

Diagnostic Utility of Immunochromatographic (ICT-rK39) Strip test in the Diagnosis of Kala Azar in Children: A Study in a Tertiary Care Hospital, Rajshahi, Bangladesh.

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Abstract:

Background: Kala-azar is a fatal disease and accurate diagnosis is vital to avoid under-diagnosis or over-diagnosis. Microscopical examination of spleen aspirates are sensitive and specific but requires expertise to carry out the aspiration safely and to read the slides accurately. Examination of bone marrow or lymph node aspirates is equally specific but less sensitive. **Objective:** To evaluate the diagnostic utility of immunochromatographic (ICT-rK39) strip test in the diagnosis of kala-azar (Visceral Leishmaniasis) in children. **Methodology:** This cross-sectional study was done to determine the comparison of sensitivity, specificity and predictive value of immunochromatographic (ICT-rK39) test with bone marrow aspiration for the diagnosis of kala-azar in children. This study was conducted in all the paediatric units of Rajshahi Medical College Hospital from June 2008 to May 2009, for the duration of one year. A total 40 children clinically suspected for kala-azar was included in the study. Bone marrow study for LD body and immunochromatographic (ICT-rK39) test were done for all the patients. **Result:** In this study results showed that ICT had 96.2% sensitivity, 53.8% specificity, 81.3% positive predictive value and 87.5% negative predictive value. Bone marrow aspiration for LD body had 81.3% sensitivity, 87.5% specificity, 96.2% positive predictive value and 53.5% negative predictive value.

Conclusion: Immunochromatographic (ICT-rK39) test for kala-azar is an effective diagnostic tool for the diagnosis of kala-azar (viscera leishmaniasis) in children.

Key Words: Kala-Azar, Fatal Disease, Diagnosis, Diagnostic Utility, Immunochromatographic (ICT-rK39) Strip Test.

Date of Submission: 17-10-2020

Date of Acceptance: 02-11-2020

I. Introduction

Kala-azar or visceral leishmaniasis is an infectious disease caused by protozoan parasites that belong to the genus *leishmania* and is transmitted by the bite of certain species of sand fly, including flies in the genus *Lutzomyia* in the new world phlebotomus in the old world¹. *Leishmania Donovanii* is the main causative parasite for visceral leishmaniasis (VL) and worldwide 5,00,000 new cases of VL occur in five countries, namely India, Sudan, Bangladesh, Nepal and Brazil each year². These five countries account for 90% of the global VL cases³. It is one of the major public health problems in Bangladesh and the disease is endemic for many decades. It was first described in 1824, in Jessore district, Bengal in what is now Bangladesh⁴⁻⁵. Considered to be a major neglected tropical disease in South Asia, kala-azar has resurged in endemic regions of Bangladesh since the 1990, with the highest rates in the districts of Mymensingh, Pabna, and Tangail⁶. From 1994 through 2004, a total of 73,467 kala-azar cases were reported to the Malaria and Vector-Borne Disease Control Unit, Directorate General of Health Services (DGHS), Government of Bangladesh⁶. Annual kala-azar case totals ranged from 3,965 in 1994 to 8,920 in 2004, with a trend of rising incidence⁶. The cardinal clinical features of Kala-azar are history of fever for more than two weeks, residing/traveling in endemic area, splenomegaly, weight loss and anaemia⁶. The World Health Organization (WHO) established the clinical case definition of VL as persistent fever (>2 weeks) and splenomegaly in a person residing in a VL-endemic area⁷. In Bangladesh, Rukunuzzaman and Rahman carried out a case control study in 2008 to determine clinical and epidemiological risk factors related to childhood visceral leishmaniasis (VL). They found that mud house dwelling, grade III malnutrition, sleeping on floor and seasonality (rainy season) are the important risk factors of VL in children⁸.

The ultimate confirmation of VL is by demonstration of *Leishmaniadonoviamastigotes* in stained smears from spleen, lymph gland or bone marrow aspirates. Splenic aspirates are more sensitive (96%) than aspirates of bone marrow lymph node (58%)⁹ but riskier as there has been chance of internal hemorrhage which may cause fatal complication. Bone marrow aspiration is also an invasive procedure but less risky than splenic aspiration, though like splenic aspiration it is not easily applicable on large scale in the rural areas and needs technical support with good laboratory facilities. There are some serological tests for the diagnosis Kala-azar. These are aldehyde test (AT), immunofluorescence antibody test (IFAT), complement fixation test (CFT), Direct agglutination test (DAT), Enzyme-linked immunosorbent assay (ELISA), Polymerase chain reaction (PCR) and immunochromatographic (ICT-rk39) test¹⁰. Among them ICT test is highly sensitive (100%) and specific (98%), its positive predictive value is 96.6% and negative predictive value is 98.3%³, its role in diagnosis of visceral leishmaniasis was evaluated in a case-control study in Mymensingh Medical College Hospital by Sarker et al.¹¹ ICT for rk39 was done for all cases and then parasitological confirmation was done by demonstration of *Leishmaniadonovani* bodies in bone marrow or splenic aspirates. The test had sensitivity 96.6%, specificity 98.3%, negative predictive value 98.3% and positive predictive value 96.6%. They concluded that the ICT strip test is easy, quick, requires no technical facilities with higher sensitivity and specificity entails it to be the ideal test for the diagnosis of kala-azar in field level¹¹. Clinical diagnosis of Kala-azar (VL) is -not always accurate. Approximately 50% of patient meeting the clinical case definition of VL has the disease, the remainder are diagnosed with other illness such as Malaria, Typhoid, Tuberculosis etc⁹. As death occurs in 90% of patient without treatment¹² and treatment consists of prolonged course of highly toxic drugs, so early diagnosis is essential. This study is made to evaluate the comparison of sensitivity, specificity and predictive value of ICT and bone marrow aspiration for the diagnosis of Kala-azar in children.

II. Methodology and Materials

This was a hospital based cross sectional study which was conducted at the department of Pediatrics, Rajshahi Medical College Hospital from June 2008 to May 2009 for the duration of 01 (one) year using purposive sampling technique. Forty suspected patients of kala-azar attended in the department of Paediatrics were enrolled in the study. Children were provisionally diagnosed as the cases of Kala-azar on the basis of clinical criteria described above. ICT (rK39) was done for all clinically diagnosed patients of Kala-azar in the department of Paediatrics, RMCH as a bed side test with strip. In the current study, detection of LD bodies in the bone marrow aspirate was considered as the gold standard. After performing ICT (rK39) test all patients were undergone bone marrow aspiration and sent the aspirate to the department of microbiology for LD body detection. Then the results were analyzed by using software SPSS version 15 to calculate sensitivity, specificity, positive predictive value, negative predictive value and accuracy of ICT (rK39) as well as for LD bodies in bone marrow aspirates in the diagnosis of Kala-azar. Finally, those were compared between rK39 and LD body group to find out the efficacy of rK39 in the diagnosis of Kala-azar.

• Inclusion Criteria

- Clinically suspected cases of Kala-azar less than 14 years old.
- Patients having complaints of-
 - Fever- or History of fever for more than 2 weeks.
 - Residing/traveling in Kala-azar endemic area
 - Weight loss.
 - Anaemia.
 - Swelling of upper abdomen.
 - Increased pigmentation
 - Ascites, oedema, anasarca.
 - Abdominal mass

• Exclusion Criteria

- Patients age above 14 years.
- Kala-azar re-infection.
- Kala-azar relapse case.
- Partially treated Kala-azar,
- Treatment failure Kala-azar.
- Kala-azar cases but bone marrow cannot be done due to any reasonable

III. Results

Maximum number of patients (52.5%) in this study were in between 5-10 years' age group followed by (300/0) 55 years age group. Mean (SO) age of the patients was 6.23±3.33 years ranging from 2-12 years. Figure I show that maximum 25 (62.50/0) studied patients were male and 15 (37.5%) were female with male female ratio 1.67:1. Housing pattern studied child (Table II) shows that, the majority children were lived in mud wall

house (70%).Others were lived in tin shade house (7.5%) and other type of houses (22.5%).Table III shows that, out of 40 cases most patients came from Rajshahi (20%), then from. Pabna (17.5%). Naogoan (15%), ChapaiNawabgonj (12.5%),Sirajgonj (12.5%) and Chuadanga (7.5%). Rest of the cases came from Nator (5%), Kushtia (5%) and Meherpur (50/0) district.Distribution of the patients by presenting symptoms and signs (Table IV) shows among them all patients were presented with fever (100%). Other presenting symptoms and signs were spleenomegaly (97.5%), hepatomegaly (80%), lump in their abdomen (65%), weight loss' (50%), gum ble.edi,nf1 (35%)./ anemia/Pallor (95%). skin pigmentation (10%).Table V shows that majority of the patients had intermittent fever (75%), others had continuous (17.5%), and remittent (7.5%). The mean duration of fever was 3.33 ± 1.77 ranging from 1 to 8 months and the mean temperature was $100.62 \pm 1.32^{\circ}\text{F}$ of ranging from 99 to 104°F . Routine investigation reports (Table VI) shows that mean Hb% was 7.93 ± 1.45 gm/dl ranging from 5 to 12 gm/dl, ESR was 84.23 ± 23.11 ranging from 38-125, TC of WBC was 3497.50 ± 910.76 ranging from 1800-6000 and mean Platelet count was 90075.00 ± 26883.78 ranging from 34000-130000.Figure II shows that, LD bodies in bone marrow aspiration were found in 27 (67.5%) of patients of kala-azar and were negative in 13 (32.5%) patients.Figure III shows the distribution of ICT for Kala-azar in studied children which describes that, ICT for Kala-azar was positive in 32 (80%) cases and negative in 8 (20%) cases.Table VII shows that out of 32 ICT positive kala-azar patient 26 patients were found LD bodies positive and among 8 ICT negative kala-azar patients only 1 patient was LO body positive. Chi-square test (χ^2) was done to measure the level of significance (p value). $\chi^2 = 13.78$, $df=1$, $p < 0.001$. So, the study result in the above table is statistically significant, and it indicates that ICT for kala-azar is statistically better than Bone marrow aspiration for LD bodies for the diagnosis of kala-azar.Table VIII shows that ICT for kala-azar had 96.2% sensitivity, 53.8% specificity, 81.3% PPV. and 87.5% NPV for the diagnosis of disease and bone marrow aspiration for LD bodies for the diagnosis of kala-azar had 81.2% sensitivity. 87.5% specificity. 96.3% PPV, and 53.50/0 NPV for the diagnosis of disease.

Table I: Distribution of the patients by age (N=40)

| Age (In year) | Frequency | Percent | Mean \pm SO (Range) |
|---------------|-----------|---------|------------------------|
| 5 | 12 | 30.0 | 6.23 \pm 3.33 (2-12) |
| 5-10 | 21 | 52.5 | |
| >10 | 7 | 17.5 | |
| Total | 40 | 100.0 | |

Figure I: Sex distribution of studied children (N=40)

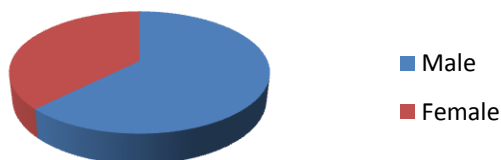


Table II: Housing pattern of the studied child (N=40)

| Housing | Frequency | Percent |
|----------|-----------|---------|
| Mud wall | 28 | 70.0 |
| Tin shed | 3 | 7.5 |
| Others | 9 | 22.5 |
| Total | 40 | 100.0 |

Table III: Geographical distribution of studied cases (N=40)

| Housing | Frequency | Percent |
|-----------------|-----------|---------|
| Rajshahi | 08 | 20 |
| ChapaiNawabgonj | 05 | 12.5 |
| Pabna | 07 | 17.5 |
| Sirajgonj | 05 | 12.5 |
| Naogoan | 06 | 15 |
| Natore | 02 | 05 |
| Kushtia | 02 | 05 |

| | | |
|-----------|----|-----|
| Meherpur | 02 | 05 |
| Chuadanga | 03 | 7.5 |
| Total | 40 | 100 |

Table IV: Distribution of the patients by presenting symptoms and signs (N=40)

| Presenting symptoms & signs | Frequency | Percent |
|--------------------------------------|-----------|---------|
| Fever | 40 | 100 |
| splenomegaly | 39 | 97.5 |
| Anaemia/pallor | 38 | 95.0 |
| Hepatomegaly | 32 | 80.0 |
| Lump in abdomen/abdominal distension | 26 | 65.0 |
| Weight loss | 20 | 50.0 |
| Gum bleeding | 14 | 35.0 |
| Skin pigmentation | 4 | 10.0 |
| Ascites | 2 | 5.0 |
| Lymphadenopathy | 1 | 2.5 |

Table V: Distribution of pattern of fever in studied children (N=40)

| Types of fever | Frequency | Percent |
|----------------------------|---------------|---------|
| Continuous | 7 | 17.5 |
| Intermittent | 30 | 75.0 |
| Remittent | 3 | 7.5 |
| Total | 40 | 100.0 |
| Duration of fever (Months) | 3.33 ± 1.77 | |
| Temperature of | 100.62 ± 1.32 | |

Table VI: Routine investigation reports of studied cases (N=40)

| Variables | Mean ± SD | Range |
|----------------|---------------------|--------------|
| Hb% | 7.93 ± 1.45 | 5-12 |
| ESR | 84.23 ± 23.11 | 38-125 |
| TC of WBC | 3497.50 ± 910.76 | 1800*6000 |
| N | 29.05 ± 8.44 | 12-45 |
| L | 58.66 ± 7.62 | 47-80 |
| M | 9.58 ± 3.41 | 2-17 |
| E | 0.63 ± 1.32 | 0-6 |
| B | 2.27 ± 2.10 | 0-8 |
| Platelet count | 90075.00 ± 26883.78 | 34000-130000 |

Figure II: Distribution of bone marrow aspiration for LD bodies in studied children (N=40)

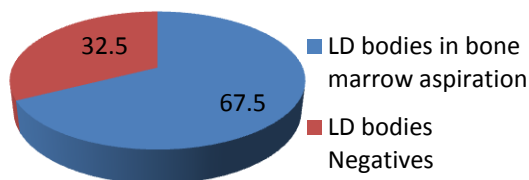


Figure III: Distribution of ICT for Kala-azar in studied children (N=40)

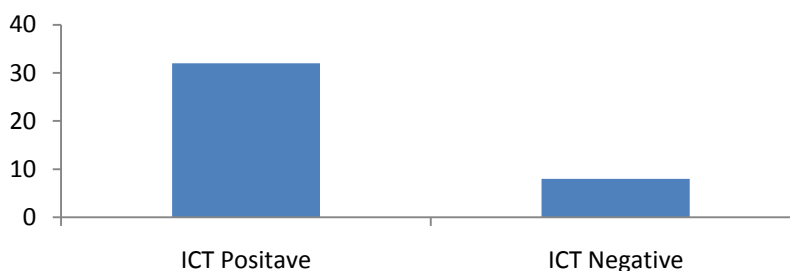


Table VII: Comparison of ICT for Kala-azar and aspiration for LD bodies (N=40)

| Bone marrow aspiration for LD bodies | | | |
|--------------------------------------|----------|----------|-------|
| ICT for kalaazar | Positive | Negative | Total |
| Positive | 26 | 6 | 32 |
| Negative | 1 | 7 | 8 |
| Total | 27 | 13 | 40 |

Table VIII: Comparison of the validity tests between rK39 and LD bodies group

| Parameter of validity test | rK-39(ICT) for Kala-azar | LD bodies in bone marrow |
|-----------------------------------|--------------------------|--------------------------|
| Sensitivity | 96.2 | 81.2 |
| Specificity | 53.8 | 87.5 |
| Predictive value of positive test | 81.3 | 96.3 |
| Predictive value of negative test | 87.5 | 53.5 |

IV. Discussion

Kala-azar was very much rampant in Indian subcontinent including Bangladesh. Kala-azar affects up to 500,000 people yearly, mostly in poor rural areas of east Africa (Sudan, Ethiopia, Kenya, and Uganda), South Asia (India, Bangladesh, Nepal) and Latin America (Brazil).¹³ In Bangladesh about 10,000 patients are reported annually from government health facilities with much more are probably remaining unreported. The current prevalence is estimated to be 45,000 cases with more than 40.6 million populations are at risk.¹⁴ Modernized investigations are able to diagnose and help in management of the patient. In this relation the study was undertaken for comparative efficacy of tests. The Current study was conducted to evaluate the usefulness of Immunochromatographic test (ICT) in the diagnosis of visceral leishmaniasis among paediatric patients. Although disease occurs irrespective of age, children aged 1-4 years are particularly at risk of infection in Mediterranean regions, and childhood infection may account for more than half of all cases in some of these countries¹⁵. In the present study mean age (\pm SD) of the children was 6.23 ± 3.33 years ranging from 2-12 years and most of them were from the age group of 5 to 70 years. In a study by Rashid et al¹⁶ among Bangladeshi paediatrics patients with visceral leishmaniasis, the age between 5-12 years was the most vulnerable and in a series in India the peak age of the disease was 5-9 years¹⁷ which are consistent with the present study. In this study sex distribution revealed male Female ratio was 1.67:1. There was a such overall predominance of male. The cattle are looked after by the male members of families. Aikat et al showed the similar reports.¹⁸ Sharma et al showed that there was no significant difference in the incidence of kala-azar in either sex or pre-pubertal age group. Findings in this study is consistent with above mentioned studies. Most of the patient in this observation came from rural areas having housing mostly made up of mud. Table-3.2 shows that 28 (70%) patients live in mud build house. Regarding housing of the kala-azar patient similar data was observed by Aikat BK et al¹⁸, Thakur CP et al¹⁹, and sarker CB et al²⁰. In this study, regarding geographical distribution most of the patients were from Rajshahi (20%), then from Pabna (17.5%), Naogaon (15%), Chapainawabgonj (12.5%) and Sirajgonj (12.5%) districts of Rajshahi division. The result is consistent with study by Bern et al.²¹ In that study among 64 districts of Bangladesh, incidence of kala-azar in Pabna was 14.2%, Sirajgonj 4.4%, Natore 3.7%, Naogaon 2.9%, Rajshahi 1.8%, Chapainawabgonj 1.7%. Regarding presenting symptoms and signs in this study, all patients presented with fever. The most common presenting symptoms were lump in abdomen (65%), Weight loss (50). Gum bleeding (5%). Among signs, anaemia/pallor was present in 38 (95%) of patients, splenomegaly in 39 (97.5%) and hepatomegaly in 32 (80%) patients. These findings are consistent with Aikat BK et al.²² findings. In this study, the pattern of fever was intermittent in nature in most of the patient 30 (75%). Continued fever was in 7 (17.5%) and 3 (7.5%) patient had remittent fever. Aiket al¹⁸ found 3 types of in kala-azar patient with predominance of intermittent type of fever. Thakur CP¹⁹ and Sarkar CB et al also found similar result, which are consistent with the present study. Normochromic normocyticaemia is a frequent and cynically

significant feature of VL and haemoglobin levels of 7-10 g/dl are commonly found. The average haemoglobin levels reported in two large series of patients were 8.3 and 7.8 g/dl^{22,23}. Al-Jurrayanet al.²⁴ reviewed 94 children with VL and found that all patients were anaemic. Manawa et al.²⁵ reviewed 23 patients with VL and found all patients to be moderately to severely anaemic (Hb = 4.3-8.1 gm/dl). In this study children had mean Hb level 7.93±1.45 gm/dl, which is consistent with above mentioned studies. Leucopenia is an early and striking manifestation of VL. There is relative lymphocytosis (58.60±7.52) with neutropenia (29.05±8.44), the differential shows an almost complete absence of eosinophils (0.63:1.32) in this study. Cartwright GE et al²³ and Aikat BK et al²² showed the mean total WBC count was 2.8X10⁹/l and 4X10⁹/l respectively. In this study the total count was 3497±910, which is also consistent with just mentioned studies. Marwaha et al²⁴ reported in their study that Mean platelet count had been found to be 109±82.3X10⁹/l, which is consistent with the present study where platelet count was found 90.07X10⁹/l. By bone marrow aspiration study, LD bodies were present in 27 (67.5%) of 40 kala-azar suspected patients. In this study it was found that the sensitivity and specificity of bone marrow aspiration for kala-azar were 81.25% and 87.5% respectively. The positive predictive value was 96.29% and negative predictive value was 53.48%. Zijlstra EE et al.²⁶ and Bern et al²⁷ found the sensitivity of bone marrow aspiration for Kala-azar 70.2% and 70% respectively which are nearly consistent with the present study. By the immunochromatographic (rK39 ICT) strip test study it was found that 32 patients (80%) were ICT strip test positive. In this study the results revealed that the sensitivity and specificity of ICT for kala-azar were 96.2% and 53.8% respectively. The positive predictive value was 81.3% and negative predictive value was 87.5%. Sundar et al²⁸, Mathur et al²⁹, and Badaro et al.³⁰ found the sensitivity of ICT strip test were 100%, 100% and 99% respectively, which are consistent with the present study.

Comparison of the bone marrow aspiration for LD body and ICT strip test shows that the sensitivity of ICT is more than bone marrow study. Positive predictive value for both tests is almost similar but negative predictive value is more for ICT strip test and *p* value for both tests is <0.001, which is statistically significant. So, in this study ICT strip test superior than Bone marrow aspiration for LD bodies in the diagnosis of Kala-azar.

Limitations of the Study

The present study was conducted at a very short period of time. This was a prospective type of study in a single community with comparatively small number of sample size. So, the study result may not reflect the exact scenarios of the whole country.

V. Conclusion and Recommendations

From the results of the present study, it can be concluded that although the bone marrow study is the gold standard for the diagnosis of kala-azar for direct evidence of LD body, immunochromatographic test (ICT-rK39) is more sensitive for the diagnosis of kala-azar in children.

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Dr. Md. Rokibul Islam, et. al. "Diagnostic Utility of Immunochromatographic(ICT-rK39) Strip test in the Diagnosis of Kala Azar in Children: A Study in a Tertiary Care Hospital, Rajshahi, Bangladesh." *IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)*, 19(10), 2020, pp. 21-27.