ABSTRACT

Following the publication of harmonized clinical trial methodologies in localized cutaneous leishmaniasis (LCL), development and validation of an outcome measurement instrument (OMI), including patient perspectives is a timely requirement. Considering the uniqueness of cutaneous leishmaniasis (CL) in Sri Lanka, *in situ* changes of predominant immune cell densities during the healing process is worthwhile to investigate.

Objectives of this study were to develop and validate a clinical score as an OMI for LCL; to describe patient perspectives of having CL using the same tool; to describe serial digital photography as supportive evidence of the healing process of LCL for OMI assessment, and to perform dual fluorescence *in situ* hybridization-immunohistochemistry (FISH-IHC) studies to assess cell densities of monocyte lineage (CD68+) and T cell lineage (CD3+) in response to standard treatment.

Forty patients were allocated for the study after confirming CL by slit skin smears (SSS) and/or polymerase chain reaction (PCR). The OMI was developed, and its validity, reliability, responsiveness, interpretability, and feasibility were assessed with appropriate statistical methods. Patient perspectives of having CL were evaluated by two open ended questions and analyzing answers with pen and paper for thematic content. Healing process of LCL lesions were photo-documented after optimizing serial digital photography. Dual FISH- IHC studies were performed on 25 paired formalin-fixed paraffin-embedded skin biopsy samples at baseline and after 4 doses of weekly intralesional sodium stibogluconate (SSG) and cells were manually counted using a software.

SSS was positive in 31/40 (77.5%) and PCR was positive in 36/36 (100%) patients. Developed clinical score accomplished content validity and face validity. Criterion validity of subjective score was “excellent” at 3 months (AUC of ROC = 0.992, 95% CI = 0.974 – 1.000, $p < 0.001$) and at 6 months (AUC of ROC = 1.000, $p = 0.018$). Both inter-rater and intra-rater reliabilities were “excellent” for objective, subjective and sequelae assessments (ICCs > 0.9; $p < 0.001$). Responsiveness measured by repeated measures ANOVA was statistically significant for subjective score [$F(2.674, 77.553) = 201.803$, $p < 0.001$] and

for sequelae score [F (2.106, 61.088) = 49.685, $p < 0.001$]. OMI was interpretable with ROC cut-off values and a subjective score ≤ 3.5 indicated a healed lesion at 3 months (92% sensitivity) and at 6 months (100% sensitivity). OMI was feasible in several aspects including completion time (mean = 2.68 minutes; SD = 0.44, range = 2.14 to 3.54 minutes). Patient perspectives in having LCL, frequently contained a psychological theme. Optimized serial digital photographs provided supportive evidence of healing process of LCL. Baseline mean cell densities of both CD68+ and CD3+ declined after 4 weeks of treatment, without statistical significance.

In conclusion, the OMI developed in this study to assess the outcome measures in LCL is statistically proven to be a valid, reliable, responsive, interpretable and feasible instrument. Patients perceived CL as a cause for concern over the course of treatment and their problems frequently contained a psychological theme. Optimized serial digital photographs can be used as supportive evidence of the healing process of LCL lesions. Baseline mean cell densities of both CD68+ and CD3+ in skin biopsies declined without statistical significance after 4 doses of weekly intralesional SSG.