

Haematological And Epidemiological Study Of Visceral Leishmaniasis In Pediatric Patients In Mid-Euphrate Area

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الخلاصة

أجريت هذه الدراسة في منطقة الفرات الأوسط في مستشفى فيات الولادة والأطفال في محافظة القادسية، النجف وكربلاء لغرض تشخيص اللشمانيا الحشوية Visceral leishmaniasis لدى الأطفال الراقدين في هذه المستشفيات لمدة من 1-6-2004 إلى 1-5-2006

شملت الدراسة عيادة 50 طفلاً لا يتجاوزون 15 عاماً بابتهاج المرض. استخدم فحص Dipstick و ELISA في عينات الدم المصابة للطفيل في مصلهم.

أثبتت الدراسة أن جميع المرضى وخاصة الأطفال معرضين للإصابة بالمرض وأن الفئة العمرية (13-24) شهراً هي الأكثر تعرضاً للإصابة بالمرض. أكدت الدراسة على وجود علاقة للعلامات السريرية للمرض (Clinical manifestation) مع نتائج ELISA و Dipstick. دخلت اكتشاف الأجسام المضادة للطفيل في مصل المريض، وإن أعلى نسبة للحالات الموجبة كانت بعد 20 يوماً من الإصابة. لوحظ خلال الدراسة الحالية وجود تغيرات في مكونات الدم Hematological changes الدم، النسبة المئوية لتراص خلايا الدم، عدد الكريات البيض والعديد التفريغيات. الدراسة إن كلا الجنسين قابل للإصابة بالمرض ولكن نسبة الإصابة أعلى في الذكور وأن المرض أكثر انتشاراً في المناطق الريفية.

Abstract

This study was conducted in the Mid-Euphrate region (Al-Qadisiya, Najaf and Karbala provinces) during the period from 1/June 2004 to 1/ May 2006.

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

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The study included 150 child suspected of having visceral leishmaniasis depending on the clinical features, haematological changes and 2 tests (Dipstick and ELISA) were used for the diagnosis the disease.

It was found that all children were susceptible for the disease but those who were 13-24 month old are the most susceptible age for all applied tests and the percentage of the positive cases according to the tests were used.

. The study was showed that all patients entered to the hospitals were suffered from increase of the fever.

All patients were admitted to hospitals have had mild splenomegally (1-5 cm) below the costal margin .

It was found that both sexes were infected, but the males were more susceptible and the disease was more prevalent in rural areas.

Introduction

Leishmaniasis forced itself 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 (1,2).. It ranks only second to malaria among human protozoan diseases(3). It is prevalent on four continents: Africa, Europe, Asia, Central and Latin America and is endemic in the tropical and subtropical regions of 88 countries(4).. Overall, it has been estimated that there are 12-13 million of cases of leishmaniasis world-wide, over 90% of cases are found in three regions: Sudan/Ethiopia/Kenya, India/Bangladesh/Nepal, and Brazil (5). with as many as 100 000 deaths every year(6). In the world, Figures of 1-1.5 million new cases of cutaneous leishmaniasis (CL), representing 50 to 75 percent of all new cases, and 500,000 cases of visceral leishmaniasis (VL) per year are likely and 350 million people are at risk(4)..This only represent the 'tip of the iceberg, since not all infected individuals develop a disease(7).

In Iraq visceral leishmaniasis became on at the common health problems. The main endemic foci are central regions, and big area particularly villages around Baghdad city. The annual number of reported cases are 1000 according to Kala-azar section of the Endemic Disease Institute. The total number of cases were reported 12038 cases between 1971 and 1989, about 90% from Baghdad region and central areas. During the last 10 years, there was a marked increased in visceral leishmaniasis cases in southern Governorates (Baghdad areas, Missan, Thi-qar, Al-Qadisya, and Mothana). It was estimated that 72.7% of all reported cases in 1996 were from these new endures face(26).

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 provinces, 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

Two tests were used in this study for diagnosis of VL patients (Dipstick strip test and ELISA test) to detect the antileishmanial antibodies in the serum of the patients.

Sampling

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

Dipstick Strip Test Method (Inbios International, Seattle,USA):As described by(9).**Test Procedure:**

- 1-The sera and chase buffer were allowed to reach room temperature prior to testing.
- 2- Remove the Kala-azar detect strip for VL from the foil punch.
- 3- Add 20 μ l 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 μ l) 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.

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).

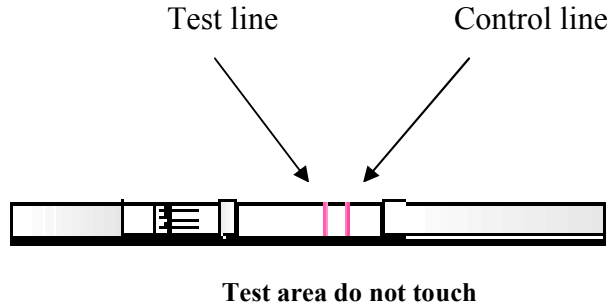


Figure (1): Positive results of dipstick strip test after added serum sample (both lines were visible).

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).

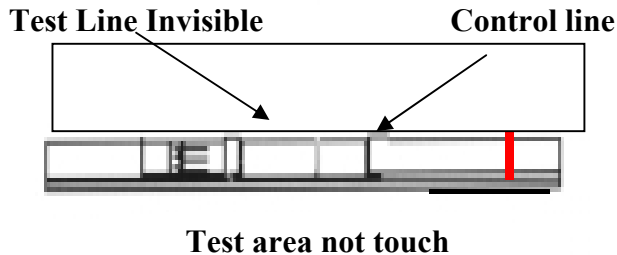


Figure (2): Negative results of dipstick strip test after added serum samples. (test line was invisible, while the control line was visible).

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.

ELISA test : Method as was described by (10).

Statistical Analysis: Chi –S test regarding (11).

Results

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 (Figure 3).



Figure (3): Child of 60 months of old showed enlarged spleen due to VL from the study area.

Hematological Finding

It was the second line for diagnosis of this disease in present study; by blood film for detection of the hematological changes such as Hb%, PCV%, WBCs count, and differential count. The results showed that, there were a decrease in Hb% level, PCV%, and decrease in the numbers of white blood cells (leucopenia) as general but increase the numbers of lymphocytes (lymphocytosis) and decrease the numbers of the neutrophils (neutropenia).

The results of this study showed highly significant changes by the applied tests used for diagnosis of the disease Tables (1,2).

Table (1): Correlation between different hematological variables with dipstick test.

Variable	No of patients	Mean	± Std. Error of Mean	P value
Positive Hb (g/dl)	107	7.9	.11	> 0.05
Negative	43	8.1	.16	
Positive PCV%	107	25.7	.35	> 0.05
Negative	43	25.9	.48	
Positive WBC /cu. mm	107	3910.9	16 .0	> 0.05
Negative	43	4084.5	133.2	
Positive Lymphocyte %	107	52.9	1.0	< 0.05
Negative	43	38.7	1.0	
Positive Neutrophil %	107	37.	1.0	< 0.05
Negative	43	53.8	1.4	

Table (2): Correlation between different hematological variables with ELISA test.

Variable	No of patients	Mean	± Std. error of mean	P value
Positive Hb (gm/dl)	101	7.92	.12	0.05 >
Negative	49	8.08	.13	
Positive PCV%	101	25.65	.37	0.05 >
Negative	49	25.94	430	
Positive WBCs/cu.mm	101	4042.67	200.2	0.05 >
Negative	49	3913.33	110.2	
Positive Lymphocyte %	101	49.96	1.35	0.05 <
Negative	49	44.84	1.23	
Positive Neutrophil %	101	40.77	1.47	0.05 <
Negative	49	46.19	1.47	

The results of the study in relation to the age of the Patients:

This study revealed that the positive cases of VL patients proceed by dipstick ,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 and ELISA, (Tables 3,4).

Table (3): Dipstick test results of VL in relate to age of the patients .

Age (month)	Dipstick result (n=150)			
	Positive patients		Negative patients	
	No	%	No	%
<12	33	30.8	18	41.9
13-24	53	49.5	16	37.2
25-36	7	6.6	5	11.6
37-48	7	6.6	1	.3
< 49	7	6.5	3	7.0
Total	107	100	43	100

p <0.05

Table (4): ELISA test results of VL in relate to age of the patients.

Age(month)	ELISA result (n=150)			
	Positive patients		Negative patients	
	No	%	No	%
< 12	33	32.7	22	44.9
13-24	52	51.5	10	20.4
25-36	8	7.9	6	12.3
37-48	4	3.9	5	10.2
< 49	4	4.0	6	12.2
Total	101	100	49	100

p < 0.05

The results of this study in relating to the residency:

The present study showed and by the applied tests that, the numbers and percentage of VL patients were high in the rural areas than the urban areas by dipstick, ELISA respectively Table (5).

Table (5): Positive results of two diagnostic tests for diagnosis of VL relate to residency.

Residency	Diagnostic test			
	Dipstick(n=107)		ELISA(n=101)	
Rural	No	%	No	%
	Urban	67	62.7	68
Total	40	37.3	33	32.7
Total	107	100	101	100

$P < 0.05$

The results of this study in relate to the sex of the patients:

Table (6), showed the positive cases and percentage of the infection by dipstick strip and ELISA tests.

Table (6): Positive results of the two diagnostic tests in relate to sex of patients.

Sex	Positive cases of VL by the diagnostic tests			
	Dipstick(n=107)		ELISA(n=101)	
	No	%	No	%
Male	63	58.9	59	58.4
Female	44	41.1	42	41.6
Total	107	100	101	100

$P < 0.05$

Discussion

Clinical manifestations and hematological changes:

The present study, showed that all 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 of (12,13).

In India, the symptoms and signs of VL vary between individuals and geographical distribution(14).

The present study observed that all suspected patients entered to hospital have splenomegally (at different grades, most of them under grade 1-5 cm), and this results similar to the results were reported by(15). and because of this dominant clinical feature, the disease was derived its name (infantile splenomegaly).

In present study, the common symptoms which appeared on the patients include fever, splenomegally, hepatomegally, weight loss, and anemia, and this symptoms were similar to the results in Sudan.(16).

Also (17) recorded that the common clinical signs of VL which seen on the most patients (children 15 months age), splenomegally, hepatomegally, and high undulating fever and the results of my study agreed with these results.

(18) recorded in their studies in India on 160 patients, all the confirmed cases of VL had persistent fever, splenomegally, anemia, and weight loss. The present results similar with these results, since the same parasite (*L. donovani*) was found in both the study areas.

The present study was recorded that, the hematological changes in VL patients which were showed decrease in percentage of the Hb%, PCV%, and the total leukocyte count (TLC), the most prominent hematological changes in the VL patients, these results agreed with the results of many studies in many areas in the world (19,20) in Europe, Bangladesh, and India, respectively.

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 also similar with the results reported (21).These results also generally were congruence with the results of many studies in different areas of Iraq (22,23,24).

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 (25,26). This may be due to many factors such as presence of the dogs (reservoir hosts, rodents, sand flies), which play role in transmission of the parasites to the human.

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 differences were non significant by all the applied tests. These results found similar to the results of many studied in different part of Iraq.(27,22,28).

The present study was showed that, splenomegally was the prominent physical finding in most patients and the most common size of the spleen at the beginning was (1-5 cm below the costal margin) and this similar to the results of (28,29,30). in Europe, Bangladesh, and India, respectively. So the diagnosis of VL is suggested by a history of the parasite exposure in endemic areas and by such clinical manifestation as fever, enlarged spleen, leucopenia, and anemia.

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