Validity of Dipstick Rapid Test in the Diagnosis of Visceral Leishmaniasis in Two Hospitals in Baghdad City during two years (2012 -2013)

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Abstract

Background: Visceral Leishmaniasis, commonly known as Kala Azar, is the most severe form of Leishmaniasis. It affects poor communities and has significant health, social and economic impact. Without treatment, it is fatal in almost all cases. The procedure of the gold standard tests for the diagnosis of Visceral Leishmaniasis, is cumbersome, time consuming, technically demanding, risky, and very difficult to apply in field conditions or remote places/Primary Health Care Centers. The dipstick rapid test based on the detection of antibodies to a recombinant antigen is used worldwide. The test requires only a drop of finger prick blood or serum, and the result can be read within 15 minutes.

Objectives:
1- To estimate the percentage of Visceral Leishmaniasis in patients attending Central Child Teaching and Child Welfare hospitals in Baghdad during 2012-2013.
2- To assess the statistical relationship between the occurrence of Visceral Leishmaniasis and certain sociodemographic variables (Gender and Age) of studied patients.
3- To assess the validity of dipstick rapid test in the diagnosis of Visceral Leishmaniasis.
Visceral Leishmaniasis (VL), commonly known as Kala Azar, the most severe form of Leishmaniasis, is a parasitic disease transmitted by the bite of infected female sandflies [1]. Months after this initial infection the disease can progress into a more severe form, called Visceral Leishmaniasis or Kala Azar [2]. It is characterized by prolonged high fever, substantial weight loss, swelling of the spleen and liver, and anaemia. It affects poor communities causing significant health, social and economic impact. Without treatment, it is fatal in almost all cases [1]. The disease is the second largest parasitic killer in the world, only malaria is more deadly [2]. In Iraq, VL is endemic and usually detected in infants and children [3]. It is usually caused by Leishmania Donovani parasite and the sand fly vector is the Phlebotomus Alexandri type [4]. Many of the cases are asymptomatic in Iraq [5]. The disease was known to be endemic since 1954 after reports from Baghdad [6], and it 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 [7]. During the last 10 years, there was a marked increase in visceral leishmaniasis cases in southern Governorates (Baghdad areas, Myssan, Thi-qar, Al-Qadisya, and Mothana) [8]. In Iraq it represents one of the serious public health problems and it is more in south and middle and lowest in the north, with highest frequency in Winter followed by Spring and lower in Summer and Autumn [9-10]. For decades, the detection of the parasite has been considered to be the definitive diagnosis of this disease. It requires invasive procedure to find the parasite in the tissue, i.e. spleen, bone

**Patients and Methods:** A descriptive cross-sectional study was done on a non-probability convenient sample of 170 patients to detect the validity of dipstick rapid test in the diagnosis of Visceral Leishmaniasis in comparison with bone marrow examination test (Gold standard test) among patients who attended two main specialized pediatric hospitals in Baghdad city, namely Central Child Teaching and Child Welfare Teaching hospitals during two years 2012 and 2013.

**Results:** The study was done on 170 patients and it was found that 60.58% of them were male, while only 39.42% of them were female. 60% of the males and 40% of the females were diagnosed as VL (by bone marrow examination). It was found that 65.29% of total number of surveyed patients were below 5 years and the disease appeared to affect mainly young children; 78.57% of patients with VL (as diagnosed by bone marrow examination) were below 5 years. The majority of the studied cases (75%) were found below the age of two years. The highest number of cases was recorded in Winter and Spring. The geographical distribution of VL showed that the highest percentage was from the central region of Iraq (26.78% from Baghdad governorate, 26.78% from Diyala governorate, 17.85% from Babylon governorate). The study showed that almost all of the clinically suspected VL patients who were admitted to the hospitals had enlarged spleen, enlarged liver, prolonged fever, and anemia. No statistically significant relation was found between the disease and the gender (P>0.05) while there was a statistically significant relation between the disease and the age of patients (P<0.05).

The percentage of the percentage of cases of Visceral Leishmaniasis diagnosed by bone marrow examination was 33%, and the percentage of the patients with negative bone marrow examination was 67%, while the percentage of positive dipstick rapid test results was 35%, and the percentage of the patients with negative dipstick rapid test was 65%.

In comparison with the result of bone marrow examination, the Validity of dipstick rapid test for diagnosis of Visceral Leishmaniasis was estimated by calculating the sensitivity of the test which was 80.35% and the specificity of it which was 86.84%.

The accuracy of the dipstick rapid test in the diagnosis of Visceral Leishmaniasis was equal to 84.7% while the Positive Predictive Value (P.P.V.) of the test was equal to 75% and its negative Predictive Value (P.N.V.) was equal to 90%.

**Conclusions:**
1. Visceral Leishmaniasis is a significant child health problem as the percentage of cases diagnosed by bone marrow examination was 33% of the total number of patients included in the study.
2. Dipstick rapid test which is the simplest, cheapest, and most rapid test is proved to be a valid test in the diagnosis of Visceral Leishmaniasis as the sensitivity of this test was 80.35% and its specificity was 86.84%.

**Key words:** Visceral Leishmaniasis (VL), dipstick rapid test.
marrow, and lymph-node. Although it is the gold standard for the diagnosis of VL, the procedure is cumbersome, time consuming, technically demanding, risky, and very difficult to apply in field conditions or remote places/Primary Health Centers. To obviate these procedures, various serological tests have been developed, evaluated, and tried and one of these tests was The rapid immunochromatographic test based on the detection of antibodies to a recombinant antigen (rK39) consisting of 39 amino acids conserved in the kinesin region of L. infantum is used worldwide. The test requires only a drop of finger prick blood or serum, and the result can be read within 15 minutes [11].

Objectives

1- To estimate the percentage of Visceral Leishmaniasis in patients attending Central Child Teaching hospital and Child Welfare hospital in Baghdad city during two years (2012 -2013).
2- To assess the statistical relationship between the occurrence of Visceral Leishmaniasis and certain sociodemographic variables ( Gender and Age ) of studied patients.
3- To assess the validity of dipstick rapid test in the diagnosis of Visceral Leishmaniasis.

Patients and Methods

1-Study Design:
A descriptive cross-sectional study to detect the validity of dipstick rapid test in the diagnosis of Visceral Leishmaniasis and compare it with bone marrow examination among patients who attended Central Child Teaching and Child Welfare Teaching hospitals in Baghdad city during two years (2012 -2013).

2-Time of the Study:
This study was conducted from the second of December 2013 to the first of June 2014 including 2 days a week and 4 hours a day (9 a.m.- 1 p.m.).

3-Place of the Study:
This study was conducted in Central Child Teaching hospital and Child Welfare hospitals in Baghdad city. These two hospitals are referral centers providing specialized health care services to all attendees from all Iraqi governorates. These centers were chosen for convenience, after approving the necessary official agreement from the Ministry of Health to conduct the study.

4-Sampling Method:
The sampling design was a non-probability convenient sample.

5- Inclusion criteria:
The study population included all patients who attended the selected hospitals for any complaint depending on the following selection criteria:
a- Both genders with age < 14 years.
b- Patients who were from Iraq.
c- All the patients with the following laboratory investigations were included :
a- Complete blood count.
b- Bone marrow examination..
c- Rapid dipstick test for Kala Azar.

6- Exclusion criteria:
Patients who did not complete all the required laboratory investigations in this study such as patients who did not have dipstick rapid test or bone marrow examination.

7- Sample size:
The sample size is convenient of 170 cases.

8- Data Collection tool:
The medical records which were surveyed and analyzed were the old medical records of the patients who attended two major secondary Child Health Care Centers in Baghdad city (Central Child teaching and Child Welfare hospitals) during two years (2012 -2013).

A specially designed questionnaire was filled from the medical records of each patient including general information on certain socio-demographic variables (like age, gender and residency), the date of diagnosis, the results of certain investigations and the main presenting clinical features of the Visceral Leishmaniasis.

9- Data analysis:
Data of all cases were checked for any error or inconsistency then transferred into a computerized database program; Microsoft Excel software was used.

Descriptive statistics were presented as frequency (number of cases) with percentages for the studied categorical variables. Then the percentage of cases diagnosed by dipstick rapid test and by bone marrow examination were found and compared with each other.

Chi-square test for independence was used to test the significance of association between certain sociodemographic variables and the frequency of cases of Visceral Leishmaniasis.

The validity of dipstick Rapid test was assessed by calculation of the Sensitivity and the Specificity of the test as follows:

1- Sensitivity: is the probability of the diseased people to give (+ve) results or it is the capacity to show positivity of the actually diseased people.
Sensitivity = [True (+ve) / True (+ve) + False (-ve)]*100%

2- Specificity: is the probability of the non-diseased people to give (-ve) results.
Specificity = [True (-ve) / True (-ve) + False (+ve)]*100%

The Accuracy of dipstick Rapid test was also assessed as follows:
Accuracy: is the proportion of true results among all results.
Accuracy = \[
\frac{\text{True (}\pm\text{ve}) + \text{True (}\mp\text{ve}) + \text{False(}\pm\text{ve}) + \text{False (}\mp\text{ve}) + \text{True (}\mp\text{ve})}{\text{True (}\mp\text{ve}) + \text{False (}\pm\text{ve}) + \text{False (}\mp\text{ve}) + \text{True (}\pm\text{ve}) + \text{True (}\mp\text{ve})}\]
\]*100% 

Finally the Positive Predictive Value (P.P.V.) and the Negative Predictive Value (N.P.V.) of dipstick Rapid test were also found as follows:

1- Positive Predictive Value (P.P.V.): is the probability of people with (+ve) result to be diseased truly.

\[
P.P.V. = \frac{\text{True (+ve)}}{\text{True (+ve)} + \text{False (+ve)}}\]

2- Negative Predictive Value (N.P.V.): is the probability of people with (-ve) result to be not diseased truly.

\[
N.P.V. = \frac{\text{True (-ve)}}{\text{False (-ve)} + \text{True (-ve)}}\]

10- Definition of Variables:

1- True (+ve) : Positive cases by dipstick rapid test and positive by bone marrow examination.

2- True (-ve) : Negative cases by dipstick rapid test and negative by bone marrow examination.

3- False (+ve) : Positive cases by dipstick rapid test and negative by bone marrow examination.

4- False (-ve): Negative cases by dipstick rapid test and positive by bone marrow examination.

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Results

The medical records of a total number of 170 patients aged 14 years old or less, were surveyed and the results include the following:

1- Description of the studied sample according to:

a- Gender: It was found that 60.58% of total number of surveyed patients were male, while only 39.42% of them were female. (Table 1).

<table>
<thead>
<tr>
<th>Gender</th>
<th>No. of patients</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>male</td>
<td>103</td>
<td>60.58</td>
</tr>
<tr>
<td>female</td>
<td>67</td>
<td>39.42</td>
</tr>
<tr>
<td>Total</td>
<td>170</td>
<td>100</td>
</tr>
</tbody>
</table>

b- Age: It was found that 65.29% of total number of surveyed patients were below 5 years. (Table 2).

<table>
<thead>
<tr>
<th>Age of the patients</th>
<th>No. of patients</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 5 yrs</td>
<td>111</td>
<td>65.29</td>
</tr>
<tr>
<td>≥ 5 yrs</td>
<td>59</td>
<td>34.71</td>
</tr>
<tr>
<td>Total</td>
<td>170</td>
<td>100</td>
</tr>
</tbody>
</table>

2- Description of cases of Visceral Leishmaniasis in the studied sample according to:

a- Seasonal distribution: The highest number of cases was recorded in Winter and Spring, less number of cases in Autumn and no cases were reported in Summer. (Table 3).
Table 3: Seasonal distribution of Visceral Leishmaniasis in the studied sample

<table>
<thead>
<tr>
<th>Month of admission</th>
<th>No. of patients</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Winter</td>
<td>31</td>
<td>55.36</td>
</tr>
<tr>
<td>Spring</td>
<td>22</td>
<td>39.22</td>
</tr>
<tr>
<td>Summer</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Autumn</td>
<td>3</td>
<td>5.35</td>
</tr>
<tr>
<td>Total</td>
<td>56</td>
<td>100</td>
</tr>
</tbody>
</table>

b- Geographical distribution: showed that the highest percentage was from the central region of Iraq (26.78% from Baghdad governorate, 26.78% from Diyala governorate, 17.85% from Babylon governorate), while the lowest percentage was from Mysan governorate (3.5%). (Table 4).

Table 4: Geographical distribution of Visceral Leishmaniasis in the studied sample

<table>
<thead>
<tr>
<th>The Governorate</th>
<th>Number of patients with VL diagnosed by Bone Marrow Examination test</th>
<th>Percentage of cases %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baghdad</td>
<td>15</td>
<td>26.78</td>
</tr>
<tr>
<td>Babylon</td>
<td>10</td>
<td>17.85</td>
</tr>
<tr>
<td>Diyala</td>
<td>15</td>
<td>26.78</td>
</tr>
<tr>
<td>Najaf</td>
<td>4</td>
<td>7.14</td>
</tr>
<tr>
<td>Thiqar</td>
<td>5</td>
<td>8.9</td>
</tr>
<tr>
<td>Mysan</td>
<td>2</td>
<td>3.5</td>
</tr>
<tr>
<td>Al-anbar</td>
<td>5</td>
<td>8.9</td>
</tr>
<tr>
<td>Total</td>
<td>56</td>
<td>100</td>
</tr>
</tbody>
</table>

c- Main presenting clinical features: The study showed that almost all of the clinically suspected VL patients who are admitted to the hospitals have enlarged spleen, enlarged liver, prolonged fever, and anemia. (Table 5).

Table 5: Distribution of Visceral Leishmaniasis in the studied sample according to clinical features of the disease

<table>
<thead>
<tr>
<th>Bone marrow examination</th>
<th>Lymphadenopathy</th>
<th>Hepatosplenomegaly</th>
<th>Anemia</th>
<th>Fever</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>+ve</td>
<td>2</td>
<td>3.5</td>
<td>53</td>
<td>94.6</td>
</tr>
<tr>
<td>-ve</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

3- The Statistical relationship of occurrence of Visceral Leishmaniasis with certain sociodemographic variables which are Gender and Age of studied patients as follows:

a- The Statistical relationship with the gender of studied patients: 60% of the males and 40% of the females were diagnosed as VL (by bone marrow examination) but no statistically significant relation was found between the disease and the gender (P ≥ 0.05). (Table 6).
Table 6: Relation of Visceral Leishmaniasis with gender

<table>
<thead>
<tr>
<th>VL as diagnosed by Bone Marrow Examination test</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Male</td>
<td>33</td>
<td>60</td>
<td>70</td>
</tr>
<tr>
<td>Female</td>
<td>23</td>
<td>40</td>
<td>44</td>
</tr>
<tr>
<td>Total</td>
<td>56</td>
<td>100</td>
<td>114</td>
</tr>
</tbody>
</table>

*P value >0.05

b- The Statistical relationship with age of studied patients: 78.57% of patients with VL (as diagnosed by bone marrow examination) were below 5 years. There was a statistically significant relation between the disease and the age of patients (P<0.05). (Table 7).

Table 7: Relation of Visceral Leishmaniasis with age

<table>
<thead>
<tr>
<th>Age</th>
<th>Number of cases of VL as diagnosed by Bone marrow examination test</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>%</td>
</tr>
<tr>
<td>&lt;5</td>
<td>44</td>
<td>78.57</td>
</tr>
<tr>
<td>≥5</td>
<td>12</td>
<td>21.43</td>
</tr>
<tr>
<td>Total</td>
<td>56</td>
<td>100</td>
</tr>
</tbody>
</table>

*P value >0.05

4- The percentage of the cases of Visceral Leishmaniasis diagnosed by bone marrow examination which was 33%, and the percentage of the patients with negative bone marrow examination was 67%. (Figure 1).

Figure 1: percentage of cases of Visceral Leishmaniasis diagnosed by Bone marrow examination
5- The percentage of the cases of Visceral Leishmaniasis diagnosed by dipstick rapid test results which was 35%, and the percentage of the patients with negative dipstick rapid test was 65%. (Figure 2).

**Figure 2: percentage of cases of Visceral Leishmaniasis diagnosed by Dipstick rapid test**

6- Validity of dipstick rapid test for diagnosis of Visceral Leishmaniasis was estimated by calculating the sensitivity of the test which was 80.35% and the specificity of it which was 86.84% in comparison with the result of bone marrow examination. (Table 8).

**Table 8: Validity of dipstick test for diagnosis of Visceral Leishmaniasis was compared with results of Bone marrow examination**

<table>
<thead>
<tr>
<th>Test</th>
<th>Bone marrow examination (Gold standard)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bone marrow examination +ve</td>
<td>Bone marrow examination -ve</td>
</tr>
<tr>
<td>Dipstick rapid Test</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Test +ve</td>
<td>45</td>
<td>80.36</td>
</tr>
<tr>
<td>Test -ve</td>
<td>11</td>
<td>19.64</td>
</tr>
<tr>
<td>Total</td>
<td>56</td>
<td>100</td>
</tr>
</tbody>
</table>

7- The accuracy of the dipstick rapid test in the diagnosis of Visceral Leishmaniasis was equal to 84.7%. (Table 8).

8- Positive Predictive Value (P.P.V.) the dipstick rapid test was equal to 75% while the negative Predictive Value (P.P.V.) of the test was equal to 90% (Table 8).
Discussion

Two tests were used in this study, dipstick rapid test and bone marrow aspiration (which is considered as a diagnostic test for diagnosis of Visceral Leishmaniasis)[1]. The present study shows that the majority of the patients were males (60.58%), while females accounted for 39.42%. 60% of the males and 40% of the females were diagnosed as VL (by bone marrow examination), but no statistically significant association was found (P>0.05) between the disease and the gender as shown. This result is similar to that of other studies done in Iraq in Hilla[12]( Al-Marzoki ), Babylon[13] ( Al-Muhammadi ), Thiqar[14],[15] ( Haidar A. et al ), (Khablus Kh. Raddam ), Baghdad[16],[17],[18] ( Sukkar F. 1976), (Sukkar F. 1972), (Murad A.M, et al ) where they reported both genders equally affected, and the result is not compatible with that of other studies done in Iraq in Al-Anbar[19] (Zaid R Al-Ani, et al.), Basrah[20],[21]( Yousif S. K, et al),( Zainab H. G, et al), Iran [22]( Soleimanzadeh G, et al.), India [23] (Kumar R, et al) in which the males were more affected than females.

It was found that 65.29% of the total number of surveyed patients were below 5 years and the disease appears to affect mainly young children as 78.57% of patients with VL (as diagnosed by bone marrow examination) were below 5 years (probably because of low immunity) and the results were in agreement with other Iraqi studies in Babylon[24]( Ahmed M. Al-Mosawy, et al), in Thi-Qar[15] (Khablus Kh. Raddam ) and Basrah[21] ( Zainab H. G, et al) where they recorded cases down to the age of two months. In Wasiti[25] (Qasim Dawood AL-Tammemi) in which the majority of cases of VL (52.41%) were between the age of (1-2) years and (32.72%) was < 1 year), Al-Anbar[19] (Zaid R Al-Ani, et al). The majority (75%) of the studied cases were found below the age of two years.

The highest number of cases was recorded in Winter and Spring, and this is in agreement with other Iraqi studies[9,10, 16] ( Qusay A et al),( Abid, Baqir K.), ( Sukker F.), and differs from studies done in THI-QAR [15] (Khablus Kh. Raddam).

The geographical distribution of VL showed that the highest percentage was from the central region of Iraq (26.78% from Baghdad governorate, 26.78% from Diyala governorate, 17.85% from Babylon governorate), while the lowest percentage was from Mysan governorate (3.5 %). This may be related to the location of the two referral hospitals that were involved in this study as both of them are located in Baghdad governorate (Center of Iraq). The result is in agreement with other Iraqi studies [26,27,28]( Abdulsadah A.Rahi, et al), (Sukker, F. 1983),( Sukker, F. 1985).

This study showed that almost all of the clinically suspected VL patients who were admitted to the hospitals have enlarged spleen, enlarged liver, prolonged fever, and anemia. These results are similar with the results of other studies in different countries, in Sudan[27] (Adler, S. et al.) ,Nepal[28] ( Bern CS, et al), and India[29]( Tilak, N., et al).

In this study, the sensitivity of dipstick rapid test was 80.35% and this result is in agreement with studies done at Mid-Euphrate Region (Al-Qadisyia, Najaf and Karbala)[30] (Hashim Raheem Tarish1, et al.), sensitivity (88.23%), Venezuela [31] 88% ( Delgado O, et al.), but less than that in Wasit[25] ( Qasim Dawood AL-Tammemi, ), where the sensitivity of the rK39 Dipstick test was 90.39%, while in India[32]( Sundar,S.,et al,) and in Nepal[33]( S. N. Jha, et al.,), there were the highest sensitivities which were equal to 100%. So in general, sensitivities of dipstick rapid test range from 67 to 100%; patients from India[32] and Nepal[33] show higher sensitivities 100% then Brazil with 90% sensitivity and Venezuela[34] (Silvo, F., et al.) in which the sensitivity is 88%.

The specificity of dipstick rapid test in this study was 86.84%, and this is less than that in the studies done in Baghdad and Wasit[7]( Jawad J, et al.,) in Wasit[25] (Qasim Dawood AL-Tammemi, ), the specificity was 100%, and in India[32]( Sundar,S.,et al,),[33]( S. N. Jha, et al.,), and higher than that in the studies in the Mid-Euphrates Region (Al-Qadisyia, Najaf and Karbala)[30] (Hashim Raheem Tarish1, et al.), in which the specificity is (60%), Sudan[27] (Adler, S. et al).

Regional variation in the results of the dipstick rapid test could be explained by the following explanations :

1- Differences in the test accuracy between subspecies of L. donovani complex as a result of variation in the recombinant antigen [35].
2- Age factor affecting the level of antibody response may explain the regional differences; Indian kala-azar occurs among individuals of all ages while in other parts of the world, such as Iraq and Iran VL is primarily of Mediterranean infantile type [36,37,38].

Conclusion

1- Visceral Leishmaniasis is a significant child health problem as the percentage of cases diagnosed by bone marrow examination was 33% of the total number of patients included in the study.
2- Dipstick rapid test which is the simplest, cheapest, and most rapid test is proved to be a valid test in the diagnosis of Visceral Leishmaniasis as the sensitivity of this test was 80.35% and its specificity was 86.84%.

Recommendations

1- Increased public awareness about this dangerous disease by encouraging family education about the prevention and control of VL including the necessity for the complete early diagnosis, treatment and follow up to save the affected children from otherwise fatal disease.
2- It is recommended the use of dipstick rapid test in the clinically suspected patients in remote areas where no expert hematologist is present, and to depend on the positive result of the test for diagnosis and treatment of VL, but if the patient has a past history of VL with a positive result of the test, or if the patient presented with negative result then the bone marrow examination is mandatory.
References


