Comparative evaluation of diagnosis of post-kala-azar dermal leishmaniasis by qPCR and microscopy in a cohort of kala-azar patients treated with three new treatment regimens

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INTRODUCTION

In India, post-kala-azar dermal leishmaniasis (PKDL) cases are a reservoir of *leishmania* parasite and may have a major role in anthroponotic transmission of visceral leishmaniasis (VL). Early diagnosis and management of PKDL cases is important for attaining the 2017 VL elimination program target in the Indian sub-continent and for its long-term sustainability. This study was conceptualized to provide evidence to the Indian National Program about the occurrence of PKDL in VL patients treated with new regimens. In India, microscopic examination and molecular tests (PCR/qPCR) are performed only at the VL referral hospital RMRI in Bihar. This study compared the results obtained from quantitative PCR (qPCR) and microscopy for confirmatory diagnosis of PKDL.

METHODS

This cohort observational study was conducted in two district hospitals of Bihar (Saran and Vaishali) and at the RMRI in Patna. All VL patients (n=1,761) who had previously been treated in the pilot implementation study in 2012-15 with new treatment regimens, single dose AmBisome® (SDA), miltefosine and paromomycin combination for 10 days (M+P), or AmBisome® and miltefosine for 8 days (A+M), were invited to participate in the study.

Ethical clearance was obtained from the RMRI Ethical Committee.

After obtaining informed consent, the subjects were assessed for history and clinical manifestation of PKDL at a follow-up visit at least 24 months after VL treatment. All clinically suspected cases (skin lesions with past history of VL treatment) were screened by rK39 rapid diagnostic test. Probable PKDL patients (skin lesions and rK39 positive test) were referred to RMRI for parasitological confirmation by microscopy and qPCR in skin slit tissue.

RESULTS

Up to 24 March 2017, a total of 1,270 VL treated patients' data had been analysed. There were 76 (6%) clinical suspected cases, of whom 60 had a positive rK39 test and were referred to RMRI for confirmatory diagnosis. 2 cases were treated earlier for PKDL based on clinical symptoms. In 12 patients, the diagnostic could not be confirmed by either microscopy or qPCR. Diagnosis was confirmed by qPCR for 36/48 patients who had qPCR performed (75%), whereas microscopy was positive for 35/60 (58.3%) who had microscopy performed. qPCR was always positive when parasites were seen by microscopy. All confirmed PKDL cases presented macular lesions.

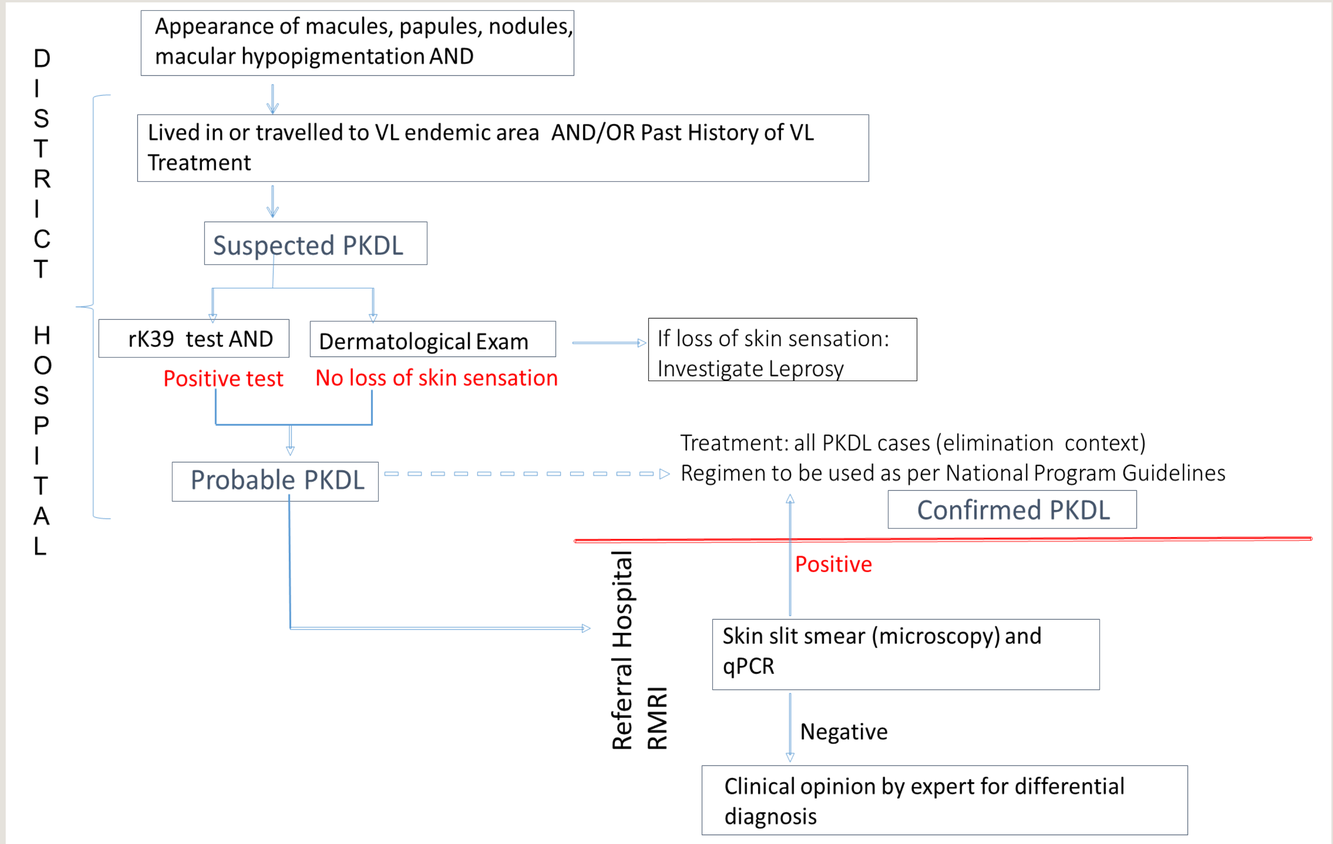
Comparative evaluation of qPCR and microscopy for detection of *L. donovani* in patients developing PKDL, previously treated with new VL treatment regimens

			No. (%) positive by	
Treatment Arm	**Parasitological diagnosis performed (n)**	**Negative by both microscopy and PC**	**qPCR (n= 48)**	**Microscopy (n=60)**
Single Dose AmBisome®	**18**	**4 (22%)**	**10 (20.8%)**	**12 (20%)**
AmBisome® and Miltefosine (A+M)	**11**	**6 (55%)**	**5 (10.4%)**	**1 (1.6%)**
Miltefosine and Paromomycin (M+P)	**31**	**2 (6.5%)**	**21 (43.7%)**	**22 (36.6%)**
Total	**60**	**12 (20%)**	**36 (75%)**	**35 (58.3%)**

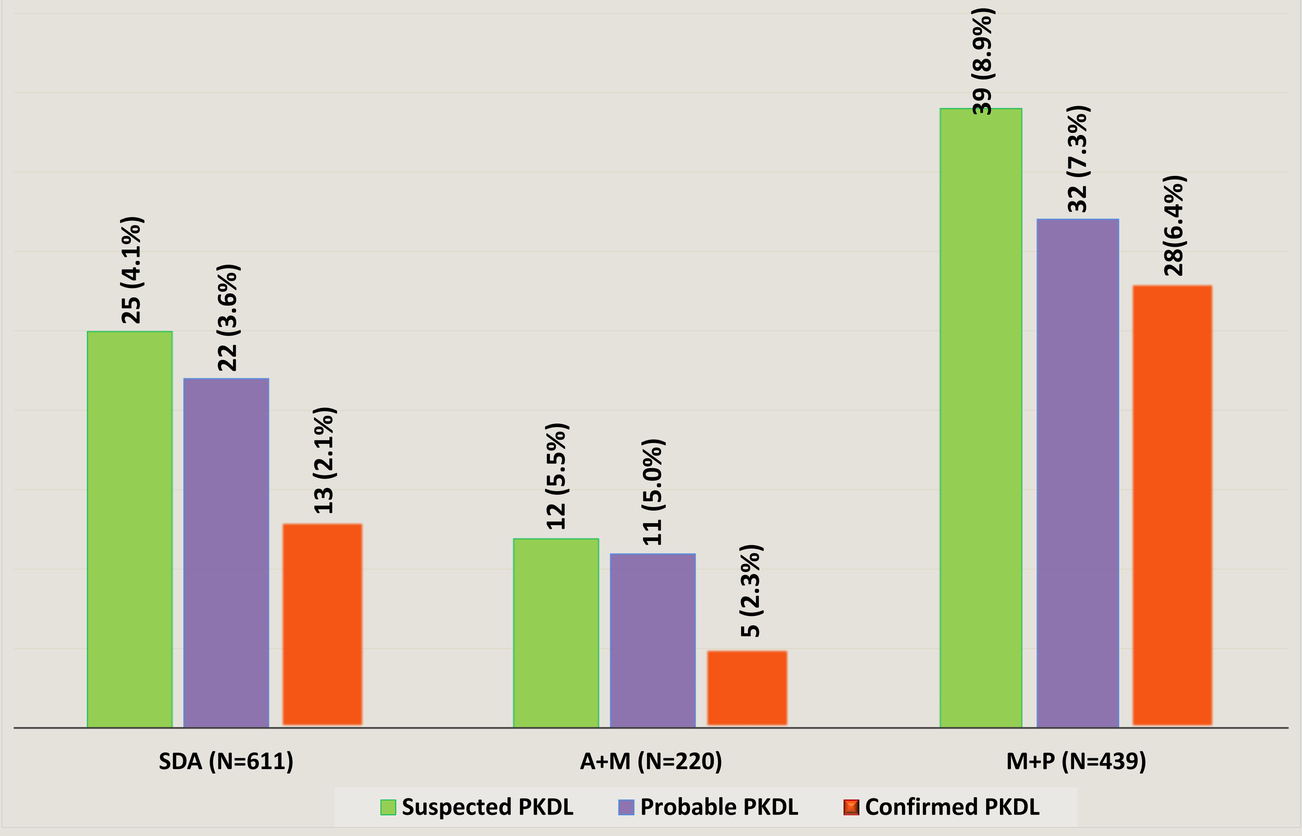
CONCLUSIONS

Preliminary results indicate that qPCR is highly sensitive for PKDL patients, particularly in macular lesions with low parasite density. PKDL is observed in patients after treatment of VL in each regimen. PKDL is a disease of major public health importance post-VL, as it is thought that PKDL could be a reservoir of *Leishmania donovani* infection and hence transmit VL. Therefore, early diagnosis and management of PKDL is an essential strategy for the goal of elimination of VL from the Indian subcontinent by 2017.

Algorithm for PKDL diagnosis



Prevalence of PKDL post-treatment of VL patients with three new regimens



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