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Sri Lankan cutaneous leishmaniasis is caused by *Leishmania donovani* zymodeme MON-37

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Abstract

Sri Lankan cutaneous leishmaniasis (CL), once considered sporadic, is fairly widespread in some parts of the country. Identification of 5 isolates from 4 CL patients by enzyme analysis during 2002 showed that they were all *Leishmania donovani* zymodeme MON-37, the parasite which also causes visceral leishmaniasis in India and East Africa.

Keywords: cutaneous leishmaniasis, *Leishmania donovani*, isoenzyme analysis, Sri Lanka

Introduction

Despite numerous reports from many parts of the country, cutaneous leishmaniasis (CL) is not widely recognized as an endemic disease in Sri Lanka. The first autochthonous case reported was from the Southern Province (Athukorale *et al.*, 1992). Since then, sporadic cases have been reported from different parts of the island (Seneviratne *et al.*, 1995; Naotunne *et al.*, 1999). During 2001, nearly 100 cases were diagnosed, mainly among patients from the north and east of the country (Siriwardana *et al.*, 2003). Little information is available on the causative agent, vector, and possible reservoir hosts. The parasite was recently cultured (Ihalamulla *et al.*, 2002). Characterization and identification of the causative agent is reported here.

Materials and Methods

During 2002, 27 clinically suspected cases referred from the General Hospital in Anuradhapura, the Military Hospital in Colombo and the National Hospital of Sri Lanka in Colombo were examined and their histories recorded. Slit-skin smears stained with Giemsa's stain were examined for amastigotes. Lesion aspirates were cultured in Evan's modified Tobie's medium (WHO, 1990) with 1% glucose saline as overlay. Punch biopsies from the edges of the lesions were aseptically transferred to sterile transport medium of 10% fetal calf serum in RPMI (Dedet *et al.*, 1999). The fluid phase of cultures positive for promastigotes from 5 isolates with parasite counts ranging from 0.07×10^6 to 0.6×10^6/mL and biopsy samples from 22 patients were sent to the Centre National de Référence des *Leishmania*, Montpellier, France (CNRL) for species identification by isoenzyme analysis (Rioux *et al.*, 1990) based on starch gel electrophoresis with the following 15 enzymes: malate dehydrogenase (MDH, EC 1.1.1.37), malic enzyme (ME, EC 1.1.1.40), isocitrate dehydrogenase (ICD, EC 1.1.1.42), 6-phosphogluconate dehydrogenase (PGD, EC 1.1.1.44), glucose-6-phosphate dehydrogenase (G6PD, EC 1.1.1.49), glutamate dehydrogenase (GLUD, EC 1.4.1.3), diaphorase NADH (DIA, EC 1.6.2.2), nucleoside purine phosphorylases 1 and 2 (NP_1, EC 2.4.2.1. and NP_2, EC 2.4.2.*), glutamate oxaloacetate transaminases 1 and 2 (GOT_1 and GOT_2, EC 2.6.1.1), phosphoglucomutase (PGM, EC 5.4.2.2), fumarate hydratase (FH, EC 4.2.1.2), mannose phosphate isomerase (MPI, EC 5.3.1.8), and glucose phosphate isomerase (GPI, EC 5.3.1.9). Isoelectrofocusing was used for greater resolution of glutamate oxaloacetate transaminase (Piarroux *et al.*, 1994).

Following laboratory confirmation of diagnosis, the patients were referred to local dermatologists for treatment and follow-up.

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Results and Discussion

Five isolates, corresponding to 4 patients, were typed, 1 from a culture (MHOM/LK/2002/L60), and 4 from biopsies (MHOM/LK/2002/L59, MHOM/LK/2002/L60, MHOM/LK/2002/L75, and MHOM/LK/2002/L78). They were all *Leishmania donovani* zymodeme MON-37. All 4 patients were treated with local application of liquid nitrogen (cryotherapy) and they responded with complete healing of lesions within 12 weeks.

Zymodeme MON-37 is close to zymodeme MON-2, the most common zymodeme in India (Thakur *et al.*, 2001); the 2 zymodemes are differentiated by a single enzyme, PGD. Zymodeme MON-37 has been reported previously from cases of human visceral leishmaniasis (VL) from Himachal-Pradesh State, India (Moreno, 1989), Ethiopia (data from CNRL) and Israel (Schnur *et al.*, 2001), and from *Phlebotomus martini* from Kenya (Moreno *et al.*, 1986) and Ethiopia (data from CNRL).

This is the first isolation of *L. donovani* zymodeme MON-37 from human cases of CL. *Leishmania donovani* is usually associated with VL in all Asian and East African foci, but it has occasionally been the cause of CL (Pratlong *et al.*, 1995). This new evidence of occasional dermotropism of *L. donovani* emphasizes the possibility of a genetic susceptibility of certain individuals that requires genetic–epidemiological study.

Owing to the paucity of information on human CL in Sri Lanka, studies are continuing to identify a possible reservoir host and the vectors, and to determine if CL in Sri Lanka is caused by a single zymodeme or if enzyme polymorphism exists, related to a more complex epidemiological structure of the focus. The local sandflies have been described (Brunneti, 1912). *Phlebotomus argentipes* and *P. stantoni* are considered putative vectors (Lewis, 1978). Our finding is significant for the proper treatment of patients, future studies, and planning of effective control measures.

Ethical statement

Ethical approval for this study was obtained from the Ethics Committee, University of Colombo, Colombo, Sri Lanka.

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