# USE OF A PCR BASED TECHNIQUE FOR DIAGNOSIS OF CUTANEOUS LEISHMANIASIS IN SRI LANKA

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Cutaneous leishmaniasis has been detected from many areas and is now considered an established disease in Sri Lanka. Laboratory confirmation of the disease is usually made by detection of *Leishmania* parasite in Giemsa stained smears and /or isolation in culture. Histology of the biopsy specimens has also been used in diagnosis .The detection rate in stained smears is estimated to be about 70-80% whilst isolation in culture is reported to be difficult with zoonotic species. Histology is time consuming and is of limited sensitivity when parasite load is low. In the last decade, molecular techniques had been developed to assist with accurate diagnosis. In this paper we present the development of a polymerase chain reaction (PCR) based technique to diagnose cutaneous leishmaniasis in Sri Lanka.

Patients tested were those who presented to the Dermatology units in Teaching Hospitals of Kandy and Kurunegala between June 2001 and July 2003 and referred to the Department of Parasitology, Faculty of Medicine, University of Peradeniya for laboratory confirmation. Biopsy samples from skin lesions in each patient were obtained under aseptic conditions. One part of the collected tissue was used for parasitology while the remainder was stored in a sterile vial at –20° C for PCR assay.

DNA was extracted using a genomic DNA isolation kit (DNAzol- GIBCO BRL) and amplified using a set of Old World *Leishmania* genus specific primers to produce an amplicon of 260 bp. A standard protocol (40 cycles,. 30 s at 94° C,30 s at 56° C and 20 s at 72° C ) was used for amplification. Negative and positive controls were also employed in each reaction. The amplified products obtained were separated in agarose gel stained with Ethidium Bromide and visualized under UV-light.

Of the 47 patients referred 17 were tested by both parasitological and PCR techniques. Four of these 17 patients were diagnosed to be negative for cutaneous leishmaniasis on both tests. All remaining 13 were diagnosed positive by PCR. In comparison, however, only 9 of 13 were diagnosed positive by parasitological examinations.

Though marginally expensive, this study has shown that the PCR is a more sensitive and relatively quick method for diagnosis of cutaneous leishmaniasis.

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