ISSN : 2309-3234

TMSS MEDICAL COLLEGE JOURNAL

Volume 05, No. 02, July 2015

An Official Publication of TMSS Medical College, Bogra

TMSS Medical College Journal

July 2015

An Offical Publication
of
TMSS Medical College, Bogra

TMSS Medical College Journal

Vol. 05, No. 02, July 2015

An Offical Publication of TMSS Medical College, Bogra, Bangladesh

Published by

Dr. Md. Abdul Mazid
on behalf of the TMSS Medical College

Graphic Designer

Md. Sirajul Islam
TMSS, Bogra

Printed by

TMSS Printing Press
Bogra, Bangladesh

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Original Articles

Role of recombinant kinetoplast 39 immunochromatographic test for diagnosis of post kala-azar dermal leishmaniasis

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Abstract

Background: Post kala-azar dermal leishmaniasis (PKDL) is a sequel of visceral leishmaniasis (VL) and PKDL patients are an important reservoir for anthroponotic transmission of VL. Therefore, diagnosis and treatment of PKDL is important for the kala-azar elimination program in South Asia, including Bangladesh. While definitive diagnosis of PKDL is still based on microscopy, despite the low sensitivity of this method of diagnosis. Recombinant kinetoplast 39 Immunochromatographic test (rK39 ICT) for detection of kinetoplast antibody against Leishmania parasites from blood is expected to be a rapid and sensitive diagnostic method. **Objective:** To see the efficacy of rapid diagnostic test of rK39-based ICT in comparison with skin biopsy specimens for LD body detection in PKDL cases endemic area. **Mathodology:** This cross sectional comparative study will explore the rapid sensitive method for diagnosis of PKDL. Both skin biopsy specimens and blood samples were collected from 91 patients suspected to have PKDL from six upozilla health complexes in Mymensingh district, Bangladesh. After smear preparation and staining LD bodies were detected from skin biopsy and antibody ware detected from blood by using rK39 ICT method. For all the statistical analysis $p<0.05$ was considered as significant. **Result:** Using microscopy, we identified 57 samples (62.6%) that were positive for Leishmania. rK39-based ICT indicated that 85 samples (93.4%) were positive for Leishmanial antibody which were statistically significant. **Conclusion:** rK39-based ICT for detection of antibody from blood is the most sensitive and easier method for diagnosis of Post Kala-Azar Dermal Leishmaniasis patients from an endemic area of Bangladesh.

Key wards: Recombinant kinetoplast 39 ICT, Leishman Donovan body, PKDL

Introduction

Kala-azar or visceral leishmaniasis (VL) is a symptomatic infection of the liver, spleen, and bone marrow that is caused by Leishmania donovani complex, L.donovani, L. infantum (syn. L. chagasi). Leishmania currently infects about 12 million people in 88 countries, causing approximately 57,000 deaths annually, with 350 million individuals at risk. More than 90% of all VL cases are from India, Nepal, Bangladesh, southern Sudan, and northeast Brazil[1]. In Bangladesh, the highest number of VL cases was recorded in the district of Mymensingh, where the average annual incidence rate between 1994 and 2004 was 5.8/10,000; currently, the incidence rate is as high as 300/10,000 in the most affected communities[2,3]. Post kala-azar dermal leishmaniasis (PKDL) is a complication or sequel of VL and occurs in nearly 10–20% of patients who have been cured of VL in India and approximately 50–56% of such patients in Sudan[4,5]. In India, the disease occurs 1–20 years after recovery from VL. In contrast, in Sudan, PKDL most often develops during treatment or within months after completion of VL treatment and the symptoms may persist for decades in some patients. Clinically, the condition is characterized by the appearance of macules, papules, or nodules in the skin[6]. Affected persons,

though clinically well, harbor the Leishmania parasite in their skin lesions, and thus become an important reservoir in anthroponotic transmission of leishmaniasis[7]. Although PKDL has been commonly diagnosed by microscopy, the sensitivity of this technique is low due to the very low number of parasites in slit skin smears and skin biopsy specimens. So, aim of this study is to find out rapid and most sensitive method for diagnosis of PKDL cases.

Materials and Methods

Study area and population

This cross sectional comparative study was conducted at the Department of Microbiology Mymensingh medical College including five upozilla health complexes of Mymensingh district since January 2011– December 2011. The total sub-centres of these health complexes are endemic for leishmaniasis in Bangladesh. A predesigned questionnaire was used to fill up regarding history of kala-azar and treatment received along with other demographic parameters after obtaining verbal consent.

Case definitions

Probable PKDL is a patient from endemic area for kala-azar with multiple hypopigmented macules, papules or plaques or nodules with no sensitivity loss. Confirmed PKDL is a patient from an area endemic for kala-azar with multiple hypopigmented macules, papules, plaques or nodules who is positive for parasite in a slit skin smear and/ or rk39 ICT test. A cure case is defined as complete disappearance of skin lesion(s) after treatment, as reported by the patient and assessed by the clinician and also negative by Microscopy and rK39 ICT.

Laboratory methods

All rk39 positive cases with or without history of kala-azar but having signs of PKDL and hence considered as probable PKDL cases were examined clinically. Slit skin a scraping was collected from lesion for diagnosis by two different methods.

Collection of slit skin scraping for smear for microscopy

The affected area of the skin was cleaned with 70% v/v alcohol and allowed to dry completely. The margin of the lesion was squeezed firmly between the finger and the thumb to drain the area of blood. Using a sterile scalpel blade, a small incision was made into the dermis. The cut surface was then scraped in an outward direction to obtain the tissue fluid and cells for smear.

Examination for LD body

Smears were prepared on clean glass slide and one part of the scraping sample was examined by two experts' microbiologist following Giemsa staining.

Examination for *M. leprae*

Another part of slit skin smears examined following Ziehl-Neelsen staining to exclude *Mycobacterium leprae*.

Data analysis: Chi-square test was used to compare the sensitivity of two diagnostic methods.

Result

rK39 ICT exhibited a considerably higher positive rate (93.4%) than microscopy (62.6%) among the macular, papular and nodular specimens, which were the most common. The detection rates in papular and nodular lesions were higher using the two detection methods (Table 1 & 2). This finding suggests that macules do not contain as much Leishmania parasite as papules and nodules. In all patient groups, regardless of time after VL treatment, the highest detection rates were obtained using ICT followed by microscopy.

Table 1: Result of rK39 ICT of PKDL cases

Forms	Total	Positive	Percent
Macular	13	10	76.9
Papular	24	23	95.8
Nodular	54	53	98.1
Total	91	85	93.4

Figure 1: Result of rK39 ICT of PKDL cases

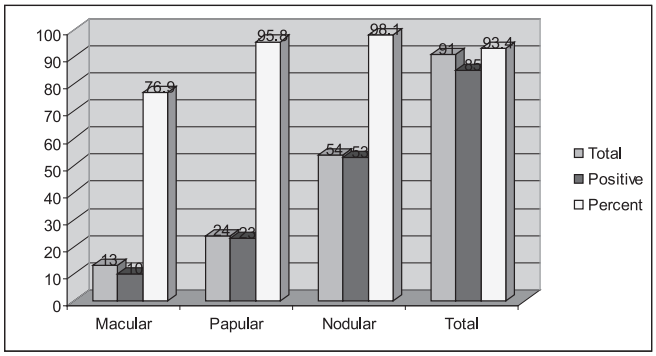
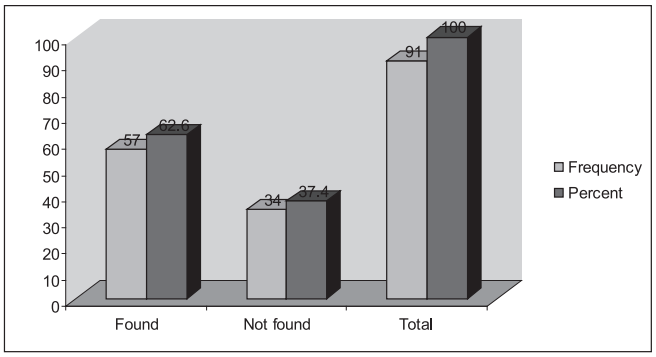


Table 2: LD body detection by Microscopy

LD body	Frequenc y	Percent
Found	57	62.6
Not found	34	37.4
Total	91	100.0

Figure 2: LD body detection by Microscopy



L.D body detection rate comparatively higher in nodular lesion than other clinical types (Table-3) and age group between 6-10 years (Table-4).

Table 3: Distribution of positive Microscopy cases (n=57) among the lesions

Form	Total sample	LD body found	Percent
Macular	13	5	38.5
Papular	24	14	58.3
Nodular	54	38	70.4
Total	91	57	62.6

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	4.816(a)	2	0.090
N of Valid Cases	91		

a 1 cells (16.7%) have expected count less than 5. The minimum expected count is 4.86.

Table 4: Age group distribution of positive Microscopy cases (n=57)

Age group	Microscopy		Total
	Found	Not found	
6-10 yrs	15(71.4%)	6(28.6%)	21
11-15 yrs	23(67.6%)	11(32.4%)	34
16-20 yrs	9(60.0%)	6(40.0%)	15
21-25 yrs	3(50.0%)	3(50.0%)	6
25-30 yrs	3(42.8%)	4(57.2%)	7
> 30 yrs	4(50.0%)	4(50.0%)	8
Total	57	34	91

Chi-Square Tests

	Value	df	Asymp. Sig.(2-sided)
Pearson Chi-Square	3.228(a)	5	0.665
N of Valid Cases	91		

a 5 cells (41.7%) have expected count less than 5. The minimum expected count is 2.24.

Discussion

There are few reports of community-based study regarding prevalence of PKDL in endemic areas of India[7,9]. In our study 57 patients developed PKDL out of 91 individuals having past history of VL with a prevalence of 62.6% which is higher than that reported from India[7,8], Bangladesh[11] and Nepal[10]. These patients have all been detected by active survey during house-to-house visit. Among 57 PKDL cases, 38 patients had nodular forms from whom parasite could be detected quite easily, which implies that these lesions are more accessible to KA vector–sandfly. The role of nodular PKDL forms in transmission of kala-azar is significant[12].

Any delay in detection of macular forms of PKDL may have double impact: further transmission of PKDL by vector and subsequent development into nodular forms where parasites are easily accessible to the sandfly. Proper diagnosis of PKDL cases in the field level which is primarily dependant on clinical manifestations is a real problem[8].

Previous reports indicated that microscopy had a detection rate between 4% to 58% for LD bodies in skin smears[6,12,13]. The sensitivity of this technique is low due to the very low number of parasites in slit skin smears and skin biopsy specimens. The rate of detection of PKDL cases by microscopy from skin scraping sample depends upon the selection of lesion site and collection of scraping sample which varies from person to person as the distribution of parasite throughout the lesion is not homogeneous. The low sensitivity of the diagnostic technique prolongs the time to diagnosis[9].

The immunochromatography test (ICT) with rK39, a recombinant antigen, has been found to be highly sensitive and specific for detection of antibodies in patients with VL and PKDL[15]. In the present study all suspected PKDL cases and those with past history of VL tested positive for anti-leishmanial antibody. Though this test is sensitive, diagnosis based on rK39 strip test is not conclusive for PKDL because of the persistence of anti-leishmanial antibodies after *L donovani* infections[16].

A PCR based diagnostic method for leishmaniasis, in which conserved sequences in leishmanial kinetoplast mini-circle DNA is amplified, has been developed in recent years. The PCR-based molecular diagnostic method is anticipated to provide a powerful approach to the diagnosis of leishmaniasis[14,17,18]. This PCR-based molecular diagnosis was done in Microbiology laboratory at MMC and detection rate that sensitivity of primary PCR was 65.4% (17 out of 26) which increased to 88.5% (23 out of 26) in nested PCR. So in cases having no past VL history, rK-39 test is sufficient for differential diagnosis but in cases with history of VL, PCR is the only way to solve the problem.

Conclusion

rK39-based ICT for detection of antibody from blood is the most sensitive and easier method for diagnosis of Post Kala-Azar Dermal Leishmaniasis patients from an endemic area of Bangladesh.

Conflict of interest: None to declare.

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