A clinical and diagnostic study of rickettsial disease in Nawalapitiya hospital
&
Application of PCR technique for rickettsial disease mapping In Sri Lanka

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A thesis submitted to the Faculty of Medicine

in fulfillment of the requirements for the degree of

Master of Philosophy

of

University of Peradeniya, Sri Lanka

January 2007

Abstract

Typhus fevers are prevalent through out the world including the Asia-Pacific region. Even though 'typhus' has been recognized as a disease entity in Sri Lanka, confirmation has not been possible due to the unavailability of reliable tests. The aim of this study was to confirm the diagnosis and develop a molecular method of diagnosis, which together with serology could be used in patient care and to map the epidemiology of the disease.

The study was carried out during a 3 year period from July 2001 to June 2004. 153 patients presenting with fever and rash to the paediatric unit, Base Hospital, Nawalapitiya were included in this study. A validated questionnaire was used to obtain clinical and demographic data from each patient. Acute and convalescent sera obtained in the first year of the study were used to determine whether the disease entity was rickettsial in origin. Using an indirect immunofluorescence assay (IFA), the Rickettsial Reference Laboratory (RRL) France tested 68 sera from 44 patients against a panel of 13 rickettsial antigens which included the typhus group (*R.typhi*), scrub typhus (*O tsutsugamushi* strains Gillian, Kato, Karp and Kawazaki) & 8 spotted fever group rickettsiae. Using criteria for interpretation developed by the RRL, 17 patients tested positive for *R japonica*, *R.honei*, *R.helvetica* and *R.felis*. 9 within this group were also positive for *R.slovaca*.

A genus specific PCR was developed using a primer designed to cover 44 rickettsial species, including the 5 spotted fever group (SFG) species positive by IFA. A positive control

(*R.montana* obtained from RRL, France) and negative controls were used in each run. All the IFA positive sera gave a positive PCR with the band size of 322bp.Four of 10 IFA negative sera were also positive by PCR.

Using the genus specific PCR and a commercial kit for detection of scrub typhus IgG and IgM, an attempt was made to document the presence of spotted group fevers and scrub typhus disease in the different provinces of Sri Lanka. 79 blood samples were received from clinicians in 12 of 54 targeted hospitals. All 9 provinces were represented. All samples received from Jaffna, Matara and Kuliyapitiya were scrub typhus IgM positive and PCR negative. In contrast, all samples tested from Nawalapitiya were PCR positive and scrub typhus serology negative. In the other provinces, mixed results were obtained. Tentative conclusions from these preliminary attempts to 'map' an infectious disease suggest that both scrub typhus and the SFG rickettsiae co-exist in Sri Lanka, though one or the other is dominant in some areas. This would concur with the possibility that vector habitats are different in the vastly different terrains seen in Sri Lanka.

This study has shown that the SFG rickettsiae and *O tsutsugamushi* cause disease in Sri Lanka and has described the tests which could be used in diagnosis as well as disease mapping. Sequencing the PCR gene product would enable identification of the precise aetiological agent within the 5 species giving positive results with the IFA. Systematic testing of patients

with 'typhus' like fevers using these 2 tests would also be helpful in understanding the epidemiology of this group of diseases in Sri Lanka.