Sensitivity and Specificity of Serological Tests (rK39 Dipstick & IFAT) in the Diagnosis of Kala-azar in Iraqi Children

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Abstract
A prospective study was conducted on (864) patients with history of prolonged fever who were admitted in hospitals of Wasit province; Iraq over (3) years in the period from 1st January 2006 to 31st December 2008. A provisional diagnosis of kala-azar (visceral leishmaniasis) was suspected depending medical history and clinical examination which was confirmed by laboratory methods (Complete Blood Count, rK39 Dipstick and IFAT). In this study (900) blood samples were tested serologically by using both rK39 Dipstick and IFAT. (864) samples out of the total were diagnosed as kala-azar by classic clinical presentation, while (36) samples were without a presumptive diagnosis of kala-azar (used to identify the specificity of rK39 Dipstick and IFAT). Eight hundred-seven samples from patients with suspected kala-azar were serologically positive; the rest (57) samples and all the (36) samples from patients without suspicion of kala-azar were negative. All seronegative patients were excluded from this study. The sensitivity of the rK39 Dipstick test was 90.39%, and the specificity was 100% while the sensitivity of IFAT was 72%, and specificity was 100%.

Key words: sensitivity and specificity, rK39 dipstick, kala-azar, Iraqi children.

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Introduction
Leishmaniasis are group of diseases caused by obligate intracellular protozoa of the genus Leishmania¹ which is transmitted by a species of sand fly of the genus Phlebotomus (in the old world). Leishmaniasis usually present in three different forms: (i) visceral, (ii) cutaneous, and (iii) mucocutaneous. The visceral leishmaniasis (VL) or kala-azar is zoonotic protozoal disease presented with a long standing fever, hepatosplenomegaly, cachexia, pallor, abdominal distension, with pancytopenia, low serum albumin, and high serum gammaglobulines. The weak immunity may predispose to severe infections which if not properly treated can lead to death. VL is endemic in many countries, but 90% of cases occurred in the Indian subcontinent and in Sudan²,³ where the aetiological agent is proved to be the Leishmania donovani complex. Clinical picture of all forms of VL vary with time, especially in AIDS patients⁴,⁵. Leishmania infections are distributed world-wide with about 12 million people currently infected all over the world and a yearly incidence of 500,000 cases² where it mainly appears in regions with a high density of the vector (sand fly)⁶. The recurrence of leishmaniasis, its occurrence in newer geographical regions and in newer hosts, with changing the clinical appearance of infected individuals, made newer challenges in the aspects of diagnosis, treatment, and disease control.

The definitive diagnosis of VL is difficult because the clinical manifestations can be similar to other commonly occurring diseases, such as malaria, typhoid, and tuberculosis. It can be established by the demonstration of amastigotes in tissue specimens or isolation of the organism by culture. Amastigotes can be identified in Giemsa-stained tissue sections, aspirates, or impression smears. Culture of a tissue biopsy or aspirate, best performed by using Novy-McNeal-Nicolle (NNN) biphasic blood agar medium, smears or cultures of material from splenic, bone marrow, or lymph node aspirations are usually diagnostic.
The resources about outbreaks of kala-azar in Iraq are limited but the disease is considered as a public health problem since early nineties of 20th century after the 2nd Gulf War and sanctions that followed where World Health Organization (WHO) reports revealed an annual incidence of more than 3000 cases, most of them in the middle and south of Iraq. The health system in Iraq showed more deterioration since 2003 after the entry of the invader Coalition Forces.

Kala-azar is regarded as an endemic disease in Iraq since 1954. During the last few years, there was an increase in kala-azar cases in some of the Middle and Southern Governorates of Iraq (Wasit, Thi-Qar, Mysan, Basrah and Al-Muthana). Most of the focuses of kala-azar located in area 100 Km around Baghdad mainly in AL-Sowaira, Al-Yousifia, Al-Mahamodia, AL-Latifia, Salman pak, and Al-Azizia. Record of Center of Disease Control in Baghdad indicated that this disease is increasing especially in the last few years. 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.

Aim of the study: To evaluate the sensitivity and specificity of different serological tests rK39 Dipstick (Kalazar Detect® Rapid Test) & IFAT in the diagnosis of kala-azar.

Patients, materials and methods
This prospective study carried out over three years (from the first of January 2006 till the end of December 2008) on children with kala-azar 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). 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) and specific tests for selected cases including rose Bengal tests, Widal test infectious mononucleosis (I.M.) test. 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 all patients admitted and provisionally diagnosed as kala-azar (864) on clinical grounds with another 36 patients (18 male & 18 female) without a presumptive diagnosis of kala-azar and used to identify the specificity of rK39 Dipstick & IFAT. The results were positive in (807) of samples confirmed to have kala-azar (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 Donova antibodies 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
Blood picture of examined samples from the patients with kala-azar revealed a decrease of all hematological parameters (Hb, WBC and platelets counts), 90.5% of patients developed anemia, 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 and 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 hypoprotenemia was elicited in 13.5% of cases and hyperbilirubinaemia with elevated liver enzymes in 6.8%. The technique used (rK39) Dipstick exhibit many advantages in comparison to IFAT in the practical aspects, because it is a simple and easy to use, cheap, and rapid so can be used in rural areas where it is difficult to perform bone marrow which needs expert practitioners, few or mostly no laboratories can perform IFAT, and transport to a referral center is difficult, and the drawback of rK39 test is that it cannot differentiate a recent from old infection.

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 kala-azar by classic clinical presentation, while 36 out of total samples were without presumptive diagnosis of kala-azar. The rK39 test revealed that 781 out of 864 with a provisional diagnosis of kala-azar were positive and all cases 36 without presumptive...
diagnosis of kala-azar 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 a provisional diagnosis of kala-azar were positive and all cases 36 without presumptive diagnosis of kala-azar were negative. Therefore, the sensitivity of the test was about 72%, and specificity was 100%. The results of rK39 and IFAT testing of serum samples from clinically suspected cases of kala-azar are summarized in (Table 1); 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%. 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%). 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. The specificity revealed by this study is equal (100%); this is in agreement with other study in patients from Nepal, Venezuela, and Brazil, while the specificity in Sudan and India is 98%.

Conclusion and recommendations
Serological tests (rK39 Dipstick & IFAT) proved effective in confirming the diagnosis of kala-azar with high sensitivity & specificity especially for rK39 test and these results encourage the use of serological tests, especially rK39 Dipstick, because of simplicity and ease of use, less cost, and rapidity of the test which 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.

Table (1): IFAT & rK39 Dipstick test for suspected V.L. (864) children

<table>
<thead>
<tr>
<th>rk39 test results</th>
<th>IFAT results</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>positive</td>
<td>negative</td>
</tr>
<tr>
<td>positive</td>
<td>596</td>
<td>185</td>
</tr>
<tr>
<td>negative</td>
<td>26</td>
<td>57</td>
</tr>
<tr>
<td>Total</td>
<td>622</td>
<td>242</td>
</tr>
</tbody>
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Chi-square test = 75.303; P-value: 0.001

References
12. CDC report. Leshmaniasis: Report on leshmania in Iraq, 1\textsuperscript{st} quarter. MOH. Communicable
Disease Control Center 2005.
102(9):891-97.
15. Peter JW. Leishmania species. In: Mandell S. Principle and Practice of infectious diseases. 4th
17. Bern C, Jha SN. Use of the recombinant rK39 dipstick test and the direct agglutination test in a
Laboratory Immunology 2002 Sept.: 951-958.