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USE OF FRACTIONS ISOLATED FROM CRUDE LYSATE OF *LEISHMANIA* IN SEROLOGY AND ANALYSIS OF CLINICAL EPIDEMIOLOGICAL DATA: A PRELIMINARY STUDY

A.U. Maha Gamage[1,2*], T. Rathnayaka[2], B. Deepachandi [2,3], S. Weerasinghe [2], T.P. Andrahennadi [1], C. Witharana [1] and Y. Siriwardana [2]

[1]Department of Biochemistry and Molecular Biology, Faculty of Medicine, University of Colombo, Sri Lanka.
[2]Department of Parasitology, Faculty of Medicine, University of Colombo, Sri Lanka.
[3]Department of Biomedical Science, Faculty of Science, NSBM Green University, Sri Lanka
[*]Udeshika9319@gmail.com

Cutaneous Leishmaniasis (CL) is the predominant local leishmaniasis. Established serological assays (rK 39 dipstick assay) result in a lower response, making it less specific for local patient diagnosis. This demanded a local Enzyme-Linked Immunosorbent Assay (ELISA) based on the whole cell lysate of the local parasite. However, using whole cell lysate of *Leishmania* species can result in non-specific binding with the ELISA plates, leading to unreliable results. The current study was carried out to obtain a fraction better than whole cell lysate. The *Leishmania donovani* parasites were cultured following established in-house protocols. Cell pellets were prepared from mass cultures and fractionated initially by cell lysis, freeze-thawing, vigorous agitation when required, and centrifugation to obtain F1- whole crude lysate and F2-Soluble fraction, F3-total soluble fraction, and F4-integral membrane antigens. Protein was estimation following a modified micro-Lowry procedure. ELISA was carried out following established in–house protocol for 60 serum samples (n = 30 laboratory-confirmed CL positive and 30 controls). Ethical approval (EC-14-154) was obtained from the Ethics Review Committee, University of Colombo. F1 (0.476) and F3 (0.444) reported the highest absorbance values. The clinical-epidemiological data study was carried out. Data was collected from patients recruited and analysed based on the absorbance values of the current study. The highest ELISA absorbance was reported from the Southern area from early reported lesions, less than 3 months. Early lesions were ulcerated and were 2-3 cm found in the body except the head, neck, and limb areas. Absorbance values of control samples of F1 (0.210) are higher than F3 (0.177), indicating F3, a better fraction than F1. However, this can be confirmed with further analysis of antigen profiling. The findings from the clinical epidemiological data study were compatible with the findings of previously published local studies indicating higher seropositivity for lesions identified from Northern Province with early and 2-3 cm long ulcerated lesions.

Keywords: Clinico-epidemiological data, Enzyme-linked immunosorbent assay, Fractionation, *Leishmania donovani*, Whole crude lysate.