Visceral Leishmaniasis (Anew Approach in diagnosis)

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Abstract

This study is to investigate the Visceral Leishmaniasis among 85 suspected infants and young children admitted to Al-Khadhimiya Pediatric Hospital in Baghdad between January 1,2005 to August 31,2005 .For each patient, a medical history was obtained and a complete physical examination was performed by physicians . The serums of the suspected cases were primarily diagnosed by using a new ,simple diagnostic method based on detecting antibodies against the recombinant K 39 antigen by immunochromatographic format (Dipstick test). The positive cases were sent to the Central Health Laboratory in Baghdad to be confirmed by indirect fluorescent antibody test (IFAT)with special chart for notifying the patient's residency in details .

The incidence of infection was (12%), most patients were from males and the majority of cases were from Baghdad .The study also showed the investigators evaluation for performance of the test and its sensitivity and specificity in different endemic areas which proved that the test is a new, simple ,and specific reliable diagnostic method and could be used for the primary diagnosis of endemic foci in remote areas which have poor access to health services .

Introduction

Leishmania parasites are flagellated protozoa that infect mammalian macrophages and cause a variety of diseases ranging from self-healing cutaneous lesions to potentially lethal visceral leishmaniasis (VL).Each disease tends to be associated with a specific species of *Leishmania* (1).VL or kala -azar caused by *L.donovani* in the Indian subcontinent and eastern Africa, by*L. infantum* in the entire

Mediterranean area and the Middle East, and by *L.donovani chagasi* in Latin America ,although the latter two species have been shown to be the same by molecular techniques (2) .More than 47 countries are currently affected by leishmaniasis , with at least 200 million people at risk and approximately 100,000 new cases annually (3) . Visceral leishmaniasis accounts for 75.000 deaths annually(4).

Past research aimed at developing a cheap and reliable serologic test that could replace parasitology. El Harith and others developed a direct agglutination test (DAT) in the 1980s and proposed it as a test for field use (5). The DAT, in which stained parasites are agglutinated by serum antibodies, is popular in Iran and Africa (6)but variation between batches and the high cost of commercially available antigen are limiting factors . In the Indian subcontinent, but less so in Europe and Africa ,a rapid strip test is used to detect antibody to rK39 (a conserved antigen of *L.infantum*) and is both sensitive(67-100%)and specific (93-100%)(7-8).

Detection of leishmanial antigen in through a latex agglutination test (katex)seems to be promising for both diagnosis and prognosis (9). Techniques based on polymerase chain reaction are potentially highly sensitive and specific (10)but they need to be made more suitable for field use in terms of cost and user skills required.

The aim of the study is to report recent visceral leishmaniasis cases diagnosed by using a new diagnostic tool which is (Kalazar ^{TM}DetectRapid test) at Al-Khadhimiya pediatric Hospital .

Materials and Methods

1.**Patients:** Between January 1,2005 to August 31,2005 Serum from 85 children admitted to the Al-Khadhimiya Pediatric Hospital complaining from signs of VL .They were examined by specialist physicians and suspected to have VL and asked for laboratory new primary diagnostic tool of VL available in the hospital since the first of January 2005 , which is Kalazar ^{TM} Detect Rapid Test .

2.Kalazar Detect ^{TM} Rapid Test: This rapid assay is for the qualitative determination of antibodies to a recombinant antigen specific for visceral Leishmaniasis caused by parasite members of the *Leishmania donovani* complex (InBios International,Seattle ,WA, USA).It is a membrane based immunoassay for the detection of antibodies of visceral Leishmaniasis in human serum. At room temperature ,25 µL of serum was added to the test dipstick in the area

beneath the arrow (fig.1) which was then placed vertically in a test tube . Two drops of the chase buffer solution provided with the test dipstick kit were added to the test tube . Following the manufacture's instructions, a test result was positive when two bands, a control band and apositive test band , appeared within 10 minutes. The test result was negative if only the control band appeared . The test is qualitative and the manufacturer indicates that a faint band should be considered apositive result (11) (fig.2).

3. Demographic information was taken from the patient's parents including :age,sex,date of hospital entry as well as geographical information including residency govermorate ,sector and city.

Results

There were 85 infatile patients admitted to the Al- Khadhimiya Pediatric Hospital complaining from signs of VL. The numbers of the male patients were 57 which represents (67%), meanwhile the number of the female patients were 28 which represents (33%) as shown in (Fig.3). The patients were divided into three age groups . The first age group was (1 month-3 years) ,the second age group (>3 years - 6years), and the third age group (> 6 years – 9 years) as shown in (Table 1). Most cases admitted to the hospital and suspected to have VL were from Baghdad which represents (84%) as shown in (Table2). From 85 sera that were diagnosed by Kalazar Rapid Test that were shown in (Fig1-2) ,only 10 cases were positive which represents (12%) and 75 cases which were negative and represent (88%) .Most positive cases for VL were from Baghdad , they were six cases which represents (60%) .The details about positive cases and their residency are shown in (Table3) .All the dipstick positive cases sera were taken to the Central Health Laboratory in Baghdad to be confirmed with indirect fluorescent antibody test (IFAT).

Discussion

This is an epidemiological study depends on small community sample from infants and young children admitted to the Al-Khadhimiya Pediatric Hospital complaining from signs of VL. They were examined by specialist physicians and suspected to have VL. Sera were taken from suspected cases and diagnosed by a new, simple diagnosing tool called Kalazar Detect ™ Rapid Tes (fig.1 and 2).

In fig.(3) the 85suspected infantile patients that complaining from signs of VL which were represnts (76%) males and (33%) femals, there were no particular reason to this male to female propotion because the hospital admission depends on parents choice especillaly there is another two govermental pediatric hospitals in Baghdad which are the Central Child Hospital and AL-Mansour Hospital for children .

In our study the Children usually presented with intermittent fever , paleness, refusal to feed or anorexia , weight loss, and abdominal distension . lymph node enlargement which they are the most common findings in pediatric leishmaniasis and according to physicians they were primarly diagnosed as VL(12),the age group of the suspected patients were extends from (1month-3years) which represents (78%) as shown in table (1)our results are in agreement with (Herwaldt,1999)in which he reported that in areas with animal reservoirs , such as the Mediterranean Basin , visceral leishmaniasis mainly affects children of 1 to 4 years of age; it is caused mainly by L. infantum, transmitted by phlebotomine sandflies , and dogs are the most important reservoir (13).

In table (2) , the vast majority of suspected cases according to sings of leishmaniasis were from Baghdad which represents (84%) naturally this percentage was high due to that most patients were from Baghdad residence.

Table (3) showed that only 10 cases were positive which represents (12%) and 75 cases were negative and represent (88%) .Most positive cases for VL were from Baghdad ,they were six cases which represents (60%). Our results revealed of the actual percentage of patients with VL diagnosed by the new diagnosing tool in a small sample in one hospital over a retalively short period. To achieve a larger goal more studies are required to be sure of the acuurecy of the dipstick test , however serodiagnosis has been widely utilized for the diagnosis of VL in laboratories and there is a need for a simple , sensitive and specific diagnostic test for screening infected humans and reservoirs in the field, especially in remote areas with poor accessibility to health facilities . The rapid assay for the qualitative determination of antibodies to an antigen specific to (k39) caused by members of the *Leishmania donovani* complex is considered a new, effective test .The use of this recombinant antigen (rk39)as a rapid tool for controlling VL is not currently performed in the countries of the region including Iraq ; meanwhile in many different endemic area around the world had been used and evaluated as a accurate tool for

diagnosis as in a previous studies showed significant variation. Sensitivity of the rk39 antigen strip test recorded (100%) occurred in patients from India and Nepal (14-15) .Patients in Venezuela had significantly lower percentages of true positive test results (88%)(16), and the rk39 antigen strip test was least sensitive in patients from Sudan and other areas(17-18) .The sensitivity of the test among Brazilian patients in the most recent study was (90%)(19).

In the previous mentioned studies the dipstick test was proved to be simple, sensitive and specific diagnostic tool for screening infected humans and reservoirs in the field in many endemic areas around the world as shown in Table (4) .In our study the rk39 dipstick test used for primary diagnosis in infants and young children then the positive results confirmed by the IFAT test done in Central Health Laboratory in Baghdad , therefore ; we recommend the use of this test as a control tool in the field in different endemic areas in Iraq as well as a primary diagnostic test in the local hospitals and health care centers.

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Table(1) Distribution of the suspected patient according to age groups and their percentages.

No.	The age group	No. of patients	(%)
1	1 month – 3 years	66	78%
2	> 3 years – 6 years	14	16%
3	> 6 years – 9 years	5	6%
Total		85	100%

Table (2) Residency of suspected patients with VL admitted to Al-Khadhimiya pediatric hospital during 2005.

Governorate	No. of patients	%
Anbar	3	4%
Babylon	2	2%
Baghdad	71	84%
Basra	1	1%
Salah Al-Deen	6	7%
Thee Qar	1	1%
Wast	1	1%
Total	85	100%

Table(3) Demographic information about positive cases diagnosed by rK39 immuno-chromatographic dipstick test for diagnosing VL

	Patients No.	(%)	Mean of age years	Gender		Residency
				Male	Female	
	6	60%	2.3	4	2	Baghdad
	3	30%	1	3	-	Salah Al-Deen
	1	10%	2	-	1	Anbar
Total	10	100%	-	7	3	-

Table(4) Performance of the recombinant K39 (rK39) antigen in the diagnosis of visceral leishmaniasis (VL)

Region	Reference	No. Of subjects enrolled	VL	rK39 positive	Sensitivity	Specificity
Nepal	Bern et al 1993 [13]	348	127	127	100%	98%
India	Sundar et al 1998[14]	127	14	14	100%	100%
Venezuela	Sundar et al 2002[15]	117	41	36	88%	100%
Other	Delgado et al 2001[16]	96	14	10	71%	100%
Sudan	Bern et al 2000 [17]	116	55	37	67%	98%
Brazil	Carvalho et al 2003[18]	188	128	115	90%	100%

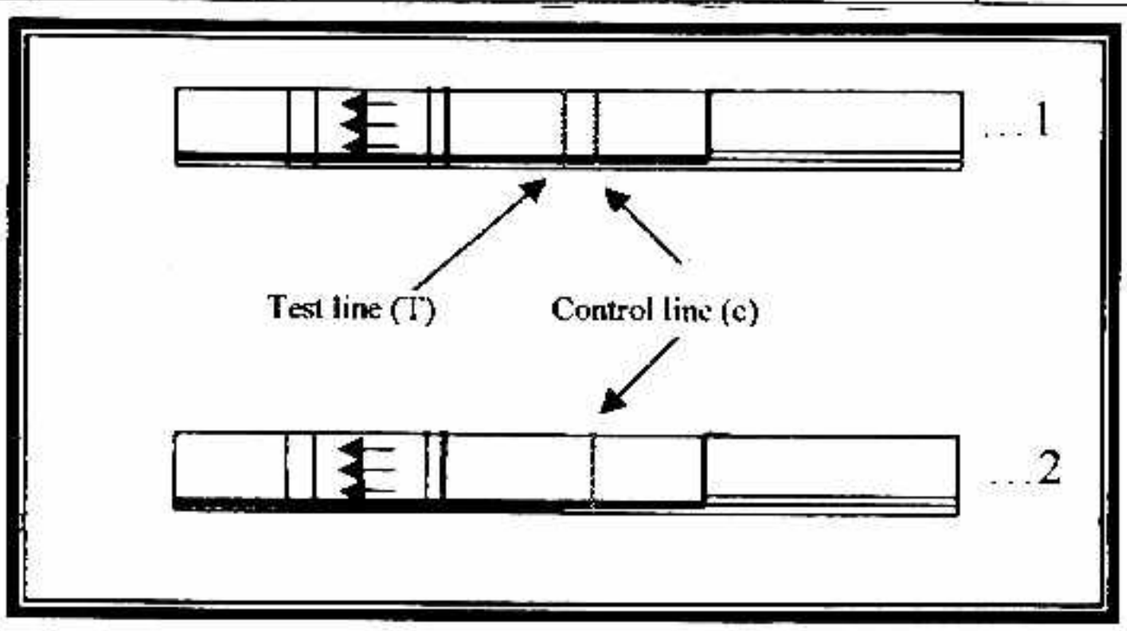

Fig. (1) A sketch represents Kalazar detect rapid test for diagnosing visceral leishmanasis.
...1 positive result
...2 negative result

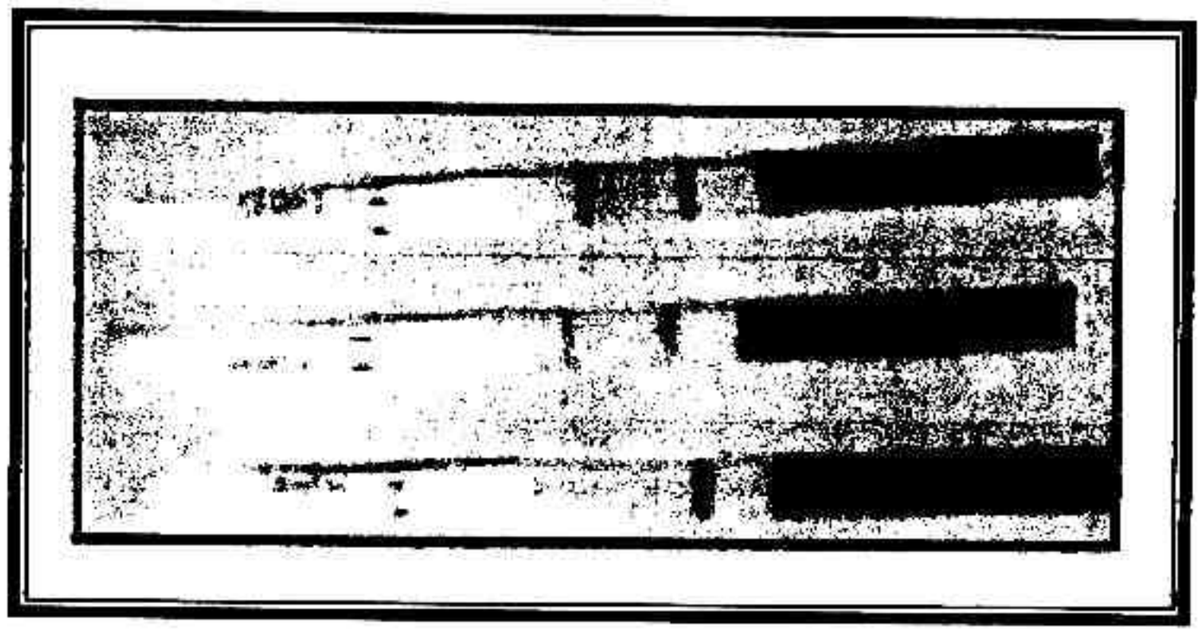

Fig. (2) Diagnostic results from three serum samples using the rapid rK39 immuno-chromatographic dipstick test for diagnosing visceral leishmaniasis. The single band represents a negative control, while the two results with a double band reflect positive diagnoses for patients with visceral leishmaniasis.

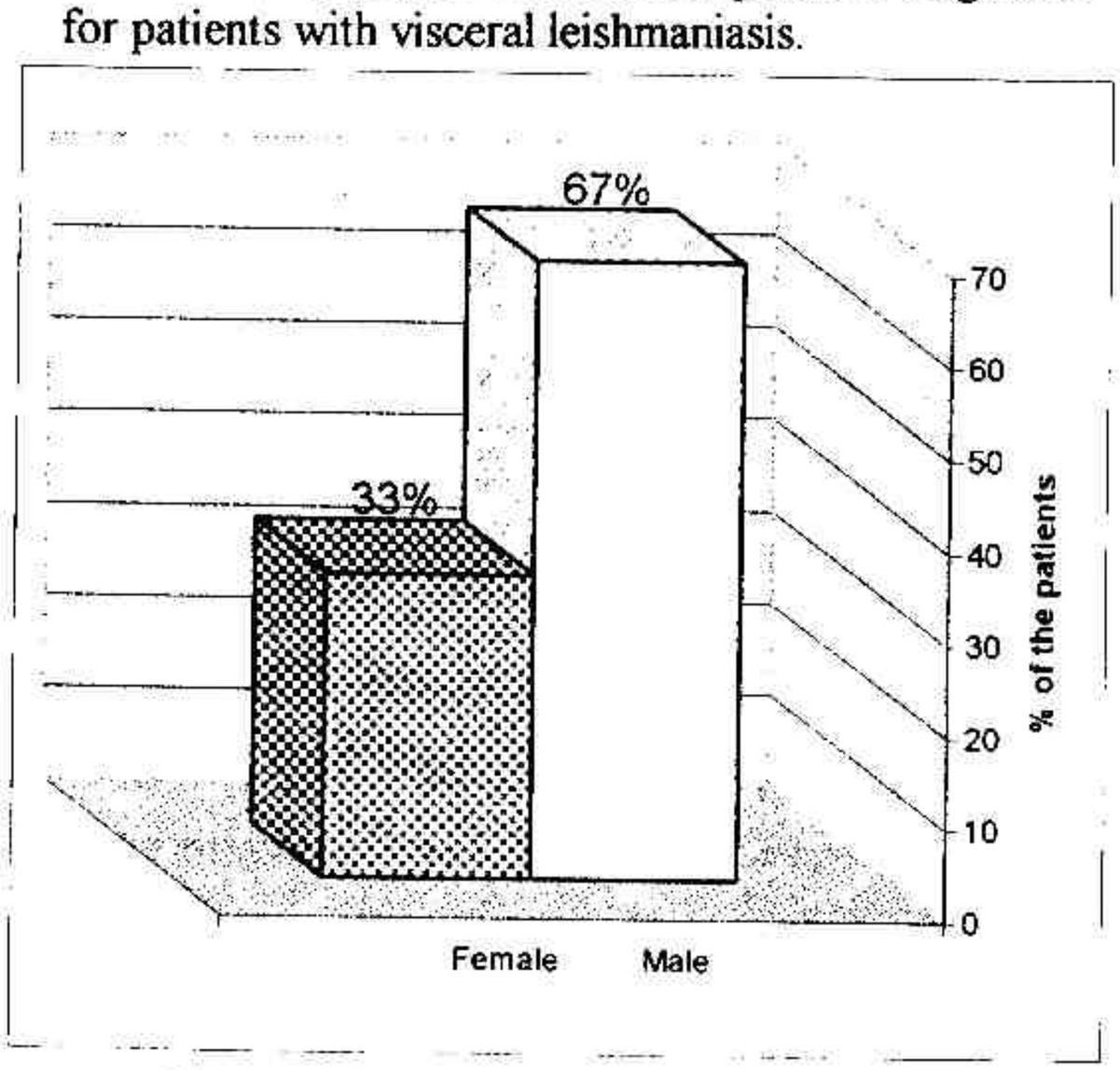

Fig. (3) Male to female proportion which suspected to have VL according to sings

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الخلاصة

أجري هذا البحث لغرض التحري عن مرض الحمى السوداء ضمن 85 حالة مشكوك باصابتها من الرضع والأطفال الصغارالذين أدخلو الى مستشفى الحميات للأطفال في الكاظمية ما بين الأول من كانون الثاني الى نهاية آب من عام2005 .تم اجراء الفحص الطبي وأخذ تأريخ الحالة لكل مريض من طباء متخصصين ، وأخذ مصل الحالات المشكوك باصابتها بالمرض وتشخيصها بوساطة اختبار جديد وسهل يعتمد على كشف الأجسام المضادة للجسم المستضد المسمى K 39 بصيغة التشخيص المناعي الصبغي . الحالات الموجبة للأختبار أرسلت الى مختبر الصحة المركزي في بغداد لغرض تأكيدها بوساطة اختبار الكشف غير المباشر عن الأجسام المناعية المصبوغة بالفلوريسنت مع ارسال كارت خاص يحوي مكان السكن بالتفصيل لغرض الأبلاغ .
كانت نسبة الأصابة المشخصة بهذا الاختبار (12%) وكانت أغلب الحالات من الذكور ومن سكان محافظة بغداد . كما يستعرض البحث الدراسات السابقة والمتعلقة بتقييم أداء هذا الأختبار من باحثين آخرين ومدى حساسيته وخصوصيته في مناطق موبوءة ومفادها أن هذا الأختبار الجديد سهل وله خصوصية ويمكن الأعتماد عليه كأداة تشخيصية للكشف عن البوءر المرضية الموجودة في المناطق البعيدة عن المراكز الصحية .