Noninvasive Sweat-Based Diagnosis of Visceral Leishmaniasis and Post Kala-Azar Dermal Leishmaniasis

Roshan Kamal Topno,^{1*†} Vikas Shankar,^{2†} Manas Ranjan Dikhit,³ Major Madhukar,⁴ Krishna Pandey,⁴

V. N. R. Das,⁴ Maneesh Kumar,⁵ Bipin Kumar,⁴ Kanhaiya Agrawal,⁴ Niyamat Ali Siddiqui,⁶

Ganesh Chandra Sahoo,³ Rishikesh Kumar,⁵ Ashish Kumar Singh,⁷ Rajeev Kumar,⁸ Dharmendra Singh,⁹ and Pradeep Das^{10*}

¹Department of Epidemiology, Rajendra Memorial Research Institute of Medical Sciences (ICMR), Patna, India; ²Department of Skin and VD, Nalanda Medical College and Hospital, Patna, India; ³Department of Bioinformatics, Rajendra Memorial Research Institute of Medical Sciences (ICMR), Patna, India; ⁴Department of Clinical Medicine, Rajendra Memorial Research Institute of Medical Sciences (ICMR), Patna, India; ⁵Department of Virology, Rajendra Memorial Research Institute of Medical Sciences (ICMR), Patna, India; ⁶Department of Biostatistics, Rajendra Memorial Research Institute of Medical Sciences (ICMR), Patna, India; ⁷Department of Immunology, Rajendra Memorial Research Institute of Medical Sciences (ICMR), Patna, India; ⁸Department of Community Medicine, Nalanda Medical College and Hospital, Patna, India; ⁹Department of Biochemistry, National JALMA Institute for Leprosy and other Mycobacterial Disease (ICMR), Agra, India; ¹⁰Department of Molecular Biology, Rajendra Memorial Research Institute of Medical Sciences (ICMR), Patna, India

Abstract. The diagnosis of visceral leishmaniasis (VL) is one of the foremost barriers in the control of this disease, as demonstration of the parasite by splenic/bone marrow aspiration is relatively difficult and requires expertise and laboratory support. The aim of the present study was to find a noninvasive diagnostic approach using the existing recombinant kinesine-39 (rK-39) immunochromatographic nitrocellulose strips test (ICT) with a human sweat specimen for the diagnosis of VL. The investigation was carried out on specimens (blood, sweat, and urine) collected from 58 confirmed VL, 50 confirmed post kala-azar dermal leishmaniasis (PKDL), 36 healthy control, and 35 patients from other diseases. The data obtained from this study reveal that 96.6% clinically confirmed active VL participants were found to be positive when tested against a sweat specimen. Interestingly, the scenario was similar when tested against a blood specimen (96.6% positive by rK-39). Moreover, a test of both sweats and blood specimens from 50 PKDL participants resulted in 100% positivity, whereas no healthy control participants were found to be rK-39 positive. The sensitivity of the rK-39 ICT in sweat specimen was 94.7%, whereas the specificity was 100% in healthy controls from endemic, nonendemic, and other infectious diseases, respectively. No difference was observed in sweat specimen of VL and PKDL cases which signifies its reliability. However, further evaluation of this method on a larger scale could enhance the reliability of the proposed model so that it could be used efficiently in VL management and eradication.

INTRODUCTION

Visceral leishmaniasis (VL; also known as “kala-azar”) is a vector-borne systemic infectious disease governed by the obligate intracellular parasite, *Leishmania donovani*. Although this tropical disease is endemic in more than 60 countries, 90% of all reported cases occur in rural areas of the Indian subcontinent and in East Africa.^{1,2} The disease is primarily associated with poverty and it can cause considerable morbidity and mortality. An approximate 0.2 to 0.4 million new cases of VL with an incidence of 50 thousand deaths occur each year; however, these numbers are extensively accredited to be a gross underestimation of the actual disease burden.³

As no successful vaccine is available and control measures are primarily dependent on chemotherapy,⁴ early detection plays a vital role in the treatment and prevention of VL.⁵ A variety of immunological tests such as the enzyme-linked immunosorbent assay (ELISA) and the direct agglutination test are available for the screening of VL. These methods have good sensitivities and specificities, but their priority for a precise diagnosis is low. For example, ELISA, which is generally used in diagnosing, often leads to cross-reactivity.⁶ In addition, the procedures are time consuming, expensive, and labor intensive. They require skilled personnel, sophisticated

equipment, a long incubation time, expensive antigens, and laboratory conditions, which delay the results.⁷ Thus, they are not very popular for routine diagnosis.⁸

In contrast to these methodologies, the recombinant kinesine-39 (rK-39) test from a blood/serum/splenic/bone marrow clinical sample is used in different endemic regions. Other than rK-39, various newer recombinant antigens such as recombinant antigen from an Indian isolate of *L. donovani* strain KE16, recombinant K28 protein, and recombinant KLO8 have been developed for the serological diagnosis of VL and post kala-azar dermal leishmaniasis (PKDL).^{9–10} But all these approaches require a blood or serum specimen. To overcome the shortcomings of the blood/serum-based diagnostic approach, a number of studies have been conducted to identify a noninvasive biological source for VL diagnosis.¹¹ As part of this effort, we develop and assess the sensitivity and competency of the sweat-based rK-39 dipstick test with the goal of presenting it as an alternative diagnosis tool in VL disease management and control.

MATERIAL AND METHODS

In this study, a total of 58 VL (confirmed by splenic or bone marrow aspiration) and 50 PKDL patients were enrolled for screening from the indoor-patient department of the Rajendra Memorial Research Institute of Medical Sciences, Patna, Bihar, India, from 2012 to 2018. An additional 30 healthy individuals from endemic regions and six from nonendemic regions were included as control subjects. The patients had clinical evidence of VL and PKDL such as fever, loss of appetite, complete blood count, liver test functions, and past kala-azar history. In total, blood and sweat specimens were

* Address correspondence to Roshan Kamal Topno, Department of Epidemiology, Rajendra Memorial Research Institute of Medical Sciences, Agamkuan, Patna 800007, India, E-mail: roshanktopno@yahoo.co.in or Pradeep Das, Department of Molecular Biology, Rajendra Memorial Research Institute of Medical Science, Patna, India, E-mail: drpradeep.das@gmail.com.

† These authors contributed equally to this work.

TABLE 1

Comparison of rK-39 strip test in diagnosis of kala-azar using sweat samples vs. blood samples from *Leishmania donovani* body-positive VL, PKDL patients, and controls

Sample type VL/PKDL	Total no of case	rK-39 strip test					
		Blood			Sweat		
		Sensitivity N (%)	Specificity N (%)	95% CI	Sensitivity N (%)	Specificity N (%)	95% CI
VL	38	36 (94.74%)	NA	82.25–99.36	36 (94.74%)	NA	82.25–99.36
PKDL	35	35 (100%)	NA	90.00–100.00	35 (100%)	NA	90.00–100.00
Healthy control (from endemic area)	30	NA	30 (100%)	88.43–100.00	NA	30 (100%)	88.43–100.00
Healthy control (from nonendemic area)	6	NA	6 (100%)	54.07–100.00	NA	6 (100%)	54.07–100.00
Other disease							
Leprosy	11	NA	11 (100%)	71.51–100.00	NA	11 (100%)	71.51–100.00
Typhoid	6	NA	6 (100%)	54.07–100.00	NA	6 (100%)	54.07–100.00
Dengue	10	NA	10 (100%)	69.15–100.00	NA	10 (100%)	69.15–100.00
Chikungunya	8	NA	8 (100%)	63.06–100.00	NA	8 (100%)	63.06–100.00

NA = not applicable; PKDL = post kala-azar dermal leishmaniasis; rK-39 = recombinant kinesine-39; VL = visceral leishmaniasis. The sensitivity of the rK-39 immunochromatographic nitrocellulose strips test in serum and in blood specimen was 94.74%, whereas the specificity was 100% in healthy controls from endemic, non-endemic, and other infectious diseases, respectively.

collected from 109 patients, and an additional 35 patients donated urine specimens along with blood and sweat specimens. The clinically confirmed active 38 VL and 35 PKDL patients gave permission for a blood specimen and skin smear to be taken, respectively, along with sweat specimens (urine specimens were not taken). The immunochromatographic nitrocellulose strips test (ICT) was performed on the rest of the 20 VL and 15 PKDL patients using blood and sweat specimens, and urine as an additional specimen was taken from the same diseased individuals. Among them, 58 confirmed VL patients by bone marrow aspiration/Splenic aspiration were included in the study.

The presence of the antibody in blood specimens was evaluated by the rK-39 strip test (Kalazar Detect™ Rapid Test; InBios International, Seattle, WA) as per the manufacturer's instructions. The test was performed by touching the absorbent pad of the rK-39 strip to either the patient's armpit or forehead (two sites where sweat is likely to be present). The same clinical activity was also carried out on 11 leprosy patients, six thyroid patients, 10 dengue patients, and eight chikungunya patients to authenticate the findings with respect to the VL and PKDL patients. The study was approved by the Human Ethical Committee of Rajendra Memorial Research Institute and Medical Sciences, and it was carried out after obtaining informed and written consent from all participants. Statistical analysis was performed by biomedical software MedCalc Statistical software version 18.2.1 (MedCalc Software bvba, Ostend, Belgium; <http://www.medcalc.org>).

RESULTS AND DISCUSSION

Clinically, VL is a devastating parasitic infection. The disease is highly prone on a large scale in endemic regions with a

high fatality rate and it is one of the largest deadly infections that require early and accurate diagnosis. Proper disease diagnosis plays a vital role in controlling this dreadful tropical disease. Although the rK-39 strip-based diagnosis approach plays a crucial role in VL/PKDL detection, it requires blood/serum, which is a barrier to diagnosis, as patients often hesitate to donate a specimen. Therefore, a noninvasive diagnosis approach is required urgently for the detection of VL and PKDL.

The rK-39 strip-test is an easy and cost-effective approach that uses chromatographic strips saturated with the rK-39 antigen. The rK-39 strip actively detects the circulation of an antibody in a diseased individual. Ideally, the rK-39 strip test is highly sensitive (82.4%), specific (100%), and affordable, as it does not require complex or extensive laboratory facilities to measure the results. Detection of VL using a urine specimen with the rK-39 strip became popular, but this approach remains unsatisfactory, as a lack of sanitation facilities and patient bashfulness make the urine-based noninvasive method difficult. With this apprehension regarding VL detection using rK-39 with a urine specimen,^{12,13} another approach is needed. Some diseases, such as cystic fibrosis, are diagnosed using sweat as a clinical sample. Thus, we aim to detect the presence of the anti-leishmania antibody in a sweat specimen using the rK-39 strip.

In this study, the advent of a red control band recorded the proper functioning of the test, and that of a lower band detected the presence of anti-rK-39 immunoglobulin G in the blood or sweat specimen. The test was considered positive if both bands were present, whereas the test was considered negative in case of the appearance of only the control band. Of the participants, 58 were BMA/splenic-confirmed VL cases,

TABLE 2

The sensitivity of rK-39 immunochromatographic nitrocellulose strips test was evaluated in blood, sweat, and urine sample obtained from same patient

Sample type VL/PKDL	Total no of case	rK-39 strip test								
		Blood			Sweat			Urine		
		Sensitivity N (%)	Specificity N (%)	95% CI	Sensitivity N (%)	Specificity N (%)	95% CI	Sensitivity N (%)	Specificity N (%)	95% CI
VL	20	20 (100%)	NA	83.16–100.00	20 (100%)	NA	83.16–100.00	20 (100%)	NA	83.16–100.00
PKDL	15	15 (100%)	NA	78.20–100.00	15 (100%)	NA	78.20–100.00	15 (100%)	NA	78.20–100.00

PKDL = post kala-azar dermal leishmaniasis; rK-39 = recombinant kinesine-39; VL = visceral leishmaniasis.

whereas 56 (96.6%) were found to be positive by rK-39 in blood specimens (Table 1). Interestingly, 100% of the active VL cases positive to rK-39 were also found to be positive against the sweat specimen. Similarly, in the healthy control participants, the rK-39 strip test appeared to be negative against both blood and sweat specimens. A study of patients previously diagnosed with leprosy, typhoid, dengue, and chikungunya were also found to be negative, which were detected in the same pattern as in VL/PKDL cases using the rK-39 test strip. The sensitivity of the rK-39 ICT in blood was 94.7%, whereas the specificity was 100% in healthy controls from endemic, nonendemic, and other infectious diseases, respectively (Table 1). Interestingly, in sweat specimen, the test showed similar kind of result as compared with blood specimens. Furthermore, the usefulness of sweat rK-39 ICT for diagnosis of VL and PKDL were compared with the results with blood. In our study, both urine and sweat specimen collected from the same patient showed 100% (95% confidence interval = 83.16–100.00) sensitivity (Table 2). The compilation of sweat sample is comparatively accessible and painless than withdrawing blood so it can help to screen *Leishmania* exposure at remote endemic areas. Based on the sensitivity and specificities, it can be arguably speculating the feasibility of the rK-39 ICT using sweat samples in the field. Further evaluation of this method in large scale can enhance the reliability of the proposed model and can be efficiently used in the control programs for VL management and eradication.

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Authors' addresses: Roshan Kamal Topno, Department of Epidemiology, Rajendra Memorial Research Institute of Medical Sciences (ICMR), Patna, India, E-mail: roshanktopno@yahoo.co.in. Vikas Shankar, Department of Skin and VD, Nalanda Medical College and Hospital, Patna, India, E-mail: drshankarvikas@gmail.com. Manas Ranjan Dikhit and Ganesh Chandra Sahoo, Department of Bioinformatics, Rajendra Memorial Research Institute of Medical Sciences (ICMR), Patna, India, E-mails: manasranjandikhit@gmail.com and ganeshiitkgp@gmail.com. Major Madhukar, Krishna Pandey, V. N. R. Das, Bipin Kumar, and Kanhaiya Agrawal, Department of Clinical Medicine, Rajendra Memorial Research Institute of Medical Sciences (ICMR), Patna, India, E-mails: madhukarcaptatna@gmail.com, drkrishnapandey@yahoo.com, drvnrdas@yahoo.com, dr.bipin.rmri@gmail.com, and kanhaiya9334@gmail.com. Maneesh Kumar and Rishikesh Kumar, Department of Virology, Rajendra Memorial Research Institute of Medical Sciences (ICMR), Patna, India, E-mails: kumar.maneesh11@gmail.com and virgo.rishii@gmail.com. Niyamat Ali Siddiqui, Department of Biostatistics, Rajendra Memorial Research Institute of Medical Sciences (ICMR), Patna, India, E-mail: niyamatalsiddiqui@yahoo.com. Ashish Kumar Singh, Department of

Immunology, Rajendra Memorial Research Institute of Medical Sciences (ICMR), Patna, India, E-mail: ashish.drug.research@gmail.com. Rajeev Kumar, Department of Community Medicine, Nalanda Medical College and Hospital, Patna, India, E-mail: rajeevkumar9952@gmail.com. Pradeep Das, Department of Molecular Biology, Rajendra Memorial Research Institute of Medical Sciences (ICMR), Patna, India, E-mail: drpradeep.das@gmail.com.

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