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The Use of rK39 Test in the Diagnosis of Visceral Leishmaniasis in Wassit Province

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Abstract: *A total of 50 suspected visceral leishmaniasis (VL) cases were diagnosed during the period from October, 2014 to April, 2015 in Wassit province, Iraq. The diagnosis of VL was done based on clinical signs, symptoms and serological test. The results of rK39 dipstick showed that 31(62%) suspected patients were positive and only 19 (38%) were negative. The present investigation revealed that the highest number of patients 15 (48.3%) was in age group (less than one year). Male were highly infected 17(54.8%) than females 14(45%) in patient groups. The highest incidence of disease 26 (71%) was observed in rural areas, and the lowest incidence rate 5 (40 %) was in urban areas. The highest frequency of disease was reported in January, 9(90%), while the lowest cases were recorded in April 1(33.3%).*

Keywords: *rK39 Dipstick, visceral leishmaniasis, human.*

1. INTRODUCTION

Leishmaniasis is amajor vector–borne Meta zoonosis caused by obligate intramacrophage protozoae of the genus *Leishmania* which is characteristic by diversity and complexity [1]. There are more than 21 species have been identified to be pathogenic to human [2]. Leishmaniasis is agroup of diseases occur between human and animals. Some form of disease is anthroponotic, while other zoonotic. These diseases transmitted by the bites of female phlebotomine sand flies; therefore transmit *Leishmania* spp widely distributed in tropicaland subtropical regions through the world [3]. Its occur in four clinical type in human including cutaneous Leishmaniasis, mucocutaneous Leishmaniasis, visceral Leishmaniasis and post kala-azar dermal Leishmaniasis [4]. Visceral Leishmaniasis or "Kala-azar" is the most severe form of common parasitic disease. It affects 1.5-2 million new cases each year[5]and it caused by *Leishmania donovani* in old world and *Leishmania infantum* or *Leishmania chagasi* in the new world[6].

Visceral Leishmaniasis is known by different names such as kala-azar,black fever,dumdum fever,whichis characterized by prolonged fever, anemia, hepatosplenomegaly, and pancytopenia [7]. Iraq has been reported to be one of the endemic area of Kala- azar[8]. During the last few years, there was an increase of VL cases in some of the Middle and Southern Governorates of Iraq (Wassit, Thi-Qar, Mysan, Basrah and AL-Muthana)[9],usually detected in infant and children than other due to environmental risk which include presence of domestic animal and rodent. Poverty and malnutrition playimportant roles in increased morbidity of the disease [10, 11]. Identification of the infected *Leishmania* species based on clinical signs and symptoms, but the need for bone marrow or spleen aspirates makes these invasive techniques potentially un save [12].A recently developed nitrocellulose dipstick test that detects antibody to the recombinant amastigote antigen K39 (rK39) is highly sensitive and specific for the diagnosis of acute VL in clinical settings, which is identify by the serum of Kala-azar patients. It is relatively cheap and results are obtained with 10 minutes. This test is read with naked eye. The test has a sensitivity of 100 % and specificity of 98% [13,14].The present study aimed to estimate of rapid immune chromatographic strip test (IC) technique in diagnosis of kala-azar patients in Wassit province.

2. MATERIALS AND METHODS

2.1. Collection of Sample

This study was performed in AL-Zahra'a and AL-Karamah Teaching Hospital in Kut city from October 2014 to the end of April 2015.

A total of 50 cases of suspected visceral leishmaniasis were included in this study. These cases were confirmed as VL patients based on clinical symptoms and rk39 dipstick test. Patient's profiles including: patient full name, age, Sex, mother's and father's education, Residence (Rural or Urban) area and presence of domestic animals (mainly dogs) or not, obtained data from one of the parents.

2.2. Kalazar Detect™ Rapid Test

The Kala-zar Detect™ Test for Visceral Leishmaniasis (VL) is a rapid immune chromatographic strip assay for the qualitative detection of antibodies to members of L. donovani in human serum. The rK39 antigen-based dipstick test (InBios International, USA) was carried out according to the manufacturer's instruction. Briefly, 20 µL of serum was added to a test strip and two drops of chase buffer solution were added. The test result was read within 10 minutes, if the antibody is present, it will react with the conjugate (protein a colloidal gold) that is pre dried on the assay strip. The mixture moves along the strip by capillary action and react with rk39 antigen on the strip, yielding a pink band, in the strip of patients who are infected. In positive patients two pinkish lines appear in the middle of nitrocellulose membrane, a control band and a positive test band appeared within 5 minutes. The test was negative if only the control band appeared. The test is qualitative, and the manufacturer indicates that a faint test band should be considered positive. If no control band appeared, the dipstick was considered invalid and a new dipstick was used.

3. RESULTS

A fifty patients were detected for Visceral Leishmaniasis was confirmed in 31(62%) patients who were positive by rk39Dipstickusing serum of patients after they were isolated from the blood from the patients of VL, and 19 (38%) give negative result by rk39 test. Table 1 shown the prevalence of VL in according to the age. The higher infection 15/31(48.3%) appeared in age group (less than one year).

Table1. *Prevalence of VL by using rk39 Dipstick test according to age group*

Age groups / Year		Kala- azar Positive	Kala-azar Negative
1	G1 (< 1)	15	4
2	G2 = 1-2	12	4
3	G3 = 2-3	3	5
4	G4 = 3-5	1	6

The infection was detected in both sexes with a predominance in male than female 17/31 (54.8%) were appeared in table 2.

Table2. *Prevalence of VL by using rk39 test according to gender*

Age groups / Year			Gender		Total
			Male	Female	
1	< 1		10	5	15
2	1-2		4	8	12
3	2-3		2	1	3
4	4-5		1	0	1
	Total		17	14	31

The prevalence of the disease 26 (52%) in rural areas and 5(10%) in urban areas which shown in table 3.

Table3. *Prevalence of VL by using rk39 test according to age, gender and residence*

No	Age year	Gender	Residence		Total%
			Urban	Rural	
1	(< 1 year)	M	1	9	10(20%)
		F	1	4	5(10%)
2	(1-2)years	M	1	3	4(8%)
		F	1	7	8(16%)
3	(2-3)years	M	1	1	2(4%)
		F	0	1	1(2%)
4	(3-5)years	M	0	1	1(2%)
		F	0	0	0
	Total		5/50(10%)	26/50(52%)	31/50(62%)

M = Male, F= Female

The results of the present study indicated that VL cases have been concentrated in many districts of Wassit province particularly rural areas. The higher infection 12(24 %) appeared in center of Kut, while the lower infection 2(4 %) appeared in Badra and Al-Sowaira(table 4).

Table4. *Prevalence of VL by using rk39 test in according to district*

No.	District	Positive cases (%)	Negative cases (%)	Total (%)
1	Center of Kut	12(24%)	2(4%)	14(28%)
2	Al-Numania	9(18%)	2(4%)	11(22%)
3	AL-Sowaira	2(4%)	3(6%)	5(10%)
4	AL-Azizia	3(6%)	3(6%)	6(12%)
5	AL-Hai	3(6%)	5(10%)	8(16%)
6	Badra	2(4%)	4(8%)	6(12%)
	Total	31/50(62%)	19/50(38%)	50/50(100%)

Table 5 shows the number of cases was highest in January 9 (18 %) and February 8(16 %), while the lowest cases were recorded in April 1(2 %).

Table5. *Distribution of VL by using rk39 test according to months*

Month	**Positive cases (%)**	**Negative cases (%)**	**Total (%)**
October	2(4%)	2(4%)	4(8%)
November	3(6%)	4(8%)	7(14%)
December	5(10%)	4(8%)	9(18%)
January	9(18%)	1(2%)	10(20%)
February	8(16%)	1(2%)	9(18%)
March	3(6%)	5(10%)	8(16%)
April	1(2%)	2(4%)	3(6%)
Total	31/50(62%)	19/50(38%)	50/50 (100%)

4. DISCUSSION

Leishmaniasis is recognized as an important public health problem in some countries of the Eastern Mediterranean Region owing to its considerable impact on morbidity, which imposes a heavy burden on national health [15]. The diagnosis of VL is complex under the most favorable circumstances. Definitive diagnosis requires demonstration of parasites by smear or culture in tissue, usually spleen, bone marrow, or lymph node, and thus entails at least one invasive procedure [16]. These procedures are less than 100% sensitive, and there is no accepted "gold standard" for VL diagnosis, while rk39 strip test provides rapid results and is easy to perform even by paramedical staff in prevailing difficult field condition [17].However it has been successfully as a diagnostic guide in suspected cases [18], in a recent evaluation it has been found quite useful and reliable indicator of VL in India, it can also used to distinguish between relapse and re-infection in treated VL patient [19]. Strip test also have limitation, as with other serologic tests patient can have antibody present for month after cure of disease and also the tests can detect antibodies in the sera of asymptomatic patient [20], rK39 strip test is a very useful diagnostic modality in the field conditions in India and Nepal [21].The current study revealed that 31(62%) positive cases out of 50 by rk39 Dipstick. In a similar study done in Southern Europe, the strip test was positive in only 71.4% cases of VL [22]. Whereas for the Jordan Valley an average of 7.5 cases of VL per 10,000 per year was calculated [23]. Among cases of VL diagnosed clinically or parasitologically, more than 90% were positive by rk39, five of 6 VL cases with negative rK39 results in Nepal [24].In the present study the highest proportion of infection 15 (30%) was recorded in less one year age group, and the lowest 1 (2%) was in the five years age group, the increase of infection among 1- < 2 years age group may be due to the movement and activity of children leading to increase possible contact with contaminated environment beside their low immune system development[25],which is in agreement with previous reports in Iraq [26, 27], while Sakru [28]found that Nearly 83% of symptomatic infection were in adult people. There was a strong tendency for cases to be more prevalent, significantly more in male than in female, but there is no proper illustration for the excess of one sex on another. It may be due to behavioral and individual risk factors [29].On the contrary of other studies that found the higher incidence of infection among female than male [30 ,31]. The present study indicated that the higher incidence rate of infection in rural areas especially in center of Kut 15 (30%) and lower incidence recorded in Badraand Al-Sowaira 2(4%) which is dis agreement with Ihsan [32] mentioned that in Wassit , the highest rate was recorded in Badra (56.3%) , followed by AL-Sowaira (51.1%),the lowest was in ALkut (16.6%).The difference

of scale of infections in sub-districts of Wassit might be due to living conditions and the deterioration of the economic and social level of the population of the rural areas so that most of the homes are made of mud and abound in these areas, rivers and farmland, fields and livestock, as well as the presence of the disease carrier animals close to their home areas [33]. The higher infection of disease were recorded in January (18%), While Zainab in Basrah [34] found most of the cases were registered in July. The differences of monthly distribution of VL patients may be related to the development of female insects and their requires of blood during their life cycle for maturation and development of eggs [35].

5. CONCLUSION

The use of rk39 in present study will open the door for other researchers to use this test as a screening tool forVL on a wide range in endemic areas. rk39 is cheap, simple, not invasive, with a high sensitivity and specificity, and does not require specialized equipment.

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