

Research Article**Seroepidemiological Studies of Visceral Leishmaniasis in Iraq**Abdulsadah A.Rahi^{1,2}, Magda A.Ali^{1,3}, Hossein Keshavarz Valian^{4,5}, Mehdi Mohebbali^{4,5}, Ali Khamesipour¹¹Center of Research and Training in Skin Diseases and Leprosy, Tehran University of Medical Sciences-International Campus, Tehran, Iran²Department of Microbiology, College of Medicine, Wasit University, Kut, Iraq³Department of Biology, College of Science, Wasit University, Kut, Iraq⁴Medical Parasitology and Mycology Department, School of Public Health and Institute of Public Health Research, Tehran University of Medical Sciences, Tehran, Iran,⁵Center for Research of Endemic Parasites of Iran (CREPI), Tehran University of Medical Sciences, Tehran, Iran***Corresponding author**

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Abstract: Visceral leishmaniasis (VL) is a vector borne disease caused by a protozoan of *Leishmania donovani* complex. After malaria VL is the second-largest parasitic killer in the world. A prospective study was done on patients with VL attending to Al-Karamah Teaching Hospital and Health Centers in different parts of Iraq, during the period from June 2012 to May 2013. The aims of this study are to assess the current situation of VL distribution and some of epidemiological aspects of the disease using immunochromatographic dipstick strip (rK39), Enzyme Linked Immunosorbent Assay (ELISA) and indirect fluorescent antibody test (IFAT) in some areas of Iraq. Peripheral blood samples were collected from 365 VL suspected patients before treatment initiation. Sera were separated and kept at -20°C until used. The diagnosis of VL was done based on clinical signs and symptoms and serological tests. The serum samples were first checked using rK39 dipstick and the positive samples were checked by ELISA and IFAT tests. The results of rK39 dipstick showed that 172 of 365 suspected patients were positive. The age of majority of the patients (69, 40.1 %) was less than one year and although not significant but the rate of rK39 dipstick positive was more in males (93, 54.1%) than females (79, 45.9%). The ELISA and IFA tests were used to check the serum samples of 172 rK39 positive patients and the results showed that 96 (85.4%) and 96 (81.25%) of the serum samples were positive by ELISA and IFAT, respectively. Eighty three (48 %) of the VL patients were resident of central parts of Iraq. The rare of VL patients were more in winter and the more common clinical manifestation was fever in all of the patients and splenomegaly was seen in 158 (91.9 %) of the patients.

Keywords: rK39, visceral leishmaniasis, serological tests**INTRODUCTION**

Leishmaniasis are complex parasitic diseases with diverse clinical manifestations, the diseases are reported from 98 countries with annual incidence of 1.5 to 2 million individuals mostly children [1]. Cutaneous Leishmaniasis (CL) and Visceral Leishmaniasis (VL) are mainly seen in 14 of the 22 countries of EMRO region [2]. Iraq is endemic to cutaneous leishmaniasis (CL) and visceral leishmaniasis (VL) [2].

Iraq is an old country with many ancient and pilgrimage cities and as such many people from different parts of the world visit Iraq throughout the year. The Iraq current situation requires presence of foreign people from all over the world and upon exposure of the travelers to the infected sand fly bites, the parasites might be transferred to the other parts of the world.

VL is the most severe form of leishmaniasis, VL is fatal if not treated; 500,000 cases of VL occurs each year, more than 90% of the VL cases reported from 5 countries; India, Bangladesh, Brazil, Nepal and

Sudan [3] and soon Iraq with 4,000-5,000 annual case will be added as 6th country to the above list [4]. A VL elimination initiative was launched in the WHO South-East Asia Region in 2005 with the aim to reduce the incidence rate below 1 per 10,000 per year at health district level [3]. In spite of long history of presence of the disease in the region and increasing numbers of reported cases, the information of VL incidence, endemic foci, clinical aspects and reservoir animals are not well known in Iraq.

Diagnosis of VL is based on clinical signs and symptoms and confirmation by serological tests and isolation of the *Leishmania* parasite. Different serological tests are used for diagnosis of VL including ELISA, direct agglutination test (DAT), indirect fluorescent antibody test (IFA) and molecular techniques PCR [5, 6]. Clinical signs and symptoms are prolonged fever, hepatosplenomegaly, substantial weight loss, progressive anemia, and even death [7]. The aims of the present study was to evaluate the rate of seropositive individuals among VL suspected cases in different parts of Iraq using rK39

(immunochromatographic) dipstick strip compared to ELISA and IFA for diagnosis of VL in different parts of Iraq.

MATERIALS AND METHODS

Blood samples were collected from suspected patients and healthy control referred to health centers from different parts of Iraq during June 2012 to April 2013.

Every suspected patient referred to a health center was interviewed and physically examined and any individual with clinical signs and symptoms was recruited.



Blood samples were collected from 365 suspected patients and 34 healthy controls and used for serodiagnosis. All samples were examined by rK39 dipstick (InBios International, USA). A 96 positive samples were confirmed by ELISA (Bordier Affinity Products, Switzerland) and IFAT tests (Biomerieux). Two ml blood sample was collected from each patient, sera was isolated and kept at -20 °C until use.

Diagnostic Methods

Three different methods of immunochromatographic strips (rK39) and ELISA were used to check the anti-

Leishmania antibodies in sera of VL suspected cases according to manufactures' instructions.

Statistical Analysis

Statistical analysis was done by using Minitab program and Excel application [8] including the followings:

- A) Chi-square (χ^2)
- B) Binomial test (Z)
- C) Evaluating the validity of the tests

Note: The comparison of significant (P-value) in any test were: S= Significant difference (P<0.05). HS= Highly Significant difference (P<0.01), NS= Non Significant difference (P>0.05).

RESULTS

The rate of positive anti-*Leishmania* antibodies are shown in table 1. As it is shown the highest rate was seen in ELISA method with 96 (85.4%) while the lowest rate was seen in 96 (72.9%) with using rK39 dipstick method.

Regarding gender differences, in the study areas, VL have been reported more frequently in males (93, 54.1%) more than females (79, 45.9% and high prevalence (18 %) in age group under one year old (Table 2).

Table 3 shows the geographical distribution of the studied Kala-azar cases. Eighty three (48%) VL patients were from central part and 6 (8%) patients from northern region of Iraq.

Regarding the seasonal distribution of the cases, the highest number of cases were recorded during January (32%) and February (28%) during study period of June 2012 to May 2013, Figure 1.

The most frequent sign & symptom was fever which present in all the patients, while the least frequent feature was abdominal pain which present in 53 (30.8%) of the patients (Table 4).

Table 1: The rate of positive anti-*Leishmania* antibodies using three different methods

Test	VL suspected cases Patients (n= 96)		Healthy individuals Control(n= 34)	
	No.	%	No.	%
ELISA				
Positive	82	85.4	0	0
Negative	14	14.6	34	100
IFAT				
Positive	78	81.25	0	0
Negative	18	18.75	34	100
rK39 dipstick				
Positive	70	72.9	0	0
Negative	26	27.1	34	100

Table 2: Prevalence of Kala-azar cases in according to age and gender

Age/Years	+Ve cases (Male)	%	+Ve cases (Female)	%
< 1	38	22.1	31	18.0
1-6	25	14.5	23	13.4
7-12	17	9.9	14	8.1
> 12	13	7.6	11	6.4
Total	93	54.1	79	45.9

Table 3: The geographical distribution of serological positive samples

Region	No. of Examined samples	VL cases positive	% VL cases positive
Middle(Baghdad, Wasit, Diyala, Najaf, Karbala, Babil, Qadisiya)	146	82	48
Southern (Basrah, Misan, Thiqar, Muthana)	130	55	32
Western (Al-Anbar)	36	20	12
Northern(Salah-Din, Ninewa, Kirkuk)	53	15	8
Total	365	172	100

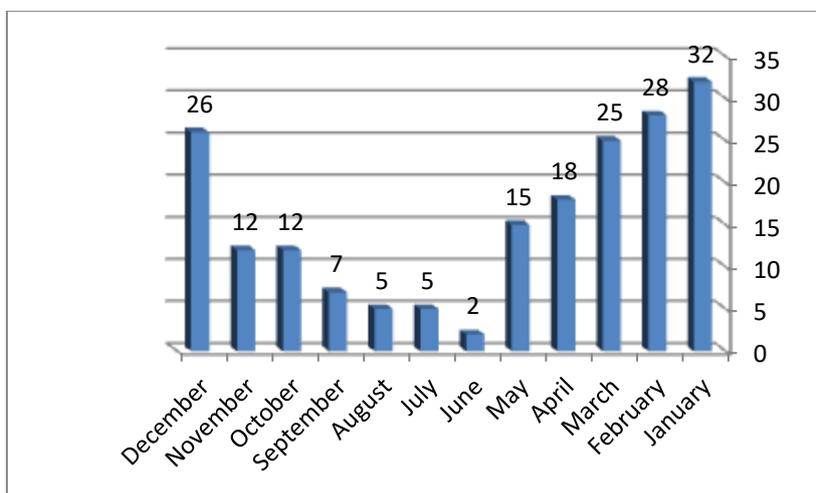


Fig. 1: Seasonal distributions of the Kala-azar cases

Table 4: Clinical signs and symptom of Kala-azar cases

Clinical features	No. of VL cases	%
Fever	172	100
Splenomegaly	156	91.9
Hepatomegaly	133	77.3
Weight loss	122	70.9
Anemia	103	60.0
Cough	98	57.0
Diarrhea	90	52.3
Jaundice	86	50.0
Leukopenia	78	45.3
Abdominal pain	53	30.8

DISCUSSION

Visceral leishmaniasis has been identified in Iraq for more than eight decades causing a serious public health problem with a high risk of morbidity, mortality and economical costs [14]. Diagnosis of VL is based on signs and symptoms, serological examinations and

parasitological approval [15], in Iraq diagnosis is done by clinical signs and symptoms and serological tests and parasitological approval is rarely performed [17]. In the present study, rK39 dipstick, ELISA and IFAT were used for diagnosis of VL and define status of the disease in different parts of Iraq.

Kala-azar is more often seen in population live in remote areas where infrastructure is poor even for diagnosis and parasitological approval is not possible. Serological tests such as ELISA require a laboratory and instrument but recombinant K39 (rK39) is easy to use in remote areas in field conditions [9].

Leishmaniasis is recognized as an important public health problem in some countries including Iraq owing to its considerable impact on morbidity, which impose a heavy burden on national health [4, 10-13]. The results of current study showed that about 2/3 of the patients were below 2 years age suggesting that small children are at higher risk. The results are compatible with the results of other studies completed in Iraq [13-16], and in Saudi Arabia and Yemen [17, 18]. The result are not compatible with the data reported from India and Africa, where the disease affect older children and adults [19, 20].

The rate of the disease although not significant is more seen in males than females which is similar to previous reports and most likely because of the more exposure to sand fly bites in males than females [21-26].

In Iraq it represent one of the serious public health problems and it is more in middle, south and west and lowest in north parts of the country. There are many factors that play important roles in the presence and distribution of VL in different parts of Iraq particularly middle regions, including the presence of animal reservoirs such as rodents, dogs, and the use of clay to build some of the houses in villages in these areas.

Furthermore, presence of agricultural areas that attracts and harbors many kinds of insects, and there population work long hours in the farms where they are more exposed to insects bites. Also, VL in our country may primarily affect families of farmers and nomads who are chiefly exposed to night biting sand flies. The presence of high gerbil population densities in the areas may be blamed as reservoir of infection that is supported by the crops for which the irrigation canals had been constructed. Furthermore, the canal embankments serve as densely populated and favored rodent and sand fly infestation areas [27].

The emergence of leishmaniasis in some foci may be the result of interruption of previously applied methods of control, like insecticide spraying on early diagnosis and treatment of positive cases. It is believed that reduction in insecticide spraying for malaria control contributes to the increase in the population of synanthropic sand flies and results of incidence of the disease in some endemic foci of VL and CL [4, 28].

Regarding the seasonal distribution of the VL cases were seen more in December (26 cases 15.1%) and in

January (32 cases 18.6%) and February (28 cases 16.3%). The incidence rate of infection then starts to decline from April and reaches its lowest in June (2 cases 1.1%), this is in accordance with other reports [13-15]. This variation in seasonal peak could be due to the existence of various dominant reservoir species in these areas as well as to the activity of the sand flies. The differences in monthly distribution of VL patients might also be related to the sand fly activities and requirement of blood meal.

In the patients, the most frequent sign and symptom was fever which predominant in all patients 100%, followed by hepatosplenomegaly, while the least frequent feature was abdominal pain which present in only 30.8%, which is in agreement with previous reports [21, 29].

CONCLUSION

VL is an endemic disease and public health threat in Iraq which needs especial attention.

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