Evaluation the benefit of kalazar detect rapid test for detection of visceral leishmaniasis in Al-Diwaniah province/Iraq

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Abstract

The aim of study is to determine the benefit of In Bios kala-azar detect rapid test in comparative with IFA test in cases with clinical suspicion of visceral leishmaniasis. Eighty four patients with clinical diagnosis of were studied in children and maternity teaching hospital in Al-Diwaniah/Iraq for children and all specimens were checked by using the Kala-azar detect rapid test and rechecked after that by using immunofluorescence antibody test, after that the patients sera were sent for immune-fluorescent antibody tests test to confirm or to exclude the diagnosis of the kala-azar. In a current study of eight four (84) patients were clinically suspected as kala-azar (visceral leishmaniasis) using the main two common laboratory investigation (InBios kala-azar detect rapid test and IFA test). The diagnosis of visceral leishmaniasis depend on clinical suspicion of the common signs and symptoms of the disease in areas highly endemic with kala-azar; hepatomegaly in 40/84 patients (7.6%) and splenomegaly founded in 81/84 (96.4%). Fever and pallor were detected in almost all patients through the first clinical attack of visceral leishmaniasis 84/84 (100%).

Kala-azar detect rapid test truly positive only in about 72/84 with sensitivity reach (85.71%) the in compares to other test; direct immuno-flourecenc antibody (IFA) test in which the sensitivity of the test reach to (98.8%); and the InBios kala-azar detect rapid test appear positive in only one case from control group thirty (30), diagnosed clinically as a typhoid fever in which the specificity of the test were (96.9 %).

In conclusion, the kala-azar detect rapid test is a sensitive, not invasive, rapid, easy to give result within minutes and low cost diagnostic method to be used for the diagnosis of kala-azar but should not use as the sole criterion for the diagnosis of the visceral leishmaniasis unless the signs and symptoms present.

Keywords: Kala-azar; Immunoflourecenc antibody (IFA); Leishmaniasis

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Introduction

Visceral leishmaniasis (VL) is a parasitic disease caused by mainly by many Leishmania species and the main type in our society are: L. donovani and L. infantum. The parasite is transmitted through the bite of an infected phlebotomine sandfly [1, 2]. Best method for diagnosis has been microscopic detection of amastigote from bone marrow& splenic & lymphatic tissue biopsy specimens [3] Serological tests for the detection of antileishmanial antibodies, including the immunofluorescent-antibody test (IFAT) ,enzyme-linked immunosorbent assay (ELISA) , and the direct agglutination test ,are well standardized and widely used [4].

However Serologic or parasitological methods & the polymerase chain reaction test (PCR) are used for the diagnosis however, serology may lack specificity due to asymptomatic infections (5 – 6) . PCR is the best diagnostic measure with limitation because it's expensive and lack of machine and experience [7, 8].

The visceral leishmaniasis(also known as black sickness or kala-azar), is characterized by persistent fever, enlarge spleen, enlarge liver, failure to gain weight, progressive pallor, leucopenia and thrombocytopenia, and other associated symptoms hypergammaglobulinemia and may complicated by superadded infections. It is the most severe form of the infection and, if left untreated, is usually fatal. Although confirmed cases of VL have been reported from 88 countries, most of the cases occur in the India and middle east and east Africa, developed nation is also affect especially opportunistic infection in AIDS patients [9-11].

Response to infection by Leishmania donovani varies a great deal, not only by the strength but also by the type of the patient's immune reaction. People with a history of infection by strains of leishmania that cause visceral leishmaniasis show a continuum of immune responses from protective to non-protective.

Those who acquired protective immunity (skin test positive) without ever having visceral leishmaniasis have a strong type 1 CD4+ response to leishmania antigens. Antigen specific interferon-gamma and proliferation, as well as the ability to kill intracellular leishmania, are hallmarks of protective immunity [12, 13].

Conventional serologic tests, such as the indirect immunofluorescent antibody test (IFAT), have high sensitivity because of the presence of high levels of IgG antibody against *Leishmania* spp. in the serum of otherwise immunocompetent visceral leishmaniasis patients [14, 15]. Spurred by the need for non-invasive diagnostic testing in the limited resource settings most affected by the disease, several field-applicable serologic tests have been developed since the 1990 [16].

The first test to be developed was the direct agglutination test (DAT); this test can be read visually without a machine reader, but requires trained technicians, specialized reagents and plates, and initially cost $10 or more per test [17].

A new latex agglutination test (KATEX) for detecting leishmanial antigen in urine of patients with VL has showed sensitivities between 68 and 100% and a specificity of 100% in preliminary trials. The antigen is detected quite early during the infection and the results of animal experiments suggest that the amount of detectable antigen tends to decline rapidly following chemotherapy. The test performed better than any of the serological tests when compared to microscopy. Large field trials are under way to evaluate its utility for the diagnosis and prognosis of VL [18].

Finally the widely two FDA approved drugs, liposomal amphotericin B (AmBisome) and the other important medication called pentostam [19]. There are no vaccines available to prevent people from visceral leishmaniasis It is important for people to avoid outdoor activities, especially in the evening and night, because this is when sandflies are most active. When outdoors in areas where visceral leishmaniasis is endemic, one should minimize the amount of exposed skin. It is important to wear long-sleeved.

In the evening, it is highly recommended to use a sleeping net to keep the sandflies out and other potentially harmful vectors. Insect repellent is another way to help prevent contracting visceral leishmaniasis [19].

The aim of study is to determine the benefit of In Bios kala-azar detect rapid test in comparative with IFA test in cases with clinical suspicion of visceral leishmaniasis.

Patients and methods

Eighty four patients with clinical diagnosis of (VL) (continuous fever, splenomegaly and pallor from different region in Aldiwaniah province endemic with kala- azar) were studied in children and maternity teaching hospital in Aldiwaniah for children in period between by descriptive prospective study (August 2015 –august 2016)**;** all specimens were checked by using The Kala-Zar Detect rapid test by experienced professionals and rechecked after that by using immunofluorescence antibody test and detect the different in results.

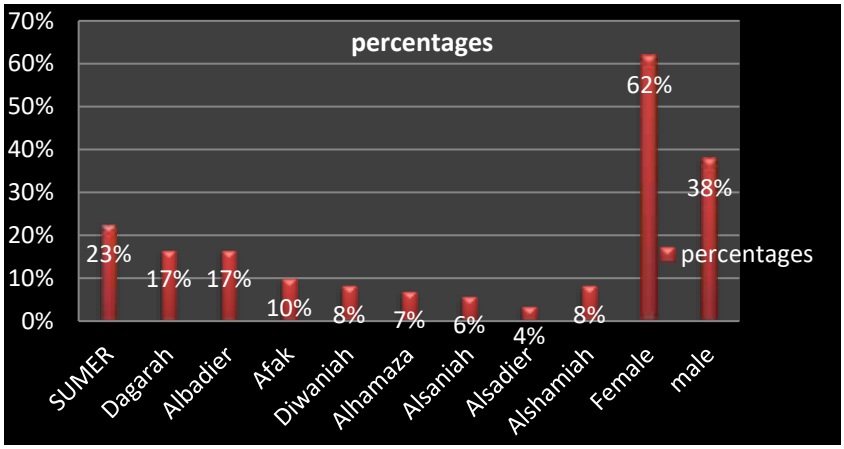
The Kala-Zar Detect Rapid Test for Leishmaniasis is a rapid method involve an immune chromatographic strip for the detection of antibodies to members of L. donovani in human serum. The rK39 antigen-based dipstick test (InBios International, USA) was carried out according to the manufacturer's instruction. In brief few drop of serum was added to a test strip and two drops of chase buffer solution were added. The test result was read within few minutes reach to about ten minute, if the antibody is available in patient sera, producing a colored band, in the strip of patients who are infected. In positive patients two band (pinkish

lines) appear in the middle of the dipstick, a control band and a positive test band appeared . The test was negative if only the control band appeared. The test is qualitative, and the manufacturer instructions shows that even light small band should be considered positive. If the control band not appear a new dipstick should be used.

After that the patients' sera were sent for immunoflourescent antibody tests (IFA) test to confirm or to exclude the diagnosis of the kala-Azar. Also, a blood sample from thirty children (from healthy with other disease) were studied for dipstick test. The sensitivity (measure the true positive rate) from those confirmed with visceral leishmaniasis depending on the IFA test and response to the Antikala-Azar treatment and the specificity (measure the true negative rate) from the samples of other disease and healthy children. The data was analyzed use SSPS program.

Results

In a comparative study eighty four (84) patients were clinically suspected as kala-azar (visceral leishmaniasis) using the main tow common laboratory investigation (InBios kala-azar detect rapid test and IFA test). A total case of 84 visceral leishmaniasis cases were included. All patients were in the age range from three months-six years (3month – 6 years) with male to female ratio about 2:1 female 52 /84 (62%) and male 32/84 (38%). the disease is more common in certain area like sumer district19/84(23%) and albadier district 14/84 (17%) region and aldagarah district 14/84 (17%) in compares' to other area in aldiwaniah province as shows in Figure -1.



In current study the diagnosis of visceral leishmaniasis depend on clinical suspicion of the common signs and symptoms of the disease in areas highly endemic with kala-azar; hepatomegaly in 40/84 patients (47.6%) and splenomegaly founded in 81/84 (96.4%). Fever and pallor were detected in almost all patients through the first clinical attack of visceral leishmaniasis 84/84 (100%), other concomitant feature like jaundice 5/84 (5. %), vomiting and other gastro-intestinal symptoms9/84 (10.7%) and ecchymosis and other clinical laboratory blood abnormalities were present in few patient 12/84(14.2%) Table -1.

In 84 patients appear that kala-azar detect rapid test truly positive only in about 72/84 with sensitivity reach (85.71%) the in compares to other test ; direct immunofluorescence antibody (IFA) test in which the sensitivity of the test reach to (98.8%) ; and the InBios kala-azar detect rapid test appear positive in only one case from control group thirty(30)[healthy and other disease] diagnosed clinically as a typhoid fever in which the specificity of the test were (96.9 %).

The sensitivity of the different antibody against visceral leishmaniasis detection methods ranged from 86to 98% (Table-2) although the differences observed were statistically highly significant (P –value below 0.05).

Table 1.
Frequency of clinical symptoms and signs of kala – Azar

No.	Symptoms and signs	No. of cases	%
1	Fever and pallor	84/84	100%
2	Splenomegaly	81/84	96.4%
3	Hepatomegaly	40/84	47.6%
4	Ecchymosis & other bleeding manifestations	12/84	14.2%
5	Leucopenia and thrombocytopenia or both	9/84	10.7%
6	Vomiting, diarrhea & other GI symptoms	9/84	10.7%
7	Jaundice	5/84	5.9%

Table 2.

Comparative study between dipstick InBios kala-azar rapid detect test and IFA test

	IFA test			P-VALUE
InBios kala-azar detect rapid test	YES	NO	TOTAL	0.010
YES	70	2	72	
NO	9	3	12	
TOTAL	79	5	84	

Discussion

The distribution of the age and gender, and clinical presentations of the present patients are nearly the same as those described in other studies on visceral leishmaniasis in Mediterranean region [20, 21].

All our patients presented with fever, and the majority with splenomegaly (96.4 %) which is similar to other studies done in Iraq, while it is lower than the incidence of hepatomegaly recorded in the same study, [22, 23]. The presence of organomegaly percent in aldiwaniah children and maternity teaching hospital /Iraq is lower than the study at King Fahad Hospital in Gizan, Saudi Arabia [24]; this common presentations makes a role in our hospital and all medical staff that any patient with prolong fever and organomegaly ,the visceral leishmaniasis should be suspected until prove other diagnosis.

The clinical hematological abnormalities in this study were pallor (anemia whether mild, moderate or severe) in most of the cases, with other laboratory changes in blood like leukopenia, and thrombocytopenia present in few cases 10.7% [25, 26, 27]. Anemia probably due to chronicity of the disease, nutritional deficiency and the age of presentation is the same age common for the development of iron deficiency anemia in our society; the anemia and other hematological abnormalities may occur due to bone marrow suppression by direct invasion of parasites. [9] The study in Gizan- Saudi Arabia where there was 98.3% of cases developed anemia ,this is nearly in line with current study [24].

In the our region, the InBios kala-azar detect rapid test now days are widely used for confirmation in any clinical suspicion of visceral leishmaniasis even in primary health center and are represent important element in the ongoing prevention effort in the endemic area this is also widely used in India other endemic region [28]. These tests have not been as widely used in kala-azar-endemic in Africa, because of reported lower sensitivity and specificity in published data's at these area [29, 30, 31].

However, our data suggest that in Iraq the sensitivity 85.71% and specificity 96.9% of the InBios kala-azar detect rapid test are at least well within the acceptable range to be used as the tool for confirmatory testing in primary health center and in the hospitals.

In our study, the specificity of the InBios kala-azar detect rapid test is the same as study during an epidemic of visceral leishmaniasis (VL) in eastern Sudan by Ritmeijer K et al 2006 [32] and also in line with study in Nepal by Bern C et al 2000 [33] while it was low in other studies [34]. The kala-azar detect rapid test is a rapid usually within ten minutes and very simple only requiring easy training of the operator, however the serological tools are an aid in the diagnosis of kala-azar and the outcome of dipstick test should always be judged judiciously in connection with clinical, epidemiological and other diagnostic data.

A study by Chowdhury et al. reported a visceral leishmaniasis in patients with positive serology but with no parasitological evidence of infection. In such situations, and because of the serious therapeutic implications of an incorrect or late diagnosis of kala-azar, there is a pressing need for an accurate laboratory test that can confirm the clinical diagnosis [35, 36].

In the immunofluorescence antibody (IFA) test in which the sensitivity of the test reach to (98.8%); has been shown to be more sensitive than In Bios kala-azar detect rapid test, and the later shows reach to 96.9% in which positive in one case from thirty control group diagnosed clinically and by other blood test as a typhoid fever respond to the treatment with antibiotics , however, aside from practical difficulties at peripheral laboratories, the sensitivities and specificities of most of the above tests have been the limiting factors. Except for the IFA test, which is used on a limited scale, these tests are rarely used for routine diagnosis of visceral leishmaniasis [37].

Also several studies in India and Brazil have concluded that the InBios rapid strip test for diagnosis of visceral leishmaniasis is highly sensitive and specific, those were in line with current study [38, 39, 40] the United States marines used the InBios rapid strip on suspected visceral leishmaniasis in returning soldiers because of satisfactory sensitivity and specificity [41, 42] most of these studies also demonstrate that the InBios rapid strip has a high sensitivity and specificity for visceral leishmaniasis diagnosis in different countries , where the species may differ [3].

Conclusion

The kala-azar detect rapid test is a sensitive, not invasive, rapid, easy to give result within minutes and low cost diagnostic method to be used for the diagnosis of kala-azar but should not use as the sole criterion for the diagnosis of the visceral leishmaniasis unless the signs and symptoms present, this test may be used for clinical treatment decision in presence of good clinical information's in area endemic with kala-azar; if the result is negative and clinical symptoms and signs present, additional test is recommended .false negative and false positive result may occur, so a negative result does not exclude the diagnosis of visceral leishmaniasis.

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