Sero-Diagnosis of visceral leishmaniasis in pediatric patients in Mid-Euphrates area

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SUMMARY

This study was conducted in the mid euphrate region (Al-Qadisya, Najaf and Karbala provices) during the period between 1/June 2004 to 1/ May 2006.

Three types of laboratory tests were used in this study for the diagnosis of visceral leishmaniasis in suspected patients attending to the pediatric hospitals in these provices.

The study included 3 groups :

The first group included 150 child (the total patients) admitted to Al-Qadisya, Najaf and Karbala hospitals and the diagnosis made on serum samples using Dipstick and Elisa tests and the results indicated the sensitivity of these tests to be(71.4 % and 67.4 %), respectively.

The second group included 33 child suspected of having visceral leishmaniasis depending on the clinical features and 3 tests (Dipstick, Elisa, and Bone marrow) were used for the diagnosis and the results indicated that the sensitivity of these tests were 21/33 (63.6%), 27/33 (81.8%), and 17/27 (62.9 %), respectively.

The third group included 17 patients who proved to be infected with VL by bone marrow test and the sensitivity and specificity by dipstick, Elisa, tests were 15/17 (88.23%), 16/17 (94.1%) respectively.

This study proved that dipstick test was the simplest, cheapest, and rapid for the diagnosis of VL. It is a safe, highly sensitive (80.2%) and specific (67.4%) and can detect the disease early.

Concerning Elisa test, it was found to be sensitive and specific (94.1% and 100%) for diagnosis but there are some difficulties that may limit its use for the time being.

It found in this study that all children are susceptible for the disease but those who are 13-24 month old are the most susceptible age was proved by all applied tests,

The present study found also that both sexes are susceptible, but the highest incidence of infection found among males (59.3% and 40.7%) among females respectively.

The present study proved that, Visceral leishmaniasis found to be more prevalent in Rural than Urban populations (68.3% and 31.7%) respectively.

الخلاصــة:

أجريت هذه الدراسة في منطقة الفرات الأوسط في مستشفيات الولادة والأطفال في محافظات القادسية ،النجف وكربلاء لغرض تشخيص اللشمانيا الحشوية(Visceral leishmaniasis) لدى الأطفال الراقدين في هذه المستشفيات للمدة من 1-6-2004 إلى 1-5-2006.

لقد تم تقسيم المرضى إلى ثلاثة مجاميع اعتمادا على نوع الفحص المستخدم في التشخيص وهي:

شملت المجموعة الأولى من الدراسة على 150 طفلاً(العدد الكلي للمرضى) يتوقع إصابتهم سريريا بالمرض واستخدم في تشخيص المرض فحص Dipstick وElisa للكشف عن الأجسام المضادة للطفيلي المسبب للمرض في مصولهم حيث وجد إن حساسية الاختبارين كانت 67.9% و 71.4% على التوالي.

في حين شملت المجموعة الثانية من الدراسة على 33 طفلاً (يتوقع إصابتهم سريريا بالمرض) .استخدمت ثلاثة فحوصات ((Dipstick, Elisa, and Bone Marrow Tests للكشف عن الأجسام المضادة للطفيلي في مصولهم أو أيجاد الطفيلي نفسه و كانت حساسية وخصوصية الفحوص المستعملة 21 / 33 (63.6%) 24/ 33 (72.7%)، 17 / 27 (62.9%) على التوالي.

إما المجموعة الثالثة فقد شملت على 17 مريضاً مصابا باللشمانيا الحشوية اعتماداً على وجود الطفيلي في نموذج نقي العظم الأحمر(Parasitological confirmed cases by bone marrow test) والعلامات والأعراض السريرية للمرض، وكانت حساسية اختبارات Dipstick, Elisa, and PCR المستعملة في التشخيص مقارنةً بنتائج فحص نقي العظم الاحمر هي 15 / 17 (88.23%)، 16 / 17 (94.1%)، 15 / 17 (88.23%) على التوالي.

أظهرت نتائج الدراسة أن فحص Dipstick هو الأسهل والأسرع والأرخص في تشخيص المرض وأنه يمتاز بحساسية عالية للكشف عن المرض (88.23%) وخصوصية (67.4%)وله القدرة على اكتشاف المرض مبكرا وخلال دقائق

أكدت الدراسة أن فحص Elisa هو أيضا من الفحوصات ذات الحساسية والخصوصية العاليتين إلا أن وجود بعض السلبيات والصعوبات تجعل منه اقل استعمالاً في تشخيص المرض وخاصةً في الوقت الحاضر.

أثبتت الدراسة أن جميع المرضى وخاصةً الأطفال معرّضين للإصابة بالمرض وأن الفئة العمرية (2 years) هي الأكثر تعرضاً للإصابة بالمرض.

أكدت الدراسة إن كلا الجنسين قابل للإصابة بالمرض ولكن نسبة الإصابة في الذكور أعلى مما في الإناث .

أثبتت الدراسة أن مرض اللشمانيا الحشوية أكثر انتشاراً في المناطق الريفية (Rural areas) من المناطق الحضرية (Urban areas) (68.3 % ، 31.7 %) على التوالي.

INTRODUCTION

The leishmaniasis is a globally widespread group of diseases caused by obligatory, intracellular, haemoflagellate protozoan parasites of the genus *Leishmania* (family Trypanosomatidae). The disease manifests it self in a variety of clinical forms, ranging from the self-healing cutaneous lesions to the more serious, potentially fatal visceralizing form, and includes the metastasising muco-cutaneous form, and the post Kala-azar dermal leishmaniasis.

Visceral leishmaniasis occurs either in a typical zoonotic or an anthroponotic pattern, depending on the species involved. *L. donovani* depends on inter human transmission **(1)**.

Leishmaniasis forced It self upon medical attention as an increasingly

significant problem over the last decade. Because of its importance,

leishmaniasis is considered as one of the 6 diseases selected by **WHO** for its

special program for research and training in tropical diseases **(2)**.

It ranks only second to malaria among human protozoan diseases **(4).**

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. All patients were admitted to hospitals have presented with clinical manifestations such as fever, loss of the weight, anemia, splenomegally and hepatomegally. The mean of their age was range from 8-120 months, from both sexes and different residency. Careful history of each patient was taken as name of the patient, sex, age, residency, duration of the fever, enlarged spleen, enlarged liver, and others notes, a fixed in the questionnaire.

Methods

Three tests were used in this study for diagnosis of VL patients, two of them were serological non invasive tests (Dipstick strip test and Elisa test) to detecting the antileishmanial antibodies in the serum of the patients, and bone marrow (BM) test to recognize the stage of the parasite in the bone marrow aspiration.

Sampling

Four ml of blood was collected by vein puncture into two sterile test tubes, in one of them put 3 ml of blood and leave it for about 2-4 hours, then collect the upper layer (serum) in clean test tube and store at 20˚C until use.

BM Technique, According to (3):

Dipstick Strip Test Method (Inbios International, Seattle, USA)(4).

It was easy, simple, rapid, and cheap test was applied for detecting the antileishmanial antibodies on cellulose membrane pre-coated with recombinant (rk39) antigen cloning from *L. donovani* and *L. infantum* **(2)**.

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:

A 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*.

A faint line is considered a positive result. As a guide of interpretation, the red in the test region will vary depending on the concentration of antileishmania antibodies present. 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 K39).

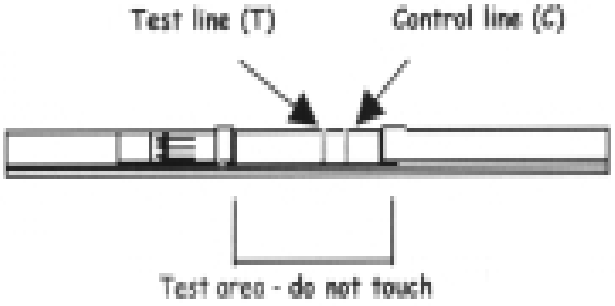

Figure (1): Positive results of dipstick strip test after added serum sample (both lines were visible).

A Negative Result:

The test is negative when only the control line appears. A negative result indicates that the Kala-azar detect dipstick did not detect antibodies of *L. donovani*. No test line is visible in Figure (2).

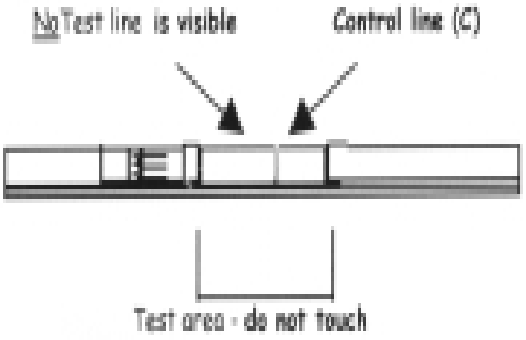

Figure (2): Negative results of dipstick strip test after added serum samples. (test line was invisible, while the control line was visible).

An 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 Kala-azar detect test for VL and fresh serum.

Enzyme-Linked Immunosorbent Assay (5):

The sensitivity: the probability that the assay will be positive when the infection is present.

The specificity: the probability that the assay will be negative when the infection is absent.

These can calculate by using the formulas:-

Sensitivity = TP (TP + FN) X1OO

Specificity = TN (TN + FP) X1OO

Where TP = true positive

TN = true negative

FP = false positive

FN = false negative

Statistical Analysis (5-6).

Results:

A total of 150 clinically suspected visceral leishmaniasis patients were entered to the 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 is the first line for diagnosis the disease by clinical diagnosis which 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 most of the patients were suffering from anemia and enlarged spleen .

The patients divided into three group according to test applied:

The First Group:

This group included all the clinically suspected patients (150) whom were diagnosed by Dipstick and Elisa tests for detection the antileishmanial antibody in their serum, 107 (71.4%) and 101

(67.4%) out of 150 examined patients were positive, respectively, and 43/150 (28.6%) and 49/150 (32.6%) were negative, respectively Table (1).

Table (1): The positive and negative cases of clinically suspected VL by dipstick and Elisa tests

Test	Positive		Negative		Total	
	No	%	No	%	No	%
Dipstick	107	71.4	43	28.6	150	100
Elisa	101	67.4	49	32.6	150	100

The Second Group:

This group included 33 out of 150 clinically suspected patients which were diagnosed by three tests, BM, dipstick strip and Elisa.

. **BM Test:**

This is the classical method used for detection the intracellular amastigote stage (L.D body) in the reticulo-endothelial system) by BM aspiration, from 27 out of 33 patients (6 patients refused the test), the positive and negative cases of this test were 17/27 (62.9 %) and 10/27 (39.1%) Table (2).

The Dipstick Strip Test:

The results of this test which was firstly used for the diagnosis of the disease in my country, showed high sensitivity and specificity of this test, 21 out of 33 clinically suspected VL patients, the percentage of positive cases were 21/33 (63.6%), and the percentage of negative cases were 12/33 (36.4%) Table (2).

The Enzyme-Linked Immunosorbent Assay Results:

The results of this test showed that, this test was highly sensitive than others tests, 27 out of 33(81.8%) patients were positive and 6 out of 33 (18.2%) patients were negative
Table (2).

Table (2): Positive and negative cases of 33 clinically suspected VL by diagnostic tests.

Test	Total No	Positive		Negative	
		No	%	No	%
Dipstick	33	21	63.6	12	36.4
Elisa	33	27	81.8	6	18.2
BM	27*	17	62.9	10	37.1

- **Six patients were rolled out of the study (rejected the test)**

The third group :

Dipstick results Versus BM Results:

From 17 parastologically positive patients by BM test, 15 patients were positive by dipstick test, so, the sensitivity, specificity, PPV, and NPV of dipstick test was (88.23 %, 70%, 83.33%, and 77.77%), respectively Table (3).

Table (3): Validity of dipstick test for diagnosis of VL was confirmed with results of BM

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		17	10	27

X2 =7 d f =1 $p < 0.05$

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 %

Elisa results versus B M results:

From 17 parasitologically positive patients were confirmed by BM test, 16 patients were positive by ELISA, so the sensitivity, specificity, PPV, and NPV of Elisa test was (94.1 %, 100%, 100%, and 90.90%), respectively Table (4).

Table (4): Validity of the enzyme linked immunosorbent assay test for diagnosis of VL was compared to parasitological confirmed results of bone marrow test.

Test		Bone marrow result		Total
		Positive	Negative	
Elisa result	Positive	16 True positive	0 False positive	16
	Negative	1 False negative	10 True negative	11
Total		17	10	27

X2 = 22.83 d f =1 , $p< 0.05$

Sensitivity = 16/17 x100 =94.1 %,

Specificity = 10/10 x 100 = 100 %,

Accuracy rate = (16+10) /27 x 100 = 96.2 %,

Predictive value for positive ELISA = 16/16 x 100 = 100 %

Predictive value for negative ELISA = 10/11 x 100 = 90.90 %

The results of the Study in relation to the Age of Patients:

This study revealed that the positive cases of VL patients proceed by dipstick test and Elisa, tests showed that the highly susceptible age group was 13-24 years old but the percentage and numbers of the patients were differentiate according to the test was applied. The positive cases and percentage were diagnosed by dipstick, Elisa, tests were 53/107 (49.5 %), 52/101 (51.5%).Table (5-6).

Table (5): Dipstick test results of VL in relation to age of the patients

Age (year)	Dipstick result (n=150)					
	Positive patients		Negative patients		Total	
	No	%	No	%	No	%
>year	33	30.8	18	41.9	51	34
2 years	53	49.5	16	37.2	69	46
3 years	7	6.6	5	11.6	12	8
4 years	7	6.6	1	.3	8	5.3
< 5 years	7	6.5	3	7.0	10	6.7
Total	107	100	43	100	150	100

X2=4.2 d f =4 p <0.05

Table (6): Enzyme linked immunosorbent assay test results of VL in relation to age of the patients

Age(year)	Elisa result (n=150)					
	Positive patients		Negative patients		Total	
	No	%	No	%	No	%
> year	33	32.7	22	44.9	55	36.7
2 years	52	51.5	10	20.4	62	41.3
3 years	8	7.9	6	12.3	14	9.3
4 years	4	3.9	5	10.2	9	6.0
< 5 years	4	4.0	6	12.2	10	6.7
Total	101	100	49	100	150	100

X2 =15.08 d f = 4 p < 0.05

Duration of the Fever:

The present study was showed that, high numbers of positive cases were occurred after 20 days of infection and high percentage were recorded by, dipstick, ELISA, (81.3%, 74.3%,), respectively Tables(7-8).

Table(7) Dipstick test results of VL in relation to duration of fever

Duration of fever (day)	Dipstick test result (n=150)					
	Positive patients		Negative patients		Total	
	No.	%	No.	%	No.	%
7-10	2	1.9	22	51.2	24	16
11-20	18	16.8	20	46.5	38	25.3
Above 20	87	81.3	1	2.3	88	58.7
Total	107	100	43	100	150	100

X2 =89.44 d f = 2 P < 0.05

Table (8): Enzyme linked immunosorbent assay test results of VL in relation to duration of fever

Duration of fever (day)	ELISA result (n=33)					
	Positive patients		Negative patients		Total	
	No	%	No	%	No	%
7-10	10	9.9	9	18.4	19	12.7
11-20	16	15.8	20	40.8	36	24
Above 20	75	74.3	20	40.8	95	63.3
Total	101	100	49	100	150	100

X2 = 16.2 d f = 2 P < 0.05

Size of the Spleen:

The results proved that the grade of splenomegally did not correlate with all applied tests, and the majority of patients having splenomegally at grade (1-5cm) by all tests (68.2%, 74.3%,), respectively Tables (9-10),

Table(9) : Dipstick test results of VL in relation to the size of the spleen

Size of spleen (cm)	Dipstick result (n=150)					
	Positive patient		Negative patient		Total	
	No	%	No	%	No	%
1-5	73	68.2	22	51.2	95	63.3
6-10	26	24.3	17	39.5	43	28.7
Above 10	8	7.5	4	9.3	12	8
Total	107	100	43	100	150	100

X2 = 4.54 d f = 2 P < 0.05

Table (10): Enzyme linked immunoserbent assay test of VL in relation to the size of the spleen

Spleen size (cm)	Elisa result (n=150)					
	Positive patient		Negative patient		Total	
	No	%	No	%	No	%
1-5	75	74.3	25	51	100	66.7
6-10	20	19.8	15	30.6	35	23.3
Above 10	6	5.9	9	18.4	15	10
Total	101	100	49	100	150	100

X2 = 8.5 d f = 2 P < 0.05

The results of this Study in relation to the residency:

The present study showed and by all the applied tests that, the numbers and percentage of VL patients were high in the rural areas than the urban areas by dipstick, Elisa and BM tests 67/107 (62.7 %) , 68/101(67.3), and 15/17 (88.2%) of the rural areas and 40/107 (37.3%), 33/101 (32.7%), and 2/17 (11.8%) of the urban areas, respectively Table (11).

Table(11): Positive results of three diagnostic tests for diagnosis of VL related toresidency

Residency	Diagnostic test							
	Dipstick(n=107)		Elisa (n=101)		BM(n=17)		Total	
	No	%	No	%	No	%	No	%
Rural	67	62.7	68	67.3	15	88.2	150	73.2
Urban	40	37.3	33	32.7	2	11.8	75	36.8
Total	107	100	101	100	17	100	205	100

X2 =7.1 d f =3 P < 0.05

The results of this Study in relation to the Sex of Patients:

Table (12), showed the positive cases and percentage of the infection by dipstick strip and Elisa, tests were 63/107 (58.9%), 59/101 (58.4%) of the males and 44/107 (41.1 %), 42/101 (41.6 %), of females, respectively, and the results were high among the males than females, but non significant at level P>0.05 .

Table (12): Positive results of the three diagnostic tests in relation to sex of patients

Sex	Positive cases of VL by the diagnostic tests							
	Dipstick(n=107)		Elisa (n=101)		BM(n=17)		Total	
	No	%	No	%	No	%	No	%
Male	63	58.9	59	58.4	11	64.7	133	59.2
Female	44	41.1	42	41.6	6	35.3	92	40.8
Total	107	100	101	100	17	100	249	100

Discussion:

Visceral leishmaniasis, is a serious health hazard disease of tropical and subtropical countries, has plagued mankind since antiquity **(9).**

The results of dipstick strip test in this study was consistent with the report of **(10-11) .**

The sensitivity and specificity of dipstick test were (88.23% and 60%) in present study and this similar to the results of same test used by (10), and this may be because of the same causative agents (*L. donovani*) of the disease.

The sensitivity dipstick and Elisa tests were 71.4% and 67.4%, this results were congurlated with the results of **(12**) in India who found the sensitivity of dipstick test was range from 67% to 80% by using the same antigen (rk39), but the results of Elisa were high than my results and this may be due to the species of the *Leishmania* parasites or type of the antigen was applied.

The results of dipstick test in present study showed (88.23%) sensitive, (60%) specific and (78.94%) PPV, and these results similar to the results of the same test by **(13)** in Sudan who showed (92%) sensitive, (59%) specific, and (81%) positive predictive value (PPV), and this may be due to use the same antigen. The sensitivity of the dipstick test in present study were less than the results of several studies from Indian subcontinent by **(15-16)** who used the same test (was to be 100% sensitive).

The present study, showed that, the sensitivity of rk39-Elisa test was (94.3%) and this similar to the results obtained by **(10)** in India who used the same test (100%) for diagnosis of VL.

In Iran, **(11)**, found the sensitivity of dipstick test was (52.27%) for diagnosis of clinically suspected VL cases.

In Brazil, **(12)**, found that, the dipstick test was shown to have 61 to 75% specificity, and 72% to77% sensitivity, compared to 100% specificity for both Elisa and 71% to 88% sensitivity for Elisa.

The present study proved fact that, the Immunochromatographic test was sufficiently sensitive and quite specific, rapid, and noninvasive pluses dose not require much expertise or elaborate equipment and it can be used for diagnosis of visceral leishmaniasis in remote endemic areas and these results were completely similar with study of **(11)** in Iran.

The sensitivity and specificity of rk39-Elisa antigen recorded in the present study, 94.1% and 100%, respectively, these results were equivalent with the results of rk39-Elisa test to detect *Leishmania* infection in Brazil by **(12-13)**. The present study showed that the high percentage of infection with *L. donovani* recorded in the children, and these present results also similar with the

results reported by **(15)**, who proved that, the most affected age group was two years of old in Kenya.

The present study recorded that, the high percentage of *L. donovani* infection was occurred mostly in age group of 13-24 months old (49.5%), and low percentage of infection was occurred in age group above 49 months, and the results were showed, non significant differences between the diagnostic tests at level P>0.05. These results generally were congruenced with the results of many studies in different areas of Iraq **(16)**. The results of present study agreed with the results of **(17)**.

The present study proved that, the higher percentage of visceral leishmaniasis infection occurred in the rural areas (68.3%), while the lower percentage of infection in the urban areas (31.7%), and these results agreed with the results of previous studies in many areas of Iraq (16-17-18)**.**

The results of this study recorded that, the high percentage of infection with *L. donovani* occurred in the males (59.3%), while the low percentage of infection with this parasites occurred in the females (40.7%) and these results found similar to the results of many studies in different areas of Iraq (19-20).

REFERENCES:

1-DeBeer, P., Harith, A. E., Van Grootheest, M., and Winkler, A. (1990). **Outbreak of Kala-azar in the Sudan. Lancet. 335: 183-224.**

2-Chang, K. P., Fong, D., and Bray, R. S. (1985). **Biology of Leishmania and leishmaniasis. In: Chang, K. P. & Bray, R. S. (eds.). Vol. 1. Leishmaniasis. Elsev. Biomed. Press, Amsterdam, pp. 1- 30.**

3-William, L., and Bradsha, W. (2004). **Technical information. I Bulletin. Volume XXXXIII Number 6.**

4-World Health Organization. (2000). Leishmaniasis control. Division of control of tropical diseases. .

5- Quinnell, R. J., Courtenay, O., Davidson, S., Garcez, L., Lambson, B., Ramos, P., Shaw, J., Shaw, M. A., and Dye, C. (2001). Detection of Leishmania infantum by PCR, serology and immune response in a cohort study of Brazilian dogs. Parasitol53-261 [medline].

6-Fleiss, J. L. (1981). Statistical methods for rates proportion, 2nd ed., p.212-236. John Wiley & Sons. New york.

7-Galen, R. S., and Gambio, S. R. (1975). Senstivity, specificity, prevalence, and incidence. In Beyond. Normality: The predictive value and efficacy of medical diagnosis. 10-14.

8-Goyal, R. K., and Mohapatra, T. M. (2004). Superiority of DAT over ELISA as a diagnostic and seroepidemiology tool for the diagnosis of India Kala-azar. India J. Med. Microbiol. **22** (1): 57-60.

9-Sundar, S., and Rai, M. (2002). Laboratory diagnosis of visceral leishmaniasis. Clin. Diagn. Lab. Immunol. **9** (5): 951–958.

10-Gholam, H. E., Shokoofeh, S., Mehdi, M., Homa, H., Setareh, M., and Philippe, D. (2003). Evaluation of rapid dipstick Rk39 test in diagnosis and serological survey of VL in human and dogs in Iran. Arch. Iranian Med. **6** (1): 29-31.

11-Richard, R., Rupert, J. Q., Bruce, A., and Clive, R. D. (2002). Rapid detection of Leishmania donovani infection. In dogs: Comparative study using an immunochromatographic dipstick test, ELISA, and PCR. J. Clini. Microbiol. **40** (7): 2352-2356.

12-Zijlstra, E. E., Nur, Y., Desjeux, P., Khalil, E. A., El-Hassan, A. M., and Groen, J. (2001). Diagnosing visceral leishmaniasis with the recombinant K39 strip test: experience from the Su. Trop. Med. Int. Health. **6** (2): 108–113.

13-Hans, V., Koert, R., Jill, S., and Robert, D. (2003). Comparison of an rk39 dipstick rapid test with DAT and splenic aspiration for the diagnosis of KA in Sudan.Trop.Med.and Intre. Healt. **8** (2): 164-167.

14-Shyam, S. (2003). Diagnostic of Kala-azar. An important stride. JAPI. **51**: 753-755.

15-Singh, S., Ayan, D., Sivakumar, R. (2005). Application of molecular methods for Leishmania controle. Expert. Rev. Mol. Diag. 5 (2): 251-255.

16-Jassim, A. M. (1998). 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.

17-Al-Mayale, H. H. M. (2004). 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.

18-Al-Tofali, R. A. (2003).The epidemiology of Leishmania and its relation with the sand fly, psychodidae Najaf provens. M. Sc. Thesis, College of Science, Univ. Kufa.

19-Niazi, A. D. (1980). Study in epidemiology and sero-epidemiology of visceral leishmaniasis in Iraq. Ph.D.Thesis. London Univ. UK.

20-Tarish, H. R. (2000). Survey study of cutaneous leishmaniasis in Najaf City. Kufa, Med. J. **3** (1): 46-49.