Clinical and Epidemiological Aspects of Visceral Leishmaniasis in Wasit Governorate

Qasim Dawood AL-Tammemi

Head of The Department of Pediatrics;College of Medicine;University of Wasit

المظاهر السريرية والوبائية لمرض اللشمانيا الاحشائية في محافظة واسط

قاسم داود التميمي/رئيس فرع طب الاطفال/كلية الطب – جامعة واسط

الخلاصة

اجريت هذه الدراسة التاملية على (864) مريضا ممن يعانون حمى طويلة الامد وادخلوا الى مستشفيات محافظة واسط وعلى مدى (36) شهرا للفترة من الاول من كانون الثاني 2006 لغاية الحادي والثلاثين من كانون الاول 800 لغاية الحادي الثلاثين من كانون الاول 800 لغاية الحادي والثلاثين من كانون الثاني 2006 لغاية الحادي والثلاثين من كانون الاول 800 لغاية الحادي والثلاثين من كانون الاول 800 مدى (30) شهرا للفترة من الاول من كانون الثاني 800 لغاية الحادي والثلاثين من كانون الاول 800 مدى (30 شهرا للفترة من الابتدائي لمرض اللشمانيا الاحشائية (الحمى السوداء) على التاريخ المرضي والعلامات السريرية وتم تاكيد التشخيص بالطرق المختبرية وهي صورة الدم الكاملة، اختبار شريط الارتشاف المناعي الملون السريع 800 مدى والعدم الابتدائي المناعي غير المباشر 150 مدى الابتدائي المرضي والعامات المناعي الملون السريع 100 مدى والتشخيص بالطرق المختبرية وهي صورة الدم الكاملة، اختبار شريط الارتشاف المناعي الملون السريع 100 مدى مدى والم 100 مدى والناي الاحشار التألق المناعي غير المباشر 160 مدى الابتدائي المرض اللابتدائي المناعي غير المباشر 160 مدى والناي الابترانية 100 مدى والعام المناعي المناعي غير المباشر 160 مدى الله الناية المناعي غير المباشر 160 مدى المباش 160 مدى المناي المباشر 160 مدى المباش 160 مدى والمباش 160 مدى و

في هذه الدراسة، تم اجراء الفحوص المختبرية السيرولوجية على (900) عينة دم اخذت من (864) مريضا يشتبه بهم سريريا كحالات لشمانيا احشائية و(36) مريضا اخرين لا توجد لديهم اعراض وعلامات لشمانيا احشائية وانما استخدموا لبيان درجة الخصوصية لهذه الاختبارات السيرولوجية. ثمانمائة وسبعة من المرضى المشتبه بهم كحالات لشمانيا احشائية ثبت مختبريا اصابتهم باللشمانيا الاحشائية بينما لم يثبت التشيص المختبري ل(57) عينة لمرضى يشتبه بهم سريريا كحالات لشمانيا احشائية ولكافة العينات ال(36) من المرضى الذين لا يشكون من اعراض وعلامات اللشمانيا الاحشائية. جميع المرضى الذين لم تثبت اصابتهم مختبريا تم استثناؤ هم من هذه الدراسة.

كانت نسبة الذكور (50.4%) والاناث (49.6%) مما يعني ان كلا الجنسين معرضان لخطر الاصابة بالتساوي معظم الحالات (52.41%) كانوا بعمر (1-2 سنة) واغلب الاصابات ظهرت في فصل الشتاء (46.96%) وتقريبا ربع عدد الاصابات في شهر شباط(فبراير) . اعلى نسبة للاصابات تم تسجيلها في قضاء الصويرة(46.47%) ثم العزيزية (35.56%) واقلها في قضاء بدره (20.5%).تبين بان معدل الاصابات في عام (2008) اقل بكثير مما كان عليه عام (2006) . معظم المرضى (48.08%) استغرقوا (6-2) اشهر بين الاصابة وظهور الصورة السريرية الكاملة لمرض اللشمانيا الاحشائية. العرض الرئيسي للمرض كان الحمى(100%) واهم علامة سريرية كانت تضخم الطحال(81.09%)، ووجد فقر الدم لدى (90.5%) من المرضى واحتاج (51.5%) منهم لنقل الدم وكان (60.5%) من المرضى تظهر عليهم علامات سوء التغذية الشديد . تبين بأن الفحص بواسطة اختبار شريط الارتشاف المناعي الملون السريع(rK39 Dipstick) هو الأفضل مقارنه بأختبار التألق المناعي غير المباشر (IFAT) للكشف عن اللشمانيا الأحشائية. بلغت نسبة الوفيات للمرضى المصابين باللشمانيا الاحشائية (4.83%) وكان مرض ذات الرئة وتسمم الدم الجرثومي ابرز الاسباب المؤدية للوفاة .

ABSTRACT

A prospective study was conducted on (864) patients with history of prolonged fever who were admitted in hospitals of Wasit governorate over (36) months in the period from 1st January 2006 to 31st December 2008. A presumptive diagnosis of visceral leishmaniasis (V.L.) was done depending on clinical examination which was confirmed by laboratory methods (Complete Blood Picture, rk39 Dipstick, IFAT). In this study (900) blood samples were tested serologically by using both rK39 Dipstick and IFAT ;(864) samples out of the total samples were diagnosed as having V.L. by classic clinical presentation, while (36) samples were without presumptive diagnosis of V.L. (used to clarify the specificity of rK39 Dipstick & .Eight hundred-seven samples from patients with suspected V.L. were IFAT) serologically positive ; the rest (57) samples and all the (36) samples from patients without suspicion of V.L. were negative. All seronegative patients were excluded from this study. In this study males constituted 50.4% and females constituted 49.6% of the total sample included, and the male: female ratio was 1.02:1. The majority of cases of V.L.(52.41%) were between the age of (1-2) years & the second major group of patients (32.72%) was < 1 year . Heavy infection appeared in Winter (46.96%) and decline in late Autumn (9.79%), with about one quarter of cases registered during February, and another peak in January. There was a decrease in the number of reported cases of V.L. in Wasit governorate in 2008 compared to 2006. The highest number of cases registered were from Al-Sowaira (46.47%) and Al-Azizia (35.56%) sub-districts ; with the least number of cases from Badra (0.25%).

The majority of patients (48.08%) took 2-6 months to present with the full blown picture of V.L. The main presenting symptom was fever which was seen in all cases (100%) and the main sign was splenomegaly which was detected in (81.9%) .A large number of patients presented with more than one sign and symptom. Pallor was apparent in 87.1% of patients with muscle wasting &malnutrition in 60.5% .Anemia was detected in 90.5% of patients which was severe to moderate in 60.5% and 416 of patients(51.5%) needed blood transfusion. Leucopenia was evident in the majority of patients (76.3%) and about 12% of patients developed severe thrombocytopenia & about half the patients with mild to moderate thrombocytopenia .

The sensitivity of the rK39 Dipstick test was 90.39%, and the specificity was 100% while the sensitivity of IFAT was 71.99%, and specificity was 100%.

Thirty-nine patients died in hospitals (4.83%); pneumonia and septicemia were the leading causes of mortality .

INTRODUTION

Although there are no available data on any recent epidemic outbreak of visceral leishmaniasis (V.L.) in Iraq, the disease is a public health threat in Iraq, especially following the Gulf War in 1990–91 and United Nations sanctions against Iraq that followed. According to the World Health Organization (WHO) [1], over 3000 cases per year were reported in Iraq following the War. The most important endemic areas before 1991 were central Iraq and the greater Baghdad area [1]. However, with the drainage of marshes in southern Iraq in 1996 and redistribution of water sources in Iraq, the majority of cases occur now in southern Iraq and the distribution of the disease has shifted south and across the country [1]. The public heath situation in Iraq further deteriorated after the invasion by Coalition Forces in March/April 2003.

Leishmaniasis, are a diverse group of diseases caused by the intracellular protozoan parasite of genus Leishmania, which is transmitted by Phlebotomine sand flies [2,3].

Visceral leishmaniasis or Kala-azar is a severe and often fatal infection of human caused by protozoal parasite of leishmania species (L.donovani Complex, L.chagasi, L. infantum). V.L. is transmitted from an animal reservoir to human by bite of a sand fly of the genus phlebotomus and it occurs in all continents except Australia[4]. V.L. is regarded as an endemic disease in Iraq since 1954[5].

WHO at 2002 [6] mentioned that there are 2.4 million new cases of Leishmaniasis occurring each year in 88 countries, with 367 million people at risk with 12 million people currently infected. The annual incidence of V.L. is estimated to be 500,000 cases per year worldwide [6].During the last few years, there was an increase in V.L. cases in some of the Middle and Southern Governorates of Iraq (Wasit, Thi-Qar, Mysan, Basrah and Al-Muthana) [3].

Most of the focuses of V.L located in area 100 Km around Baghdad mainly in AL-Sowaira ,Al-Yousifia, Al- Mahamodia, AL-Latifia, Salman pak , and Al-Azizia[7].Record of Center of Disease Control in Baghdad indicated that this disease is increasing especially in the last few years [8]. Most diagnoses are only genus-specific, being based on symptoms, the microscopic identification of parasites in Giemsa-stained smears of tissue or fluid, and serology [9,10].

AIMS OF THE STUDY

1. To study selected epidemiological aspects of visceral leishmaniasis in the districts of Wasit governorate and to evaluate the effectiveness of control measures .

2. To identify the blood pictures parameters of cases with V.L in Wasit governorate.

3. To evaluate the sensitivity and specificity of different serological tests [rK39 Dipstick (Kalazar Detect® Rapid Test) & IFAT] in the diagnosis of this disease.

Qasim Dawood AL-Tammemi

PATIENTS, MATERIALS AND METHODS

This study is a prospective study that was carried out over 36 months (from the first of January 2006 till the end of December 2008) on V.L. cases who were admitted to Wasit hospitals (Wasit governorate located in southeast of Baghdad with its districts, including about one million population, most of them live in rural areas) [11]

The following information was obtained for each child: age, sex, place of residence, date of admission and discharge, clinical features on admission and outcome of cases enrolled in the study. The total number of cases was 864 patients. The diagnosis was established on basis of history, clinical examination and laboratory investigations including [complete blood count (CBC) with blood film, serum bilirubin & liver function tests(LFT), total serum protein(TSP), widal & rose Bengal tests, infectious mononucleosis (I.M.) test, and other specific tests for selected cases]. The definitive diagnosis was confirmed by serological tests for kala-azar [rK39 Dipstick & immunoflourescent antibody test (IFAT)]. The Dipstick test rK39 and IFAT were done for the total number of admitted cases provisionally diagnosed as V.L.(864) on clinical grounds with another (36) patients (18 male & 18 female) without a presumptive diagnosis of V.L. and used to clarify the specificity of rK39 Dipstick & IFAT. The results were positive in 807 cases and confirmed to have V.L. (93.4%). The cases with negative Dipstick test rK39 & IFAT results were excluded from this study.Bone marrow aspirate was done for only (15) patients and it was positive for Leishmania Donovani bodies in (12) samples (80%) and it was not included in this study.

STATISTICAL ANALYSIS:

The results of the study were analyzed statistically by using descriptive statistics of frequency and percentage and evaluating the validity of the tests by using the Chi-square test and P-value.

RESULTS AND DISCUSSION:

In this study males constituted 50.4% and females constituted 49.6% of the total sample included, and the male: female ratio was 1.02:1.(**Table 1**.). This may indicate that both males and females are equally exposed to the risk of transmission of the disease. These results are consistent with other studies done in Iraq(Sukkar F1978 [12] Al-Alousi1980[13], and Al-twejri2004 [14]) who found equal percentage of infection which is similar to what we found .While Muhammad Uzair et al [15], and Rahim F. et al[16] found that only 40% of patients with V.L. were males. Kidvar & Moslehi[17] found that 70% of patients

were males mainly from rural areas. There is no real explanation for the increase of one sex on another and they attributed that for the highest exposure of male children for the vector & for the type of clothes of rural female children with more covering of the body & protection from the insect bite.

2007,2008							
Year	Male	(%)	Female	(%)	Total		
2006	198	47.25	221	52.75	419		
2007	120	52.17	110	47.83	230		
2008	89	56.33	69	43.67	158		
Total	407	50.4%	400	49.6%	807		

Table(1). No. of	f cases of V.L.	by gender in W	asit g	governorate for the	years 2006 ,
		2007 200	00		

*Chi-square test = 4.169; P- value : 0.124 (no significant difference)

The age has adverse effect with infection in children. It was found that the majority of cases of V.L.(52.41%) were between the age of 1-2 years & the second major group of patients (32.72%) was < 1 year(**Table.2**) ; this suggests that younger children are at higher risk probably because of low immunity. The increase of infection among 1- < 2 years age group may be due to the movement and activity of children leading to possibility to contact with contaminated environment beside their immune system is not well developed while in > 4 years is well developed in addition to the continuous exposure with low level of parasite so the infection decreases.

Our results were compatible with the result of other studies carried out in Iraq; AL-Kassar 2005[18] found that the highest infection(54.25%) appeared between 1-2 years of age; AL-Miali 2004[19] found that the percentage of V.L. cases under 3 years old was 92.43% from the total sero- positive cases; and Kidvar & Moslehi[17] found that the percentage of V.L. between 1-2 years was 61%. While Al-twejri 2004 [14] found that infants under one year were more affected (65%) with V.L. and Pearson & Sousa 1990 [20] found the disease is more common in adolescents and adults in India & Africa.

Age (years)	2006		2007		2008		Total	
	No.	(%)	No.	(%)	No.	(%)	No.	(%)
< 1	142	(33.9)	73	(31.74)	49	(31.01)	264	(32.72)
1-<2	222	(53)	119	(51.74)	82	(51.9)	423	(52.41)
2- < 3	31	(7.39)	18	(7.83)	18	(11.4)	67	(8.3)
3 - < 4	15	(3.57)	5	(2.17)	3	(1.9)	23	(2.85)
4 - 5	4	(0.95)	4	(1.74)	5	(3.16)	13	(1.61)
> 5	5	(1.19)	11	(4.78)	1	(0.63)	17	(2.11)
Total	419	100%	230	100%	158	100%	807	100%

Table(2). Distribution of the cases of V.L. according to age in Wasit governorate

The season of infection coincide with the growth and propagation of vector, in Wasit heavy infection appeared in Winter (46.96%) and decline in late Autumn (9.79%)(**Table. 3**). The monthly distribution of cases was variable from one month to the other (**Table. 4**) with about one quarter of cases registered during February, and another peak in January.

Season	2	2006		2007		2008		Total	
	No.	(%)	No.	(%)	No.	(%)	No.	%	
Winter	179	(42.72)	120	(52.18)	80	(50.63)	379	(46.96)	
Spring	131	(31.27)	70	(30.43)	56	(35.44)	257	(31.85)	
Summer	55	(13.13)	27	(11.74)	10	(6.33)	92	(11.4)	
Autumn	54	(12.88)	13	(5.65)	12	(7.6)	79	(9.79)	
Total	419	100%	230	100%	158	100%	807	100%	

Table(3). Seasonal distribution of V.L. in Wasit governorate

* Chi-square test = 17.809; P- value : 0.007 (there is a significant difference)

Month	2	.006	2	007	20	008	Т	otal
	No.	(%)	No.	(%)	No.	(%)	No.	(%)
January	58	13.84	63	27.4	25	15.82	146	18.1
February	111	26.5	44	19.13	42	26.58	197	24.41
March	51	12.17	38	16.52	17	10.76	106	13.14
April	34	8.11	21	9.13	16	10.13	71	8.8
May	46	10.98	11	4.78	23	14.56	80	9.91
June	18	4.3	11	4.78	4	2.53	33	4.09
July	14	3.34	7	3.04	3	1.9	24	2.97
August	23	5.49	9	3.91	3	1.9	35	4.33
September	14	3.34	7	3.04	4	2.53	25	3.1
October	17	4.06	5	2.18	4	2.53	26	3.22
November	23	5.49	1	0.44	4	2.53	28	3.47
December	10	2.38	13	5.65	13	8.23	36	4.46
Total	419	100%	230	100%	158	100%	807	100%

 Table(4). Monthly distribution of V.L. in Wasit governorate

The outbreak of infection in different parts of the world related to the prevail of favorable environmental condition for the sand flies, our results in accordance with observation of workers in Iraq especially in the Middle and South [18,21]. Khlabus [22] found one third of cases registered during November and December, and another peak in July and August ; Jassim1998 [23] found that the peak incidence of V.L in children appeared during January & February while it was very low or absent during Summer; Lewis &Peters 1977 [24] found that the density of sand fly is decreasing during Winter then reappeared and increased between March and October so that the fluctuation in the rate of the seasonal distribution of the disease is mainly due to the fluctuation in the density of the vector throughout the year; Sukkar [12] stated that the number of V.L patients continue to increase with the increased density of sand fly which is affected by the

level of water in the rivers & the rain fall which help the formation of organic materials that the sand fly feed on, and the mixing between reservoirs such as dogs and humans, and the presence of rodents in houses and near farms, increase the rate of occurrence of the disease; and the presence of high density of sand fly in a certain area especially those carrying the leishmania parasite to be an important factor for occurrence of the infection in different times of the year.

There was a decrease in the number of reported cases of Kala-azar in Wasit governorate in 2008 compared to 2006. This could be attributed to many factors like:

- Agriculture deterioration and lack of irrigation due to dryness & low water levels in rivers and tributaries with subsequent decrease in the populations of vector and reservoir.

- Improvement of the educational programmes that could be offered to the population and medical staff especially in rural areas with poorly constructed houses.

- Improvement of health and vector control facilities .

-Improved awareness of the doctors about the disease with early diagnosis of cases and availability of treatment.

The highest number of cases registered were from Al-Sowaira (46.47%) and Al-Azizia (35.56%) sub-districts (**Table 5**), these areas are mainly agricultural areas; with the least number of cases from Badra (0.25%). The difference of level of infections in all categories in sub-districts of Wasit may be due to that highly endemic areas may suffer more destruction of construction level, the ignorance of controlling and eradication of sand fly and rodents, behavior of people, type of residence and others. Strains difference may play a role, the latter needs further investigation.

The presence of domestic animals and/ or rodents had direct effects on the infection with V.L. **[18,25]**. The domestic animals played as a reservoir of the leishmania so as the rodents, the presence of the farmers and the negligence of eradication of the rodents led to the increase of infections, the same results had been observed in Thi-Qar and Babil [18,26]. While Ihsan M.[26] mentioned that in Wasit, the highest rate was recorded in Badra 65.3% followed by AL-Sowaira 51.1%, the lowest was in AL-Kut 16.6%.

District	2	2006		2007		2008		(%)
	No.	(%)	No.	(%)	No.	(%)		
Al-Sowaira	210	50.12	88	38.26	77	48.73	375	46.47
Al-Azizia	123	29.36	114	49.57	50	31.65	287	35.56
Al-Nuamania	42	10.02	16	6.96	19	12.02	77	9.54
Al-Hai	30	7.16	9	3.91	6	3.8	45	5.58
Al-kut	12	2.86	3	1.30	6	3.8	21	2.60

Table(5). Geographic distribution of V.L. in Wasit governorate

Badra	2	0.48	-	-	-	-	2	0.25
Total	419	100%	230	100%	158	100%	807	100%

The duration of illness before the first clinical presentation of patients was different from 2 weeks to more than 6 months (**Table.6**) and this explained the wide range of incubation period between the exposure to the insect bite and the development of the disease. A large group of patients (48.08%) took 2-6 months to present with the full blown picture of V.L., while another major group (44.86%) presented within less than 2 months. Positive family history of kala-azar was elicited in 118 patient(14.62%) and this was of help in reaching the diagnosis and it may confirm that the disease in highly endemic in Wasit governorate . Khlabus [**22**] found that family history of Kala-azar was positive in (18%) of cases ; 59.52% were presented within 2-5 months & 13.69% were presented after 5 months.So that the earlier presentation of patients from Wasit districts indicate the severity of cases which may be due to malnutrition and bad health care.

Duration	No. of cases	%
2 weeks - < 2 months	362	44.86
2-6 months	388	48.08
> 6 months	57	7.06
Total	807	100

Table (6). Distribution of cases of V.L. according to duration of illness before hospitalization

The clinical features associated with V.L. were different (**Table.7**). The main presenting symptom was fever which was seen in all cases (100%) and the main sign was splenomegaly which was detected in (81.9%), this is in agreement with other study in Thi-Qar and Babil [2,27]. A large number of patients presented with more than one sign and symptom. Pallor was apparent in 87.1% of patients with muscle wasting & malnutrition in 60.5% and oedema in 5.3% of patients which all indicate the severity of V.L. Signs of pneumonia (cough &/ dyspnea) developed in 37.5% which was a leading cause of morbidity & mortality among patients with V.L. Vomiting and/or diarrhea and bloody diarrhea complicated more than 17% of cases of V.L. Jaundice was seen in 39 patients (4.8%) and bleeding tendency in 5.7% of total number of cases; both are common findings in late presentation of the disease.

Symptoms and signs	No. of cases	%
Prolonged fever	807	100
Pallor	703	87.1
Splenomegaly	661	81.9
Hepatomegaly	565	70
Lymphadenopathy	46	5.7
Jaundice	39	4.8
Generalized Oedema	43	5.3
Ascites	4	0.5
Bleeding tendency	46	5.7
Convulsions	16	2
Vomiting and/or diarrhea	107	13.4
Bloody diarrhea	30	3.7
Cough and/or dyspnea	303	37.5
Failure to thrive	114	14.1
Muscle wasting	488	60.5
&malnutrition		

Table(7). Distribution of clinical symptoms and signs for cases of V.L. in Wasit governorate

Blood picture of examined samples from the patients with V.L. (Table 8) revealed decreasing of all blood parameters of the patients which were more severe in Hb, WBC and platelets counts, 90.5% of patients developed anemia which was severe to moderate in 60.5% and 416 of patients(51.5%) needed blood transfusion. Studies in some parts of the world confirmed the decrease in Hb and blood cells due to the effect of leishmania parasite [2,18,28]. The cause of anemia is a reflection of autoimmunity, macrophage engulf the red blood cell. Sen et al at 2000 [29] indicated that the destruction of R.B.C is due to precipitation of the complement (C3) and increase the permeability of the R.B.C wall to the Ca ions leading to its fragility and subsequently its destruction, beside the disturbances of blood producing organs (spleen and bone marrow) [30]. Leucopenia was evident in a large number of patients (76.3%) which might be due to bone marrow infiltration by the parasite, while leucocytosis was seen in 11.4% of patients which might be due to secondary bacterial infections for these immunocompromised patients .About 12% of patients developed severe thrombocytopenia & about half the patients with mild to moderate thrombocytopenia and this indicates how much the bone marrow is infiltrated by the parasite. Severe hypoproteinemia was elicited in 13.5% of cases and hyperbilirubinaemia with elevated liver enzymes in 6.8%; these findings were more than what was detected by Khlabus [22] which indicates the severity of cases in Wasit.

The technique used (rK39) Dipstick has several major advantages compared with IFAT in the field setting, the simplicity and ease of use , less cost, and rapidity of the rK39 dipstick are especially important in a setting such as rural areas in Iraq, where bone marrow can be performed by only a few expert practitioners, few or mostly no laboratories can perform IFAT, and travel to a referral center is difficult [**31**], and the disadvantage of the rK39 test is inability to differentiate between recent and old infection [**32**].

Results of lab. findings	No. of cases	%
Serological tests	864	
rK39 Dipstick test	781	90.39
IFAT	622	71.99
Hb% (gm/dl)	•	•
Severe anaemia (< 5)	88	10.9
Moderate anaemia (5-8)	400	49.6
Mild anaemia (>8)	242	30.0
Normal Hb.	77	9.5
WBCs count (cell/cu.mm)		
Leucopenia (< 4000)	616	76.3
Normal WBC count (4000-11000)	92	11.4
Leucocytosis (> 11000)	99	12.3
Platelets (cell/cu.mm)	•	•
Severe thrombocytopenia (<50000)	97	12.1
Mild to moderate thrombocytopenia (50000-	402	49.8
<150000)		
Normal platelets count	308	38.1
Total serum protein (g/l)		
Severe hypoproteinemia <42	109	13.5
Liver function tests		
Hyperbilirubinemia with elevated liver enzymes	55	6.8

Table (8). Distribution of cases of V.L. according to laboratory findings

In this study 900 samples were tested serologically by using both rK39 and IFAT ; 864 samples out of the total samples were diagnosed as having V.L. by classic clinical presentation, while 36 out of total samples were without presumptive diagnosis of V.L., The rK39 test revealed that 781 out of 864 with presumptive diagnosis of V.L. were positive and all cases 36 without presumptive diagnosis of V.L. were negative Therefore, the sensitivity of the test was 90.39% and the specificity was 100 %.

Serological testing by IFAT was performed to the same number of samples& revealed that 622 out of 864 with presumptive diagnosis of V.L. were positive and all cases 36 without presumptive diagnosis of V.L. were negative .Therefore, the sensitivity of the test was 71.99%, and specificity was 100%.

The results of rK39 and IFAT testing of serum samples from clinically suspected cases of V.L. are summarized in (**Table 9**); 596 samples were positive by both rK39 and IFAT testing; furthermore, rK39 test was positive for another 185 suspected, but these were negative by IFAT test and only 26 samples were tested positive by IFAT test and negative by rK39 test; 57 samples were tested negative by both tests. The rK39 test was more sensitive than IFAT test.

A comparison of our results with those of previous studies showed significant regional variation .Sensitivities of the rK39 antigen strip test range from 67% to 100 % [32,33]. The highest sensitivities 100% occurred in patients from India and Nepal, and the rK39 antigen strip test was least sensitive in patients from Sudan 67% [33,34]. The sensitivity revealed by this study 90.39% is more than that in Sudan and Venezuela and slightly lower than that in Brazil 95%.This regional variation may be due to the causes mentioned above [33,34]. The specificity revealed by this study is equal 100%; this is agreement with other study in patients from Nepal, Venezuela, and Brazil, while the specificity in Sudan and India is 98% [33,34].

 Table (9). Comparison between IFAT & rK39 Dipstick test using serum samples from suspected V.L. (864 children)

rK39	test	IFAT results			Total
results			Positive	Negative	
		Positive	596	185	781
		Negative	26	57	83
Total			622	242	864

*Chi-square test = 75.303 ; P- value : 0.001 (significant difference between IFAT and rK39 Dipstick test)

The average stay in hospital was 8-9 days. All patients were given pentostam for 30 days.Blood transfusion was needed in 416(51.5%) of patients & antibiotics in 482 (59.7%). The duration of response to pentostam was less than 7 days in 614 (76%) of cases and more than 7 days in 193(24 %)of cases. Thirty-nine patients died in hospitals (4.83%); pneumonia and septicemia were the leading causes of mortality (**Table10**). Mortality rate was higher than what was found by Khlabus [22]. which indicates the severity of cases in Wasit and the associated malnutrition ;it was nearly equal to mortality in Malaysia (4.88%) [35]. and less than that in India(8.81%) [36].

Table(10): Cause of death in patients	
Cause of death	No. (%)
Pneumonia (chest radiography)	18 (2.23)
Septicemia (blood culture)	11 (1.36)
Hepatic failure (liver enzymes&bilirubin)	3 (0.37)
Renal shutdown (renal function tests)	2 (0.25)
Gastro-enteritis with severe dehydration	4 (0.5)
Intracranial hemorrhage (C.T.scan)	1 (0.12)
Total	39 (4.83)

Table(10). Cause of death in patients with V.L.

The fifty-seven seronegative patients for V.L. out of the total number of patients 864 who were suspected on clinical grounds to have V.L.; were exluded from this study; but they were thoroughly investigated and diagnosed as detailed in(**table.11**).

Table(11).Differential diagnosis of seronegative patients suspected to have V.L.(All patients were below five years of age).

Diagnoses for non V.L cases (57 patients)	
Typhoid fever (blood culture)	22
Brucellosis (IFAT)	7
Infectious mononucleosis (serology)	8
Miliary TB (chest radiograph)	1
Acute leukaemia (bone marrow aspirate)	3
Histiocytosis X (bone marrow biopsy)	1
Congenital infection (TORCH serology)	4
Thalassaemia (Hb.electrophoresis)	2
Autoimmune hemolytic anaemia (Coomb's test)	1
Gausher disease(bone marrow aspirate & enzyme essay)	1
Galactosemia (enzyme essay)	1
Acute hepatitis A (serology)	6
Total	57

CONCLUSIONS AND RECOMMENDATIONS

From this study it can be concluded that

1. Visceral leishmaniasis is still endemic in Wasit , mainly in the rural and agricultural areas with the main age group affected was less than 3 years with no sex predominance for this disease which appeared mainly in Winter months . The

fatal outcome of the disease can be minimized by early supportive and specific treatment.

2. Serological tests (rK39 Dipstick &IFAT) proved effective in confirming the diagnosis of V.L. with high sensitivity & specificity especially for rK39 test .

3. Although there was a clear reduction in the prevalence of V.L. in Wasit from 2006 to 2008 ; there is still a great work to be done for eradication of the disease and reducing it s dangerous sequelae .

Thus we recommend that

1. Regular educational programmes should be carried out to the general population and medical staff about methods of prevention of this disease like periodic application of insecticides with residual action in order to control the vector sand fly, rodents control measures, stray dogs control measures and the use of protective clothes and use of insect repellents when exposure is unavoidable & distribution of bed nets to high-risk areas for V.L.

2. Doctors should suspect kala-azar in young children with prolonged fever, a comprehensive community health education campaign to create community awareness about V.L; and lectures on diagnosis and treatment of VL for primary health care personnel. Providing the specific & supportive treatment at the appropriate time to lessen the morbidity & mortality of the disease.

3. Encouraging the use of serological tests, especially rK39 Dipstick , because of simplicity and ease of use , less cost, and rapidity of the test are especially important in a setting such as rural areas in Iraq, where bone marrow can be performed by only a few expert practitioners, few or mostly no laboratories can perform IFAT, and travel to a referral center is difficult .

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