

Comparison of PCR analysis with the Routine Histopathological Diagnostic Technique in Detecting Leishmania Donovanii in Clinically Suspected Cutaneous Leishmaniasis Samples

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Cutaneous leishmaniasis is a rapidly emerging endemic disease in Sri Lanka that is caused by the specific strain, *Leishmania donovani* zymodeme MON-37. The condition shows a broad spectrum of clinical and histopathological manifestations making the disease discrimination hard. This study focuses on comparing the diagnostic ability of PCR based molecular analysis with the routine histopathological diagnostic technique in detecting *Leishmania donovani* in clinically suspected cutaneous leishmaniasis samples. Hematoxylin and eosin-stained tissue sections were prepared using fifty clinically suggestive patient sample wax blocks. The presence of amastigotes was recorded by observing under high power ($\times 40$) magnification. Sample DNA was extracted from the respective formalin fixed paraffin embedded biopsy wax block sections and samples with less than 20 $\mu\text{g/ml}$ of DNA were re-concentrated. PCR amplification was carried out using primers specific to the *internal transcribed spacer 1* (ITS-1) of the ribosomal operon of *Leishmania* (kDNA) and analysed using 1.5% Agarose gel electrophoresis. Out of the 50 samples only 13(26%) of the samples were having amastigotes while the rest demonstrated consistent histopathological reactions. Upon DNA quantification only (24) 48% of the 50 samples had adequate amount of DNA extracted (greater than or equal to 20 μg). This included all the 13 samples that had visible amastigotes in their respective H&E-stained tissue sections. Out of the 24 samples with adequate extracted DNA, 21 samples gave positive results with PCR (87.5%) and only 03 samples gave negative results (12.5%). From the 21 PCR positive samples 10 (47.61%) had clearly visible amastigotes in their respective H&E-stained tissue sections. The samples that lacked adequate amount of DNA even after re-concentrating the extracted DNA, showed a statistically significant relationship (P-value=0.029), where the amount of extractable DNA decreased with the processed period. Samples that were processed less than one year ago had high amounts of extracted DNA (26%) and only 02% of samples that were processed three or more years ago had adequate DNA. The calculated sensitivity, specificity, positive predictive value and negative predictive values for kDNA based PCR diagnostic method compared to the routine histopathological observations were 76%, 70.30%, 47.60% and 89.65% respectively. A statistically significant difference between the two diagnostic methods were observed with calculated DF=2 and P-value of P=0.0001. A Kappa value (k) of 0.46 showed a moderate agreement between the two diagnostic methods. Results of the study concluded PCR as a highly sensitive diagnostic method in detecting *Leishmania donovani* in clinically suggestive cutaneous leishmaniasis patient samples when adequate amount of sample DNA is extractable.

Keywords: Cutaneous Leishmaniasis, *Leishmania Donovanii*, Sri Lanka, PCR