OP-09: Usefulness of different fractions of cell lysate of *Leishmania* in serodiagnosis of leishmaniasis: a preliminary study

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Introduction: Leishmaniasis is a vector-borne parasitic disease. Three major clinical forms exist: visceral leishmaniasis (VL), cutaneous leishmaniasis (CL) and mucocutaneous leishmaniasis (MCL). CL is predominant in Sri Lanka. Established serological assays (rK 39 dipstick assay) result in a lower response for local patients, demanding a local parasite-based assay. Therefore, an in-house enzyme-linked immunosorbent assay (ELISA) was developed using whole cell lysate of parasites. Use of whole cell lysate of *Leishmania* species in ELISA can result in non-specific binding with the ELISA plates leading to unreliable results. We examined the usefulness of different fractions of antigens in ELISA as a better diagnostic approach in leishmaniasis.

Methods: The promastigotes of *Leishmania donovani* were cultured following in-house protocols. Whole cell lysate was prepared and fractionated to obtain whole crude lysate as fraction one (F1), supernatant of whole crude lysate as fraction two (F2), total soluble antigens (F2 and mostly peripheral membrane antigens) as fraction three (F3) and insoluble antigens as fraction four (F4). A modified micro-Lowry assay was carried out to estimate protein content. ELISA was carried out using 60 serum samples (n=30 laboratory-confirmed CL positive and 30 controls) using the in-house protocol. Ethical approval (EC-14-154) was obtained from the Ethics Review Committee, University of Colombo. A literature-based antigen study was carried out considering 20 *Leishmania* antigens to identify the distribution of antigens in each antigen fraction.

Results: Higher ELISA values were obtained for F1 (0.476) and F3 (0.444). F4 (0.158) reported the lowest values. ELISA absorbance values of control samples of F1 (0.21) were higher in comparison to F3 (0.177). According to literature-based antigen studies, F1 (20) and F3 (14) consist of a higher number of antigens which are mostly immune-dominant compared to F2 (12) and F4 (9).

Conclusions: F3 may be better than F1 for patient diagnosis. Antigen profiling can be carried out for confirmation.

Keywords: *Leishmania donovani*, cutaneous leishmaniasis, whole cell lysate, antigen fractions, enzyme-linked immunosorbent assay