar.

In Iraq, a study was carried out on Iraqi children VL by using dipstick rK39. The results showed that the sensitivity and specificity of dipstick test were 63.6% and 88.23%, respectively [23]

Carvalho and colleagues (2003)[24] reported that the sensitivity was 90% while the specificity was 100%, Goswami and colleagues (2003)[25] recorded that the estimated sensitivity was 100% and the specificity was 98.18%. Other studies recorded that the sensitivity was 95% and specificity of the strip test was 98 % ([26][27][28]. While Dawood *et al.* (2009)[29] recorded that the sensitivity was 88.23% and the specificity was 60%.

Other sensitivities and specificities of the ICT were 93% and 97% [16][17].

Cañavate *et al*. (2011)[18] mentioned the sensitivity was 93.9% and the specificity 95.3%.

The performance of serologic tests may vary depending on the characteristics of the circulating parasites, differences in the populations tested, and the rapid test formulations [18]. The ability to make most VL diagnoses without the need for specialized equipment or invasive procedures makes the ICT a major advance for peripheral health care facilities. Therefore these data form the basis for recommending the use of an rK39 ICT as the first-line test to confirm VL in patients meeting the appropriate case definition in Iraq.

It was concluded that this test is comparable to parasitology in terms of their sensitivity and can replace parasitology as the basis for a decision to treat visceral leishmaniasis at peripheral health centers in endemic areas.

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