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SEROLOGICAL STUDY OF VISCERAL LEISHMANIASIS IN WASSIT GOVERNOTATE

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ABSTRACT
A study was carried out on Patients suspected infection with Visceral Leishmaniasis (VL) of both sexes, in Wasit governorate during the period from 1st January to 31 March 2013. This study aimed to compare some epidemiological features of visceral Leishmaniasis in Wasit governorate, and to determine the prevalence of visceral Leishmaniasis. In addition to evaluate the sensitivity and specificity of rK39rK39 test for diagnosis. The study samples included 57 suspected positive VL patients and 30 suspected negative VL patients diagnosed according to clinical singes in addition to 10 healthy person considered control group. A presumptive diagnosis was done depending on clinical examination which was confirmed by laboratory methods (rk39 Dipstick test). The results revealed that the prevalence of Visceral Leishmaniasis in Al Hay District (91.6%) was higher than the prevalence in both districts Al Swera and Al Kut (66.6%). The female slightly suffered more than male so that the differences in prevalence were not significant. The younger children were more affected. The peaks of infection noticed on February 88.2% (in Winter). Most obvious symptom is haepatosplenomegaly (89.3%) of the cases; the lowest symptom was the jaundice. The rK39 is a rapid, sensitive and specific diagnostic tool for symptomatic cases of VL in remote areas with poor accessibility to health services. The technique of rK39 proved to be a better test to detect VL. The presence of domestic animals had positive effects on severity of infection. The severity of infections indicated the presence of different parasitic strains.

KEYWORDS: Serological study, Visceral Leishmaniasis, rK39.

INTRODUCTION

Visceral leishmaniasis (VL), caused by L. (L.) *donovani complex*, is also known as "Kala azar" or "Dum dum fever". VL is the most severe form of leishmaniasis, and the clinical symptoms include irregular bouts of fever, substantial weight loss, fatigue, anemia and substantial swelling of the liver and spleen (Bryceson, 1996). The disease is usually fatal if untreated, and is the second-largest parasitic killer in the world with an estimated 500,000 new cases and more than 50,000 deaths each year (Desjeu, 2004). Some 90% of VL cases occur in just five countries; Bangladesh, India, Nepal, Sudan and Brazil (Desjeux, 2004). Humans are considered to be an accidental host, although anthroponotic transmission without animal reservoirs is reported in some Leishmania species (Desjeux, 2001). In the old world, sand fly vectors belong to the genus Phlebotomus (*e.g*., *P. Papatasi* and *P. Sergenti* are the most important) while in the new world, it belongs to the genus *Lutzomyia* (Bryceson, 1998). In Iraq the responsible vector is *P. Papatasi*, which is widely spread in central and southern parts of Iraq (Abdul Rahim, 2004; Abulhab and AL-Hashimi, 2001). Sukkar *et al.*, (1985) found about 17 types of sand flies and phlebotomaspapatasi was the predominant type all over Iraq. The role of stray dogs and wild canines was presented by the sero-epidemiological study of VL in Basrah, Southern Iraq conducted by Gani *et al.* (2010). VL is caused by two leishmanial species, *L. donovani* or *L. infantum*, depending on the geographical area. *L. infantum* Infects mostly children and immunesuppressed individuals, whereas *L. donovani* infects all age groups.

MATERIALS & METHOD

The Wasit governorate located in southeast of Baghdad with its districts. Most of the peoples lived in rural areas (Abdul Rahim, 2004). The number of population is about 1,205,512 persons in the Al Kut which represents the center of the governorate and Located on Tigris River (Iraqi Ministry of Planning, 2010).

Study population sampling technique and sample size

The study was included all report cases as suspected to have VL that were admitted to Wasit governorate hospitals. All cases were tested serologically with rk39 test. These cases admitted to hospitals, from 1st of January to March 31, 2013. The following information was collected by questionnaire data sheets distributed to patient's parents or relatives: Name in full, Age in months, Sex, date of diagnosis by months, Patient situation after admitted (Healing or Death), Address in full (Governorate district, sub district, village/quarter, *etc*.), Life style (Rural or Urban) area, Presence of domestic animals (mainly dogs) or not, Presence of some clinical signs (haepato-spleenomegaly and Jaundice).

Study Group

Blood samples are collected and divided into three groups. The first group included 57 samples are suspected as positive cases diagnosed by classical clinical singes, the second group included 30 samples are suspected negative cases diagnosed by classical clinical singes, and the third

group included 10 healthy people considered as control groups. All samples were tested serologically by rK39, a presumptive diagnosis of VL which is often made on the basis of the classic clinical presentation of haepato spleenomegaly, fever, splenomegaly, hepatomegally, jaundice, pallor and hematological abnormalities in endemic areas as described by Jeronimo (2004). This test was considered as standard for diagnosis of visceral Leishmaniasis.

Laboratory test

The rK39 test (Kalazar Detect® Rapid Test): Blood samples were collected in special container without anticoagulant. Sera were separated and stored at -20°C until used. Sera were brought to room temperature prior to the testing (Sen *et al*., 2004 and Baballo *et al.*, 2001). The tests were carried out in all governorate laboratories by using the commercially available diagnostic kit (rK39 test).

Statistical Analysis

Statistical analysis was performed using SAS (Statistical Analysis System -version 9.1). Proportions were compared by chi-square test. $P < 0.05$ was considered statistically significant (Daniel, 1999).

RESULTS & DISCUSSION

Blood samples from Baghdad and Wasit governorates were examined using dipstick rK39, the positive result appear in highest rate was recorded91.6% in Al Hay district and respectively, 88.4% in Al Numania district, 71.4% in Al Azizyia district and lowest was in both districts Al Swera and Al Kut 66.6% (Table1),(Fig.1)

TABLE 1: Prevalence of VL in Wassit Governorate by using rk39 Dipstick according to Districts

Districts	No. of sera tested	Positive(%)
Al Kut	6	4(66.66%)
Al Hay	12	11(91.66%)
Al Swera	6	4(66.66%)
Al Azizyia	7	5(71.42%)
Al Numanyia	26	23(88.46%)
Chi square value	4.00	
P	0.40	

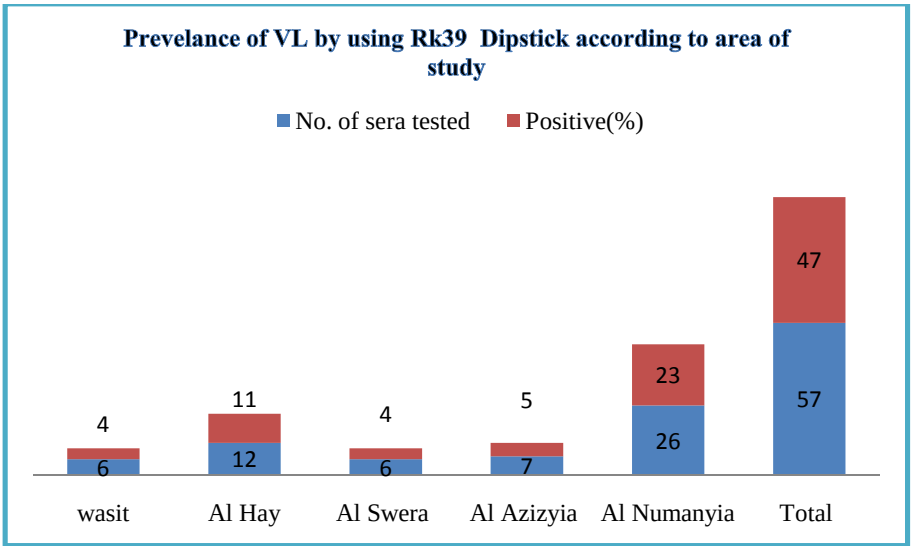

FIGURE 1: Prevalence of VL in Wassit Governorate by using rk39 Dipstick according to Districts

Leishmaniasis is widely spread in different parts of the world, Iraq regarded as an endemic place, especially in the middle and south parts (AL-Saqur *et al*., 2008), the main reasons, is due to adaptation of the vector sand fly in these areas. The registered infected cases Wasit governorate is high; this may be due to many reasons, the way and standard of living, the presence of reservoir and / or domestic animals and above all is the viability of infected sand fly (Khadim, 2005).

The distribution of V.L. according to gender in Wasit governorate showed nearly similarity of infection in male and female which was 82.1% and 82.7 % respectively (Table 2). There is no real explanation for the increase of one sex on another as some authors recorded an increase in male than female (AL-Taie, 2002 and AL-Kassar, 2005) while AL-Alousi *et al*. (1980) reported that there was no significant differences due to age. The age group has adverse effect with infection in children less than 1-3 years old it was 44/54 (81.4%) in less than during rat 3/3 (100%) in age group 4-6years old (Table 3). However, the difference was not significant. Al-Hamash, (2012) found about 2/3 of patients were below 2 years of age. 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 4-5 year is developed in addition to the continuous exposure with low level of parasite so the infection decreases(AL-Kassar, 2005).

The season of infection coincide with the growth and propagation of vector. In Wasit Governorate heavy infection appeared in winter (February, 88.2%) and decline in start of spring (March, 66.6%), (Table 4).

TABLE 2: Prevalence of VL in Wassit Governorate by using Rk39 Dipstick according to Gender

Gender	No. of Tested	Positive (%)
Male	28	23(82.14%)
Female	29	24(82.76%)
Chi square value	0.003	
P	0.95	

TABLE 3 :Prevalence of VL in Wassit Governorate by using Rk39 Dipstick according to Age group

Age Group	No. of Sera Tested	No. of human seropositive for rK39 (%)
<1-3 years	54	44(81.48%)
4-6 Years	3	3(100%)
Chi square value	0.67	
P	0.41	

TABLE 4 : Monthly Prevalence of VL IN Wassit Governorate by using Rk39 Dipstick according to Months

Month	No. of sera tested	Seropositive for Rk39 (%)
January	25	22(88.0%)
February	17	15(88.2%)
March	15	10(66.6%)
Chi square value	3.50	
P	0.17	

TABLE 5: Prevalence of VL IN Wassit Governorate by using Rk39 Dipstick according to presence of important clinical singe symptom

clinical singes	No. of Sera tested	Seropositive (%)
Haepatospleenomegaly	47	42(89.3%)
Jaundice	47	8(17.02%)
Chi square value	23.12	
P	<0.0001	

TABLE 6 : the sensitivity and specificity of rK39 dipstick and ELISA to diagnosos in three governorates

Assay	sensitivity		specificity		Control group
rk39	47/57	96%	2/30	74%	0/30

TABLE 7: Prevalence of VL by using rk39 Dipstick in Wassit province according to presence of Domestic animals

Area	No. of Sera tested	Seropositive for Rk39 (%)
Presence of Domestic animals	48	40(83.33%)
Absence of Domestic animals	9	7(77.77%)
Chi square value	0.16	
P	0.68	

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 previous results in Iraq especially in the Middle and South (AL-Kassar, 2005 and Abul-hab, 1988). Regarding the clinical features associated with V.L. in Wassit were different, between the haepatospleenomegaly in 89.3% and the cases of Jaundice 17.2%, this is in agreement with other study in Babil, Thi-Qar and Baghdad (Hussein, 2004; AL-Marzoki, 2002 and AL-Saqur *et al.*, 2008)(Table 5).

The technique used (rK39) dipstick has several major advantages 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(AL-Marzoki,2002), and the disadvantage of the rK39 test is inability to differentiate between recent and old infection (Neouimine,1996).

Therefore, the sensitivity of the test was 96%, specificity was 74 %. The results of rK39 testing of serum samples from clinically suspected cases of VL are summarized in (Table 6) 47 samples were positive by rK39 from 57 suspecting positive samples; and only two samples were tested positive by rK39 test from 30 suspected negative samples and no positive cases during 10 Healthy persons control group that diagnosed by rK39 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 % (Neouimine, 1996 and Bern, 2000).

The specificity revealed by this study in Baghdad and Wasit governorate 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% (Bern, 200; Sundar and Rai, 2002). The cost of the rK39 dipstick was approximately Us $ 5per test more cheaper as

compare with other costly tests , therefore the appropriate use of rK39 dipstick could help to ensure that the scarce health care resources of Iraq are used in the most effective manner. The presence of domestic animals(mainly Dogs) had direct effects on the infection with V.L (83.3%) in patients with VL while (77.7%) (Table 7) recorded in people that not contact with domestic animal this is in agreement with most of the workers in different parts of the world (AL-Kassar, 2005 and WHO, 2003). 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 especially in Wasit led to the increase of infections, the same results had been observed in Babil and Thi-Qar (AL-Kassar, 2005 and AL-Maamori, 2004). The difference of level of infections in all categories in Wassit districts may be due to Wassit could be suffered more destruction of construction level. The ignorance of controlling and eradication of sand fly and rodents, behavior of people, type of residence, migration movement, increase level of rainfall, non-completion of sewage projects and other factors. Also the strains difference may play a roll in increasing of infection.

In conclusion, rK39 could be used as a rapid, sensitive and specific diagnostic test for visceral leishmaniasis in symptomatic cases living in remote areas where there is poor accessibility to health services. The test is quick (results are obtained in 5–10 minutes) and no special equipment is needed. In disease endemic countries, VL can easily be misdiagnosed on a clinical basis, therefore, screening populations using rK39 test is recommended for early diagnosis and treatment of the disease, thereby reducing its morbidity and mortality.

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