Case Report

Identification of atypical dermal leishmaniasis resolved by restriction fragment length polymorphism

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Abstract

This case report series alerts to the atypical manifestations of dermal leishmaniasis in an area endemic for post kala-azar dermal leishmaniasis, the sequel to visceral leishmaniasis. We have reported two cases with multiple skin lesions, wherein the rK39 strip test, polymerase chain reaction and parasite load confirmed the presence of *Leishmania* parasites. The causative parasite was identified as *Leishmania major* by restriction fragment length polymorphism of the ribosomal DNA Internal Transcribed Spacer-1, overruling the clinical suspicion of post kala-azar dermal leishmaniasis. The third case presented with fever and extensive hypopigmented patches in the upper extremities; parasites were identified in blood and skin by polymerase chain reaction and typed by restriction fragment length polymorphism as *Leishmania donovani*, establishing this as a case of visceral leishmaniasis concomitant with dermal leishmaniasis, secondary to dissemination of viscerotropic *L. donovani*. The present case series emphasizes the importance of molecular tools to identify the *Leishmania* species in order to ensure appropriate treatment.

Key words: Cutaneous, Internal Transcribed Spacer-1 polymerase chain reaction, leishmaniasis, parasite load, polymorphism, post kala-azar dermal leishmaniasis, restriction fragment length

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Introduction

Leishmaniasis, caused by the parasite *Leishmania* demonstrates clinical pleomorphism with regard to the causative species, disease reservoirs, vectors and host-immune responses, thus causing cutaneous leishmaniasis or visceral leishmaniasis which may be followed by a dermal sequel termed as post kala-azar dermal leishmaniasis.[1] Generally, in cutaneous leishmaniasis, the causative species are *L. (L.) major*, *L. (L.) tropica* and *L. aethiopica*[1] whereas in South Asia, species include *L. tropica*[2] and *L. donovani*.[3]

Post kala-azar dermal leishmaniasis generally develops after apparent successful cure from visceral leishmaniasis in 10% of cases and the manifestations are limited to macular, papular or nodular skin lesions.[4] Among the diagnostic tests, rK39-based immunochromatographic strip test or enzyme-linked immunosorbent assay are ineffective for post kala-azar dermal leishmaniasis, as the antibodies could be from a prior episode of visceral leishmaniasis. Accordingly, molecular diagnosis by polymerase chain reaction that typically targets the Internal Transcribed Spacer-1 is more effective, which when followed by defining the causative

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species by restriction fragment length polymorphism can clinch the diagnosis.[5]

We report two cases with a strong clinical suspicion of post kala-azar dermal leishmaniasis, where polymerase chain reaction was insufficient and warranted identification by restriction fragment length polymorphism. In another case, the patient presented with multiple episodes of fever, concomitant with extensive hypopigmentation and was identified as a case of visceral leishmaniasis with dermal leishmaniasis. The study was approved by the Institutional Ethical Committee, and peripheral blood or skin biopsy (4 mm) collected after informed consent.

Case Reports

Case 1

A 40-year-old man from West Bengal, residing in Saudi Arabia since 2014, presented to us with multiple ulcerated lesions on the face, trunk and superior extremities that had developed four months ago. He had no fever, hepatosplenomegaly or lymphadenopathy and denied any past history of visceral leishmaniasis. The rK39 dipstick was positive, enzyme-linked immunosorbent assay was performed and considered positive when OD_{405} was at least two-fold higher than the mean of 25 endemic and nonendemic controls (mean ± standard deviation being 0.15 ± 0.15); the OD_{405} of 1.06 was considered positive.[6] Internal Transcribed Spacer-1 polymerase chain reaction and quantitative polymerase chain reaction were performed from a 4 mm skin biopsy, following DNA extraction (QIAamp DNA Blood mini kit, Qiagen, Germany).[5] For *Mycobacterium leprae* infection, a restriction length *M. leprae*-specific repetitive element polymerase chain reaction was performed using *M. leprae*-specific primer sequences, PS1: 5′-TGCATGTCATGGCCTTGAGG-3′ and PS2: 5′-CACCGATACCAGCGGCAGAA-3′.[7] A 129 bp product was obtained for leprosy cases but absent in this case [Figure 1a]. Internal Transcribed Spacer-1 polymerase chain reaction was performed using *Leishmania*-specific primers with a *L. donovani* World Health Organization reference strain MHOM/IN/1983/AG83 being the positive control and DNA from the foreskin of healthy individuals being the negative control; an Internal Transcribed Spacer-1 polymerase chain reaction 320 bp product was obtained.[5] However, as ulceration was present, which is not a feature of classical post kala-azar dermal leishmaniasis, and the patient resided in Saudi Arabia, polymerase chain reaction restriction fragment length polymorphism was performed and was confirmed to be a case of cutaneous leishmaniasis [Figure 1b].[8] The parasite load as calculated by real-time polymerase chain reaction was 8654 parasites/µg of gDNA [Table 1].[9] The patient received liposomal amphotericin b (LAmB), 3 mg/kg body weight intravenous for 6 days, but was lost to follow-up.

Case 2

A 51-year-old woman from Bangladesh, residing in Saudi Arabia since 2016, presented at our out patient department with erythematous plaques on the face, left forearm and left middle finger [Figure 2a and b], without any past history of visceral leishmaniasis. She was diagnosed with cutaneous leishmaniasis in May 2016, when a single plaque appeared on her arm, and received sodium stibogluconate, 20 mg/kg body weight intramuscularly for 15 days, and self-reported subsidence of the lesions. Later, multiple papules developed on her nose and right cheek followed by left forearm and left middle finger, which subsequently coalesced to form large plaques with central clearing, while some developed erosions. The features mimicked cutaneous tuberculosis (lupus vulgaris), and the possibility of borderline leprosy with lepra reaction, sarcoidosis and plaque stage of cutaneous T-cell lymphoma had to be eliminated. There was no fever, lymphadenopathy or hepatosplenomegaly. Internal Transcribed Spacer-1 polymerase chain reaction was positive, restriction length *M. leprae*-specific repetitive element polymerase chain reaction was negative, and the polymerase chain reaction restriction fragment length polymorphism corresponded to *L. major* [Figure 1b]; accordingly, it was confirmed as a case of cutaneous leishmaniasis, parasite load being 6692/µg gDNA. Histopathological analysis showed a lymphohistiocytic dermal infiltrate with an ill-formed granuloma, and Giemsa staining demonstrated

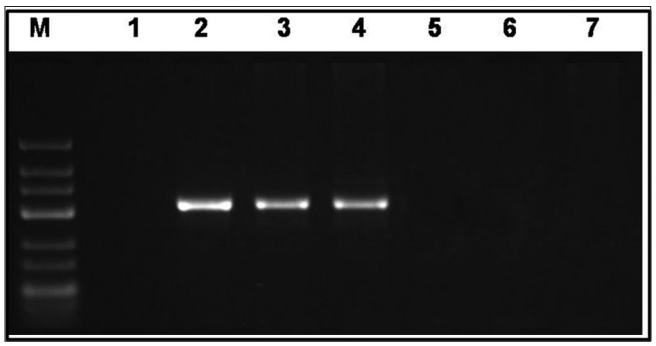

Figure 1a: Restriction length *Mycobacterium leprae*-specific repetitive element polymerase chain reaction. Lane M: Low molecular weight marker; Lane 1: Blank; Lanes 2–4: Skin of leprosy cases; Lanes 5–7: Skin of cases 1, 2 and 3 respectively

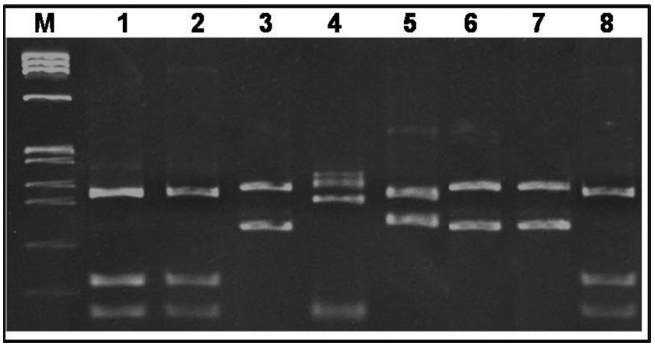

Figure 1b: Restriction fragment length polymorphism of Internal Transcribed Spacer-1 region amplified from *Leishmania* species. Lane M: Low molecular weight marker. Lanes 1, 2: Reference strains of *L. donovani*; Lanes 3–5: Reference strains of *L. major*, *L. tropica* and *L. amazonensis* respectively; Lanes 6–8: Skin of cases 1, 2 and 3 respectively

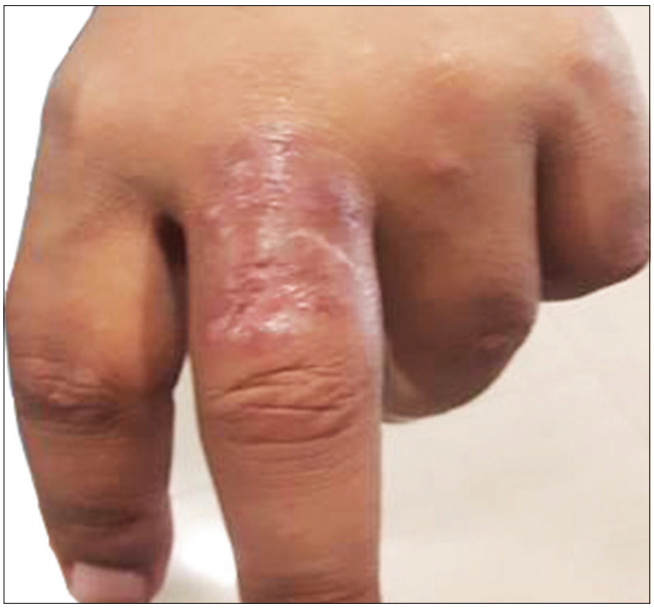

Figure 2a: Case 2: A 51-year-old woman with erythematous plaques on the left middle finger

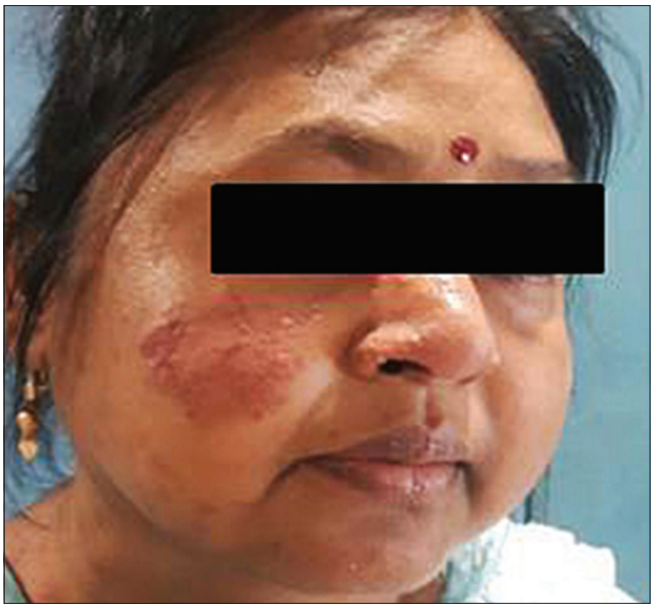

Figure 2b: Case 2: A 51-year-old woman with erythematous plaques on the face

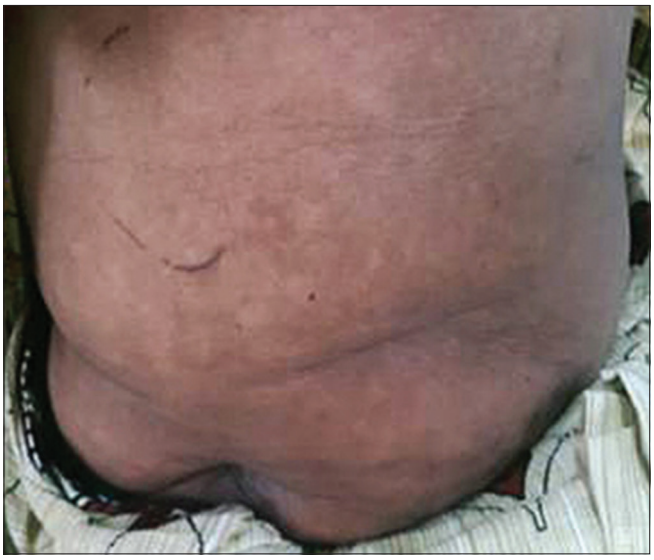

Figure 2c: Case 3: A 50-year-old woman with hypopigmented lesions on trunk

amastigotes [Figure 3a-c]. The patient did not agree to undergo treatment.

Case 3

A 50-year-old woman from West Bengal presented at the outpatient department with fever for 15 days without chill and rigor. In 2000, she was treated for visceral leishmaniasis (sodium antimony gluconate) but relapsed in 2008 and received miltefosine. Clinical examination revealed hepatosplenomegaly but no lymphadenopathy. Alongside, multiple hypopigmented patches appeared on the face, trunk and upper extremities 3–4 years ago [Figure 2c] devoid of photosensitivity, sensory abnormality or thickening of peripheral nerves. Slit-skin smear did not reveal any acid fast bacilli or Leishman Donovan bodies. The rK39 test was positive and enzyme-linked immunosorbent assay OD_{405} was 1.46 [Table 1] which was attributed to the previous history of visceral leishmaniasis; blood and splenic aspirates were collected, as also a skin biopsy based on a suspicion of macular post kala-azar dermal leishmaniasis. The splenic aspirate showed the presence of Leishman Donovan bodies, blood Internal Transcribed Spacer-1 polymerase chain reaction was positive, and parasite load 20,560/μg of gDNA, whereas restriction length *M. leprae*-specific repetitive element polymerase chain reaction was negative [Table 1]. The skin biopsy was Internal Transcribed Spacer-1 polymerase chain reaction positive, parasite load being 562/μg of gDNA [Table 1] and the polymerase chain reaction restriction fragment length polymorphism matched *L. donovani* [Figure 1b]. Accordingly, it was considered as a case of visceral leishmaniasis concomitant with dermal leishmaniasis, or para kala-azar dermal leishmaniasis. The patient initially received a single dose of LAmB (10 mg/kg body weight intravenous), followed by 15 mg/kg body weight intravenous in three divided doses within one week. A repeat Internal Transcribed Spacer-1 polymerase chain reaction and quantitative polymerase chain reaction in blood was negative, but skin biopsy remained Internal Transcribed Spacer-1 polymerase chain reaction positive, parasite burden being 1151/μg of gDNA.

Discussion

For cases 1 and 2, the lesions resembled post kala-azar dermal leishmaniasis, but as they resided in an area endemic for cutaneous leishmaniasis, establishing its etiology was important. Both were rK39-positive corroborating with

Table 1: Diagnostic and hematological features of the study population

Case no.	rK39 strip test	ITS-1 PCR	ELISA ($OD_{405\ nm}$)	Parasite load (parasites/µg gDNA)	Hb (g/dl)	WBC (cells/mm^3)	Platelet count (cells/mm^3)	Albumin/ globulin ratio
1	+ve	+ve	1.06	8654	14.5	2300	152,000	1.87
2	+ve	+ve	0.32	6692	11.8	4200	137,000	-
3	+ve	+ve	1.46	Blood: 20,560 Skin: 562	8.3	2100	110,000	0.60

rK39: Recombinant kinase 39, ITS-1: Internal Transcribed Spacer Sequence-1, +ve: Positive for *Leishmania*-specific rK39 strip test and ITS-1 PCR, ELISA: Enzyme-linked immunosorbent assay, Hb: Hemoglobin, WBC: White blood corpuscles, PCR: Polymerase chain reaction, OD: Optical Density

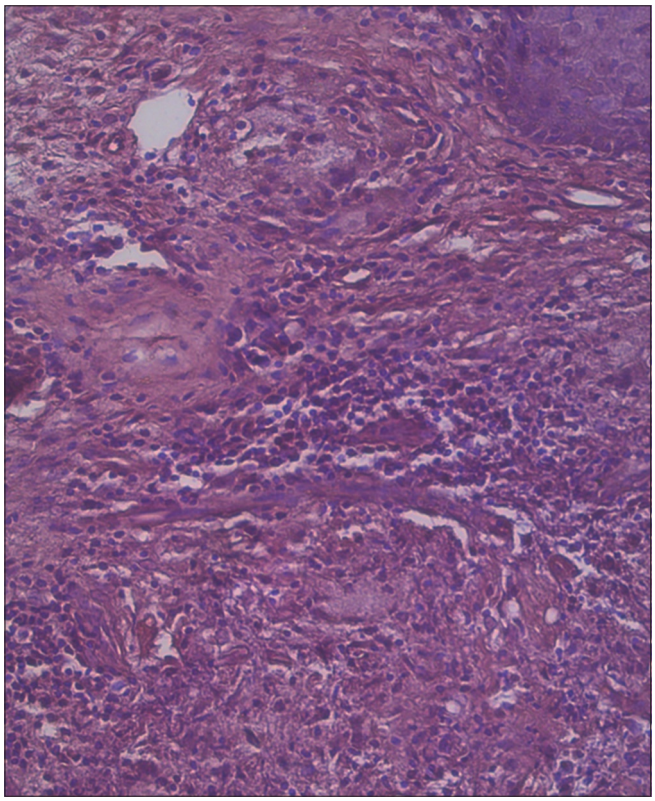

Figure 3a: Representative stained skin section showing severe lymphohistiocytic dermal infiltrate [hematoxylin and eosin(H and E), ×400]

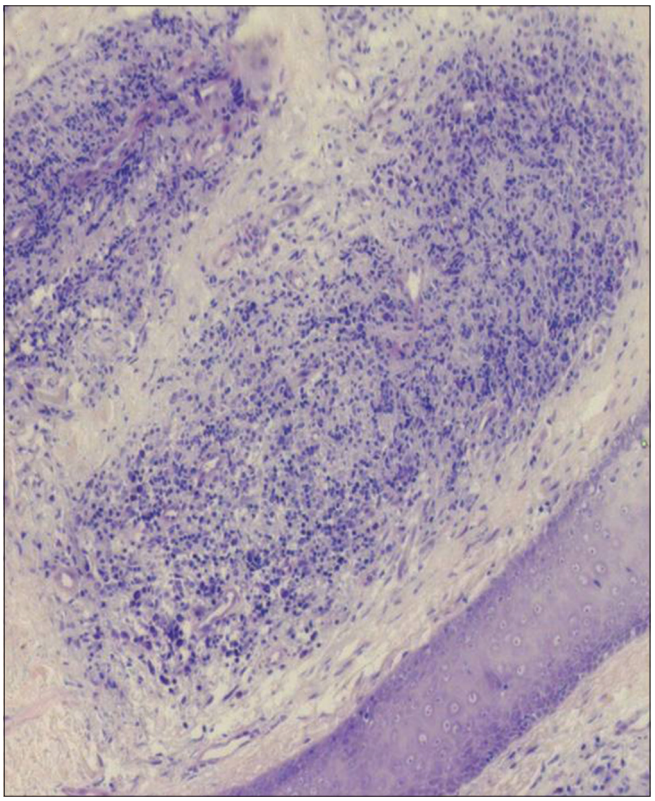

Figure 3b: Representative stained skin section showing amastigotes [Giemsa, black arrow, ×100]

previous studies, endorsing that rK39-positivity is not restricted to *L. donovani*.[10,11] It is also possible that as these individuals were residing in visceral leishmaniasis-endemic areas, they may represent the asymptomatic visceral leishmaniasis population.[12] For Case 3, the symptoms posed a diagnostic dilemma as fever and hepatosplenomegaly was concomitantly present with hypopigmented patches, suggestive of macular post kala-azar dermal leishmaniasis. An important limitation was that the patients were lost to follow-up and therefore, the study failed to provide the treatment outcome. Taken together, polymerase chain reaction restriction fragment length polymorphism is an invaluable tool to confirm disease etiology, emphasizing development of a referral system for establishing the causative organism and ensuring appropriate management of leishmaniasis.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patients have given their consent for their images and other clinical information to be reported in the journal. The patients understand that names and initials will not be published and due efforts will be made to conceal identity, but anonymity cannot be guaranteed.

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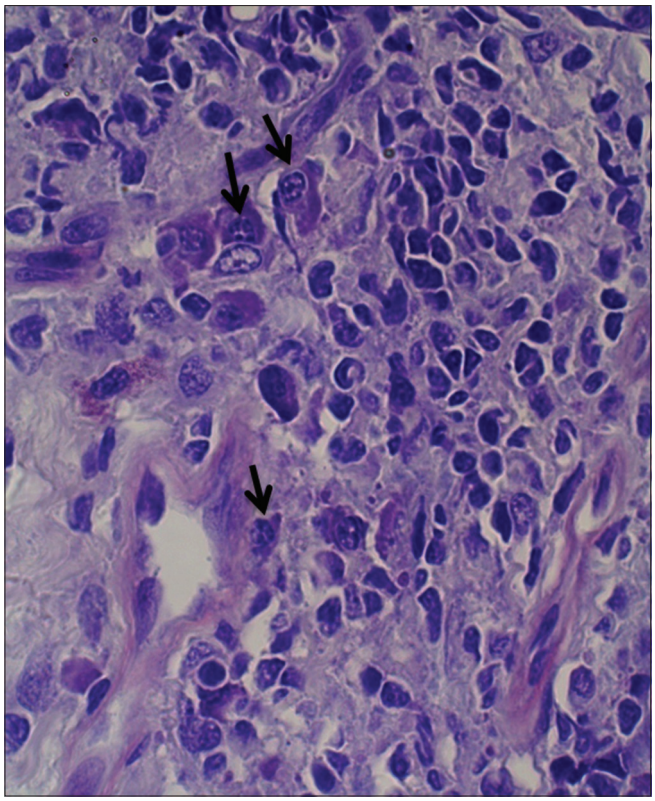

Figure 3c: Representative stained skin section showing amastigotes (Giemsa, *black arrow*, ×1000)

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Conflicts of interest

There are no conflicts of interest.

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