

#### OP 4

### **Restriction Fragment Length Polymorphism (RFLP) of the Internal Transcribed Spacer 1 (ITS1) region of cutaneous leishmaniasis causing *Leishmania donovani* in Sri Lanka**

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**Objectives:** To isolate PCR quality DNA from punch biopsy samples of 35 suspected cutaneous leishmaniasis (CL) lesions, carry out Internal Transcribed Spacer 1 (ITS1) PCR, analyse Restriction Fragment Length Polymorphism (RFLP), sequence of ITS1 region of 10 randomly selected patient samples and to determine the genetic variation among the causative parasites.

**Methods:** Punch biopsies (3mm) from CL lesions (n=35) were taken and stored in NET buffer at -20C. DNA was extracted using a commercially available kit. ITS1 PCR was carried out using previously described primers. PCR products were digested with HaeIII, run in a 1.7 % ethidium bromide gel and visualized under UV light. Same ITS1 PCR products of 10 randomly selected samples were sequenced commercially. Analysis of sequences was carried out with CLUSTALW2 multiple sequence analyzing software.

**Results:** All 35 CL samples showed the same *Leishmania donovani* ITS1 RFLP pattern. The BLAST search confirmed that the 10 sequenced Sri Lankan strains belong to *L. donovani*. Multiple sequences analysis showed that Sri Lankan *L. donovani* strains are highly homogenous in the ITS1 regions. However, the Sri Lankan strains showed, few indels in the ITS1 region when compared with the *L. donovani* ITS1 sequences originated from India, Sudan and Ethiopia.

**Conclusions:** Cutaneous leishmaniasis in Sri Lanka is still caused only by *L. donovani* and ITS1 region of the *L. donovani* strain of Sri Lankan origin is highly homogenous and conserved.