

Evaluation of a rapid diagnostic test method in kala-azar & Pkdl cases in a tertiary care centre of Eastern India

Dr.Saswati Chattopadhyay¹, Dr.Purbasha Ghosh², Dr. Indrajit Gupta³,
Dr.Tapajyoti Mukherjee⁴, Dr. Poulomi Nandi⁵, Dr. Mousumi Kar⁶,
Dr. Santanu Pramanik⁷, Dr. Asraful Islam⁸.

¹Assitant Professor, Department Of Microbiology, Burdwan Medical College, Burdwan, West Bengal,India;

²Assitant Professor, Department Of Microbiology, Burdwan Medical College, Burdwan, West Bengal,India;

³Post Graduate Trainee, Department Of Microbiology, Burdwan Medical College, Burdwan West Bengal,India;

⁴Assitant Professor Department Of Microbiology, Burdwan Medical College, Burdwan, West Bengal,India;

⁵Assitant Professor, Department Of Microbiology, NRS Medical College, Kolkata;

⁶Demonstrator, Department Of Pathology, Burdwan Medical College, Burdwan, West Bengal, India;

^{7,8}Post Graduate Trainee, Department Of Microbiology, Burdwan Medical College, Burdwan, West Bengal, India.

Abstract: Background & objectives: Definitive diagnosis of kala-azar requires demonstration of parasites in tissue or bone marrow. In the present study, the diagnostic utility of rK-39 immunochromatographic test (ICT) strip for the diagnosis of kala-azar and post kala-azar dermal leishmaniasis (PKDL) at a tertiary care centre of Eastern India was done.

Methods: The study was conducted from January 2011 to December 2011 on 100 suspected kala-azar and PKDL cases.

Results: Of the 100 samples, 36 were positive by ICT; of which, 32 were diagnosed as kala-azar (25 males mainly in children of age group 0-20 yrs –[7]) and 4 as PKDL (all 4 males) The rest 64 controls included individuals with malignancies, haemolytic disorders, chronic liver diseases, etc. All had fever of duration ranging from <1 month to 1.5 yr (median 4.5 months). All PKDL patients gave past history of kala-azar and their slit skin test smears were microscopically positive for Leishman-Donovan (LD) bodies. The strip test was positive in all the cases of kala-azar and PKDL (sensitivity 100%) and negative in all control sera (specificity 100%).

Conclusion: The rK-39 ICT is a highly sensitive and specific test, and may be suitable for a rapid and reliable field diagnostic method of kala-azar and PKDL.

Key words: - Immunochromatographic test, kala-azar, PKDL, rK-39, visceral leishmaniasis.

I. Introduction

Visceral leishmaniasis (VL) or kala-azar, a systemic infection of the reticulo-endothelial system, is a vector borne parasitic disease caused by the protozoa parasite *Leishmania donovani* and transmitted by the bite of infected sandfly *Phlebotomus argentipes* in India.^[1] Globally the incidence and prevalence of the disease is 0.5 and 2.5 million; ^[2] of this more than 90 per cent of the world's VL cases are in India, Bangladesh, Nepal, Sudan and Brazil with the incidence of the disease being highest in India.^[2,3] In India about ninety per cent of all the cases are reported from Bihar alone and now also from several adjoining districts of West Bengal and Uttar Pradesh.^[1]

The disease carries a high mortality rate ranging from 80% to 100% and even with treatment, case fatality rates >10% is common ^[4] and so an early diagnosis of kala-azar is very much needed. Isolation of the parasite in culture or in the tissue extracts of spleen, bonemarrow or lymph node is the "gold standard" and gives the definitive diagnosis but is cumbersome, time consuming and shows low sensitivity in occult and sub-clinical infections. Serological tests like enzyme linked immunosorbent assay (ELISA), direct agglutination test (DAT) and immuno-fluorescent assay, shows variable sensitivity and low specificity. ^[5,6] Recently, a highly sensitive kinesin related gene has been detected in *Leishmania chagasi* that encodes for a 39 amino acid residue (k-39), which is also present in the amastigote forms of VL causing Leishmania strains (*L. donovani*, *L. infantum* and *L. chagasi*). ^[7] Detection of antibodies against this recombinant antigen K-39 (rK-39) was found to be highly sensitive in diagnosing this condition. The study is an attempt to evaluate the diagnostic performance of rK-39 strip test for the diagnosis of kala-azar at a tertiary care hospital in eastern India.

II. Material & Methods

In 2011, a study was conducted from January 2011- December 2011 on 100 patients of pyrexia of unknown origin (PUO), with or without splenomegaly to rule out kala-azar (fever for >2 weeks with wasting, and splenomegaly or lymphadenopathy)/ after exclusion of malaria. Informed consent was taken from the patients/guardians and ethical committee approval was also taken.

A detailed history including clinical features, physical and laboratory findings [haemogram, serum albumin/globulin (A/G) levels, liver and kidney function tests were recorded for each patient.

A finger prick sample (20 µl blood) was collected and added to the well of immunochromatographic test strip and interpreted. The test was considered positive when two bands (a control and a test band) appeared within 10 min and negative when only the control band appeared. All the strips were read independently by two medical personnel and one technical staff.

The diagnosis of kala-azar and PKDL was confirmed in clinically suspected patients by identification of Giemsa stained Leishman-Donovan (LD) bodies.

II. Result

Of the total 100 clinically suspected Kala-azar cases, 36 were positive for rK-39 antibodies. Of these, 32 were diagnosed as cases of VL and 4 as PKDL. Of the remaining - 40 patients had other diseases (confirmed malaria, malignancies, dengue, SLE, typhoid cases) and 24 were healthy. [TABLE-1] All the 36 cases had bone marrow aspirate positive for LD bodies which were confirmed by microscopy. The rk39 strip test shows negative result for all forty patients with confirmed other diseases and also for the 24 healthy controls. [Table-1] Age and sex wise distribution of Kala-azar cases are mentioned below. [TABLE-2 and TABLE-3] Clinical And Laboratory Findings were correlated in the Kala-Azar cases, PKDL and the controls [TABLE 4]

Table-1-- The Study Population according to +ve rk39 test

rK-39 test	Clinically suspected case(N=36)	Patients with other diseases(N=40)	Healthy (N=24)
positive	36	0	0

Table-2: Sex Prevalence of +ve rk39 cases in different months

Month	No. of cases	+ve	male	female
Jan	5	2	2	-
Feb	9	7	5	2
Mar	7	3	2	1
April	5	1	1	-
May	14	1	1	-
June	13	3	2	1
July	14	7	4	2
Aug	14	8	7	1
Sept	2	1	1	-
Oct	9	-	-	-
Nov	7	-	-	1
Dec	1	1	1	-
TOTAL	100	34	26	8

Table 3:-Age Prevalence Of +Ve Rk39 Cases

Age	No. of cases	Male	Female
0-10	10	6	3
11-20	12	9	2
21-30	6	5	1
31-40	2	3	-
41-50	1	1	1
51-60	3	2	1
61 & >	-	-	-
Total	34	26	8

Table 4:-Clinical and laboratory findings in cases of Kala-azar, PKDL and controls

Clinical/laboratory parameters	Kala-azar (n=32)	PKDL(n=4)	Control(n=64)
Fever	32	0	64/64
Weight loss	32	0	64/64
Splenomegaly	25	0	54/64
Hepatomegaly	18	0	43/64
Pancytopenia	12	1	19/64
Lymphadenopathy	5	0	24/64
Positive rK-39 strip test	32	4	0/64

III. Discussion

Visceral leishmaniasis continues to be an important public health problem with significant morbidity and mortality in the endemic areas. The included patients were from different endemic zones like Bihar (18) West-Bengal (13) and Jharkhand (5). In our study, there was a male predominance in the kala-azar cases and the maximum number of cases belonged to 11-20yrs age group followed by 0-10yrs group. This reveals that there is an increasing incidence of paediatric kala-azar cases. [8] All the patients were treated with antileishmanials and discharged after they all responded to the treatment.

IV. Conclusion

In Indian patients with a clinically suspected disease profile, this test is simple, highly sensitive, specific, rapid, field applicable and cost-effective method for the diagnosis of VL and PKDL.

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