J Vector Borne Dis 49, December 2012, pp. 266–267

Short Research Communication

Viscerotropic potential of parasites isolated from post-kala-azar dermal leishmaniasis cases : An experimental evidence

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Key words India; *Leishmania donovani;* leishmaniasis; viscerotropism

Post-kala-azar dermal leishmaniasis (PKDL) is marked by the appearance of non-ulcerative skin lesions with *Leishmania* parasites, which develop after apparent recovery of kala-azar (KA), though less commonly it has been known to occur in patients who have not suffered previously from visceral leishmaniasis (VL)[1, 2]. This skin reflection of visceral disease (i.e. PKDL) is characterized by hypopigmented macules and erythematous eruptions leading to formation of papules and nodules[3] in skin without involvement of viscera. The pathology caused by *Leishmania* parasites in PKDL, is known to be confined to only dermis in the host which raises one of the possibilities for the loss of viscerotropic nature of the parasites. In India, 10% of the patients of kala-azar develop PKDL and now the frequency is declining[3]. The viscerotropic nature of parasite of PKDL case is poorly understood till date and WHO in its technical report series 949 has recommended that studies are needed to determine the role of PKDL's reservoir for transmission of kala-azar[3]. The present study was, therefore, undertaken to elucidate this fact and to know whether the parasites which cause dermatotrophic lesions still retain viscerotropic or not. The study was approved by the Animal Ethics Committee of the Institute.

Biopsy of dermal lesion (preferably nodular type) of clinically diagnosed PKDL patients was inoculated in modified NNN medium overlaid with Locke's and incubated the culture at 25 ± 1ºC in BOD incubator for one month. Wet smear was examined microscopically on 2–3 days interval for isolation of promastigotes. Newly isolated promastigotes of PKDL cases (n =13, 10 with and 3 without previous history of KA) were adapted by subpassage and proper inoculum size for establishment of infection in susceptible animal.

Promastigotes (1×10^8 cells) of stationary phase of PKDL were inoculated intraperitoneally in inbred Balb/C mice (male, 20–22 g) in a batch (n=5) for each isolate. Animals were sacrificed between 3 and 4 months to observe the establishment of infection. Dabbed smears of spleen biopsy were stained with leishman stain and examined microscopically for amastigotes. A chopped portion of spleen and liver was inoculated in modified NNN medium overlaid with Locke's medium and wet smear was examined microscopically up to one month at 2–3 days interval for isolation of the promastigotes.

Amastigotes were found in stained dab smears of spleen of all the 13 isolates, as well as promastigotes were also recovered in culture of spleen biopsy of mice of all the isolates. This showed that laboratory inbred Balb/C mice were infected with the PKDL and the PKDL isolates exhibited existence of viscerotropic nature as a change of biological behaviour of parasites in animals. Hence, parasites may not loose their capability to infect viscera and corroborate that the *L. donovani* causing VL and PKDL are the same[4].

The finding that PKDL cases got VL infection after getting the immunosuppressive infection like measles and repeated attack of malaria and tuberculosis in human[5] supports our finding of viscerotropic nature of PKDL isolates in animals. In PKDL cases, some scientists have reported the presence of parasites also in bone marrow and lymph node[6, 7] which may indicate the visceralization of parasites. The presence of DNA of *L. donovani* in bone marrow and peripheral blood of PKDL but no evidence of visceral disease[8] also confirm the transit phase of parasites in between skin and viscera that might be due to immunological hindrance in human. Therefore, our findings confirmed that parasites causing PKDL, retain the biological behaviour of viscerotropism. Thus, in the absence of apparent animal reservoir like in India, PKDL is an important source of parasites to transmit the disease. The work emphasizes the treatment of PKDL to check the transmission of disease[9]. Further studies are warranted to determine the time period of onset of PKDL after treatment of kala-azar cases and how long the parasite remain viable for viscerotopic conditions. There is

also need to ascertain/strengthen the occurrence of cutaneous leaishmaniasis by *L. donovani* in Himachal Pradesh (India)[10], Sri Lanka[11] and Kenya[12] which may elucidate the link between CL and PKDL.

ACKNOWLEDGEMENTS

Authors are thankful to Mr N.L. Kalra and Dr R.C. Dhiman (NIMR, Delhi) for valuable advice and to Messrs S.K. Chaturvedi, S.K. Sinha and B.N. Roy for technical assistance.

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Received: 3 July 2012 *Accepted in revised form:* 14 October 2012