DIPSTICK STRIP TEST VERSUS BONE MARROW TEST FOR DIAGNOSIS OF VISCERAL LEISHMANIASIS IN PEDIATRIC INPATIENTS MID-EUPHRATE AREA

Hashim Raheem Tarish[1], Fuad Muhsen Jawad[2] & Khairy Abdulla Dawood[3]

ABSTRACT

This study was conducted in the Mid-Euphrate Region (Al-Qadisya, Najaf and Karbala provinces) during the period between first/June 2004 to first/May 2006. Two types of laboratory tests were used in this study for the diagnosis of visceral leishmaniasis in suspected patients attending pediatric hospitals in these province's.

The patients were divided into two groups; the first group included 150 children admitted to Al-Qadisya, Najaf and Karbala hospitals and the diagnosis was made on serum samples using Dipstick test, and the results indicated the positive cases by this test were 107/150 (71.4%). The second group included 27 children suspected of having visceral leishmaniasis depending on the clinical features, and microscopical smear finding by Bone marrow examination), the results indicated that the positive cases by this test were 17/27(62.9%). Fifteen out of 17 VL patients (microscopically confirmed) were found positive by dipstick test. The test was proved to be the simplest, cheapest, and rapid for the diagnosis of VL. It is a safe, highly sensitive (88.23%) and specific (60%) and can detect the disease early. It was found that all the age groups are susceptible for the disease but those who are 13-24 months old are the most susceptible. Both sexes are susceptible, but the highest incidence of infection more males (59.3%) were affected than female (40.7%). Visceral leishmaniasis was found to be more prevalent in rural than urban populations (68.3% and 31.7%) respectively.

Statistical method: Chi- square test and percentage of infection were applied. P-value 0.05

INTRODUCTION

The *Leishmania* parasite is a protozoan belonging to the order Kinetoplastida and the family of Trypanosomatidae.[1] The genus *Leishmania* includes more than 20 species. The parasite exists in two morphological forms: the non flagellated amastigote (3-5 µm in diameter) living intracellular in macrophages and monocytes of the mammalian host, and the flagellated promastigotes (15-30 µm in length), in the vector and culture.[1] Leishmaniasis is present in Iraq and was contracted by a number of the troops involved in the 2003 invasion of the country and the subsequent occupation.[2,3] Confirming Visceral leishmaniasis, is a serious health hazard disease of tropical and subtropical countries, has plagued mankind since antiquity.[4] The main problem to control visceral leishmaniasis is to establish a definite diagnosis. Till date the demonstration of the parasite in splenic/bone marrow aspirate is considered to be the gold standard.[5] But these invasive procedures have their own drawbacks. These tests may sometime be found to be false negative, if the parasite density is low. Hence, there is a need for an alternative approach for diagnosis of visceral leishmaniasis.[6,7] The clinical signs and symptoms of visceral leishmaniasis or Kala-azar are not pathognomic. Visceral leishmainasis may be confused or shared with similar diseases such as malaria, tropical splenomegaly, schistosomiasis, and others conditions.[8,9] The confirmatory diagnosis of visceral diseases relies on either the microscopic demonstration of the *Leishmania* parasite (L.D. bodies) in the BM aspirate,[10] or in the peripheral blood (Buffy coat). But these and other old tests became unreliable because of their risks as pain, bleeding, low sensitivity and specificity and also may be rejected by the patients and requires skill or expertise.[11]

MATERIALS AND METHODS

Patients

A total of 150 clinically suspected visceral leishmaniasis patients were entered to Pediatric and Maternal Hospitals of Al-Qadisya, Najaf, and Karbala provices, during the period from June 2004 to May 2006. Most the patients were admitted to the hospitals have presented with clinical manifestations such as fever, loss of the weight, anemia, hepatosplenomegaly.

Methods

Dipstick strip test and bone marrow were used in this study for the diagnosis of VL patients.

[1]Department of Medical Microbiology, College of Medicine, University of Kufa, Iraq
[2]Department of Pediatric, College of Medicine, University of Kufa, Iraq
[3]College of Vet. Medicine, University of Al-Qadisiya, Iraq

Sample collection

Four ml of blood was collected by vein puncture into sterile test tubes and left about 2-4 hours, then serum was collected in clean test tube and store at 20˚C until use.

BM Technique, and Geimza Stain of BM Biopsy Smear. The BM test this is the golden classical method used for detection the intracellular amastigote stage (L.D body) in BM aspirate tested microscopically, Immunochromatographic dipstick Strip Test Method (Inbios International, Seattle, USA):

Test Procedure:

1. Allow the sera and chase buffer to reach room temperature prior to testing
2. Remove the Kala-azar detect strip for VL from the foil punch.
3. Add 20 µl of the serum to the test strip in the area beneath the arrow.
4. Place the test strip into well of a 96 well microtiter plates so that the end of the strip is facing downward as indicated by the arrows on the strip.
5. Add 3 drops (150 µl) of chase buffer solution provided with the kit.
6. Read the result in 10 minutes. It is significant that the background is clear before reading the test, especially when samples have low titer of antileishmanial antibody, and only a weak band appears in the test region (T). Results interpreted after 10 minutes can be misleading.

Interpretation of the Results:

Positive Result: The test is positive when a control line and the test line appear in the test area as shown in Figure (1), a positive result indicates that the Kala-azar dipstick detected antibody to *L. donovani*.

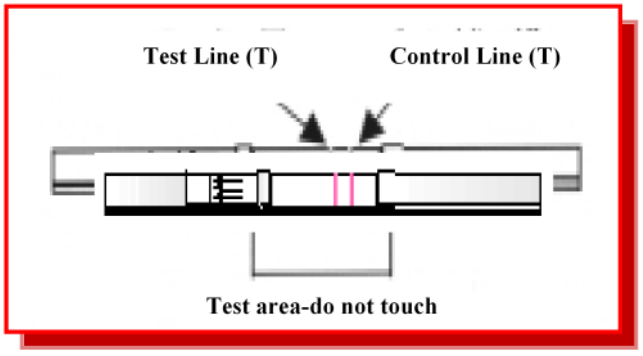

Fig 1. ***Positive results of dipstick strip test after added serum sample (both lines were visible).***

Negative Result: The test is negative when only the control line appears. A negative result indicates that the dipstick did not detect antibodies of L. *donovani*. Figure (2).

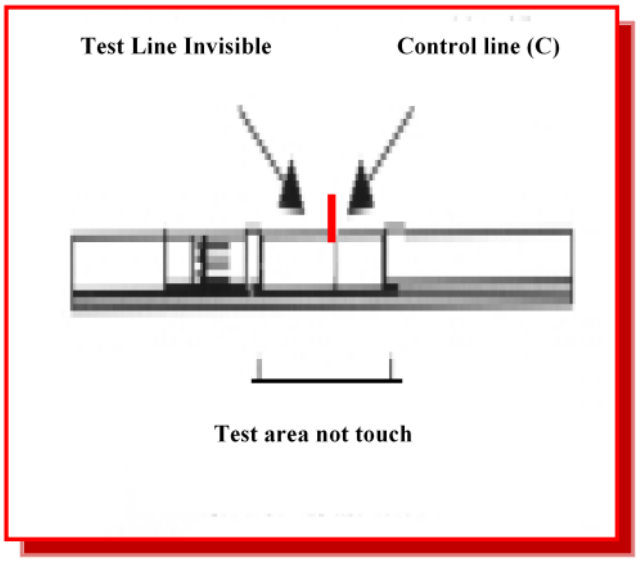

Fig 2. ***Negative results of dipstick strip test after added serum samples. (test line was invisible, while the control line was visible).***

Statistical Analysis: Chi-sequare test and percentage of infection were applied to find the significant difference between the data. P-value < 0.05 was considered significant.

RESULTS

A total of 150 clinically suspected visceral leishmaniasis patients were admitted to Pediatric and Maternity Hospital of Al-Qadisya, Najaf, and Karbala, during the period from June 2004 to May 2006. Most the patients were presented to hospital with anemia, weight loss, splenomegaly, hepatomegaly, and fever. The steps of diagnosis of the disease were based on the following.

Clinical Diagnosis: It depends on the peculiar signs and symptoms related to this disease such as anemia, weight loss, enlarged spleen, enlarged liver, and duration of fever. This study was showed that of the patients were suffered from anemia and enlarged spleen (Figure-3).

Fig 3. *Child of 60 months old with enlarged spleen due to VL (photo was done after the agreement of his family).*

Fig 4. *Bone marrow aspirates from a child with VL. Numerous amastigotes (arrows), with a round nucleus and flat kinetoplast, is present within the cytoplasm of a macrophage (X100) (Geimsa stain).*

The patients were divided into two groups according to the tests applied:

The First Group: This group included all the clinically suspected patients (150) who diagnosed by Dipstick test; the positive cases were 107/ 150 (71.4%).

The Second Group: This group included 27 out of 150 clinically suspected VL patients which were tested by BM test. Seventeen out of 27 patients were found infected (positive) 17/27 (62.9%). (Table-1). Figure (4).

The Dipstick Strip test compared with positive result of BM examination:
The results of this test were highly sensitive 15/17(88.23%) and specific, 6/10(60%) compared to the result of the BM examination. (Table-1).

Table 1. Validity of dipstick test for diagnosis of VL was compared with results of BM examination.

Test		Bone marrow examination		Total
		Positive	Negative	
Dipstick result	Positive	15 True positive	4 false positive	19
	Negative	2 false negative	6 True negative	8
	Total			27

P<0.05
Sensitivity = 15/17 x100 = 88.23%
Specificity = 6/10 x 100 = 60%
Accuracy rate = (15+6) /27 x 100 = 77.77%
Predictive value of positive dipstick = 15/19 x 100 = 78.94%
Predictive value for negative dipstick = 6/8 x 100 = 75%

Relation of the age of patients with dipstick test:

This study revealed that the positive cases of VL patients, by dipstick test, have shown that the highly susceptible age group was 13-24 months and the lowest susceptible age groups above 49 months. (Table-2)

Table 2. Dipstick test results of positive VL in relation to age of patients.

Age (months)	Dipstick result (n=107)	
	Positive patients	
	No.	%
<12	33	30.8
13-24	53	49.5
25-36	7	6.6
37-48	7	6.6
< 49	7	6.5
Total	107	100

P<0.05

The frequency of VL related to residency:

The present study showed that, the positive cases of VL patients were higher in the rural areas than the urban areas.(Table-4)

Table 3. Positive results of VL patients related to residency.

Residency	Diagnostic test			
	Dipstick (n=107)		BM (n=17)	
	No.	%	No.	%
Rural	67	62.7	15	88.2
Urban	40	37.3	2	11.8
Total	107	100	17	100

P < 0.05

The frequency of VL in relation to the sex:

The present results were showed that, the positive cases were high among the males than females. (Table-4).

Table 4. Positive results of VL patients related to sex.

Sex	Positive cases of VL by the diagnostic tests			
	Dipstick (n=107)		BM (n=17)	
Males	No.	%	No.	%
	63	58.9	11	64.7
Females	44	41.1	6	35.3
Total	107	100	17	100

P<0.0

DISCUSSION

Two tests were used in this study these include dipstick strip test and bone marrow for detection the *Leishmania* parasite (L.D. bodies) in the aspiration of BM and also was considered as confirmatory test to evaluate the results of above tests.[11] Since visceral leishmaniasis occurs mainly in areas of the world where health services are poorly developed, research has focused on the development of a simple, cheap and reliable diagnostic tests for diagnosis the disease.[12] The present study has included two tests for diagnosis suspected visceral leishmaniasis and compared the results of dipstick strip test with the results of BM test (parasitological confirmed results) for the same patients (17 parasitologically VL patients by bone marrow aspirate) in order to evaluate the performance of each tests.

Dipstick Strip Test Results Versus BM Results:

Immunochromatographic dipstick test for *Leishmania* diagnosis has recently been developed and are all based on recombinant k39 (rk39), a protein predominant in *L. infantum* and *L.donovani* tissue amastigote.[13] The sensitivity and specificity of dipstick test were (88.23% and 60%) respectively in present study and this results is similar to the results of same test used in other studies,[15-17] and this may be because of

the same causative agents (*L.donovani*) of the disease. The results of dipstick strip test in this study was consistent with the report of [17] for detecting the infection early in India and with the results of the study in Iran by[18] and this similarity may be due to using the same test and presence the same causative agent of the disease.

The present study, showed that most the clinically suspected VL patients admitted to the hospitals have enlarged spleen, enlarged liver, prolonged fever, and anemia, these results were similar with the results many other study in different countries.[13-17,19] The symptoms and signs of VL vary between individuals and geographical distribution,[18] this similarity may be due to immune response of the children and poor hygiene. The present study has recorded the hematological changes in VL patients which had revealed a decrease in percentage of the Hb%, PCV%, and the total leukocyte count (TLC), the most prominent hematological changes in the VL patients, these results are in agreement with the results of many studies in many areas in the world because of the parasite effect on the reticulo-endothelium system.[20-24]

Children are one of the targets of infection with VL in the Mediterranean country, the present study showed that the high percentage of infection with *L. donovani* recorded in the children, and these present results are also are similar with the results reported by other authors,[3,29-31] this may be related to immune response of the children.

The present study proved that, a higher percentage of visceral leishmaniasis infection occurred in the rural areas (68.3%), and these results are consistent with the results of previous studies in many areas of Iraq[3,24,25] and this may be due to the presence of the vector of this parasite.

The results of this study recorded that, the high percentage of infection with *L. donovani* occurred in the males (59.3%), these results are similar to the results of many studied in different part of Iraq because males are more prone to exposure to the sand flies (vectors) than females because of their outdoor activities.[26,27]

REFERENCES

1. Alexander J, Satoskar A, Russel D. Leishmania species. J. Cell Science. 1999; 112: 2993-2002.
2. William L, Bradsha W. Technical information. I Bulletin. 2004; 43(6).
3. Al-Mayale HHM. The evaluation and using of some of immunological tests in epidemiological study of leishmaniasis in Al-Qadisia province. Ph.D. Thesis, Al-Qadisiya Univ. Coll. Edu. 2004.
4. Goyal RK, Mohapatra TM. Superiority of DAT over ELISA as a diagnostic and seroepidemiology tool for the diagnosis of India Kala-azar. India J. Med. Microbiol. 2004; 22(1):57-60.
5. Chulay JD, Bryceson AD. Quantitation of amastigotes of Leishmania donovani in smears of splenic aspirates from patients with visceral leishmaniasis. Am. J. Trop. Med. Hyg. 1993; 32 (3): 475-479.
6. Fleiss JL. Statistical methods for rates proportion, 2nd ed.,.John Wiley & Sons. New york. 1981; 212-236.
7. Davidson RN. Science and clinical practice: visceral leishmaniasis in clinical practice. J. Infect. 1999; 39: 112-116.
8. Shyam S.Diagnostic of Kala-azar. An important stride. JAPI. 2003; 51: 753-755.
9. Tavares CAP., Fernandes AP, Melo MN. Molecular diagnosis of leishmaniasis. Expert. Rev. Mol. Diagn. 2003; 3(5): 657- 667.
10. Singh S, Ayan D, Sivakumar R. Application of molecular methods for Leishmania controle. Expert. Rev. Mol. Diag. 2005; 5(2):251-255.
11. Singh S, Sivakumar, R. Recent advances in the diagnosis of leishmaniasis. J. Postgraduate Medicine. 2003; 49 (1): 55-60.
12. Boelaert M, Safi EI, Jacquet S, Muynck D, Van der A, Stuyft P, et al. Operational validation of the direct agglutination test for diagnosis of visceral leishmaniasis. Am. J. Trop. Med. Hyg. 1999; 60 (1): 129-134.
13. Bern CS, Jha N, Joshi AB, Thakur GD, Bista MB. Use of recombinant k39 dipstick test and the direct agglutination test in a setting endemic for visceral leishmaniasis in Nepal. Am. J. Trop. Med. Hyg. 2000; 63: 153-157.
14. Jelinek T, Eichenlaub S, Loscher T. Sensitivity and specificity of a rapid immunochromatographic test for diagnosis of visceral leishmaniasis. Eur. J. Clin. Microbiol. Infect. Dis. 1999; 18 (9): 669-670.
15. Burns JM, Shreffler WG, Benson DR, Ghalib HW, Badaro R, Reed SG. Molecular characterization of a kinesin-related antigen of Leishmania chagasi that detects specific antibody in African and American visceral leishmaniasis. Proc. Natl. Acad. Sci. USA. 1993; 90: 775-779.
16. Delgado OM, Feliciangeli D, Coraspe V, Silva S, Perez A, Arias J. Value of a dipstick based on recombinant rk39 antigen for differential diagnosis of American visceral leishmaniasis from other sympatric endemic disease in Venezuela parasite. 2001; 8: 355-357.
17. Zijlstra EE, Nur Y, Desjeux P, Khalil EA, El-Hassan AM, Groen J. Diagnosing visceral leishmaniasis with the recombinant K39 strip test: experience from the Su. Trop. Med. Int. Health. 2001; 6 (2): 108–113.
18. Gholam HE, Shokoofeh S, Mehdi M, Homa H, Setareh M, Philippe D. Evaluation of rapid dipstick

Rk39 test in diagnosis and serological survey of VL in human and dogs in Iran. Arch. Iranian Med. 2003; 6(1): 29-31.
19. Sundar S, Rai M. Laboratory diagnosis of visceral leishmaniasis. Clin. Diagn. Lab. Immunol. 2002; 9 (5): 951–958.
20. World Health Organization. (2000). Division of control of tropical diseases. Leishmaniasis control home page (updated 2000).
21. Vermal N, Dash S. Reappraisal of underlying pathology in adult patients presenting with pancytopenia. Trop. Geog. Med. 1992; 44: 322-327.
22. Tilak N, Jain R. Pancytopenia. A clinical haematological analysis of 77 cases. Indian J. Pathol. Microbiol. 1999; 42 (4): 399-404.
23. Carillo J, Chinchilla M, Valverde B, Porras O, Mora L. Visceral leishmaniasis in Costa Rica: first case report. Clin. Inf. Dis. 2003; 29(3): 678-679.
24. Jassim AM. A direct agglutination test for the diagnosis and sero-epidemiological survey of visceral leishmaniasis in Basrah Governorate, Iraq, Ph.D.Thesis.Col.Med. Univ. Al- Mustansiriyah. Iraq, 1998.
25. Al-Tofali RA. The epidemiology of Leishmania and its relation with the sand fly, psychodidae Najaf provens. M. Sc. Thesis, College of Science, Univ. Kufa, 2003.
26. Niazi AD. Study in epidemiology and sero-epidemiology of visceral leishmaniasis in Iraq. Ph.D.Thesis. London Univ. UK.1980.
27. Tarish HR. Survey study of cutaneous leishmaniasis in Najaf City. Kufa, Med. J. 2000; 3(1): 46-49.