MOLECULAR CHARACTERIZATION AND PHYLOGENETIC IDENTIFICATION OF HUMAN RICKETTSIAL SPECIES FROM THE PATIENTS

K. G. A. D. Weerakoon[1], R. P. V. J. Rajapakse[2], S. A. M. Kularatne[3], S. Wikramasinghe[4], S. B. Adikari[5], K. B. A. T. Bandara[2] and S. L. C. A. Dharmarathna[2].

[1] *Department of Parasitology, Faculty of Medicine and Allied Sciences, Rajarata University of Sri Lanka,* [2]*Department of Veterinary Pathobiology, Faculty of Veterinary Medicine and animal sciences,* [3]*Department of Medicine, Faculty of Medicine,* [4] *Department of Parasitology, Faculty of Medicine,* [5]*Department of Anatomy, Faculty of Medicine, University of Peradeniya,*

Rickettsioses are emerging vector borne infectious diseases. Rickettsiae are a group of alpha-proteobacteria found as an obligatory intracellular parasite of eukaryotic cells. The genus *Rickettsia* is classified in the order *Rickettsiales* and in the family Rickettsiaceae within the á-sub division of the class Proteobacteria. Rickettsiacea family has two genera, "*Rickettsia*" and "*Orientia*" composed of three antigenically distinct groups. These include scrub typhus, spotted fever and murine typhus. Many different species of these three antigenic groups of rickettsiae have been identified in the world[1]. Furthermore, studies have shown cases of scrub typhus, spotted fever and murine typhus from all nine provinces of Sri Lanka[2,3]. However, only serological evidence is available to confirm the existence of these pathogens in Sri Lanka. Moreover, molecular characterization of genetic make-up and the identification of rickettsial organisms occurring in the local settings have not been done thus far. Therefore, the objective of the present study is to identify the different rickettsial species present in Sri Lanka.

Patient recruitment and sample collection for the study were done at medical unit, Teaching Hospital, Peradeniya from November, 2009 to October, 2011 Patients clinically diagnosed as having rickettsial infections were included in the study group and clinical data and sample collection were done after obtaining the informed written consent. Seropositivity of the infection was confirmed using immunofluorescent antibody assay. Of them, cutaneous biopsy samples were obtained from six patients. Samples were stored at -20^{0}C until used. DNA was extracted using QIAGEN spin column kit (Qiagen Sciences, Maryland, 20874, USA).

Nested polymerase chain reaction (nPCR) assay was performed on extracted DNA to amplify the 17 kDa antigen gene. The primers used for primary and nested PCRs were R17-122, forward (5'- *CAG AGT GCT ATG AAC AAA CAA GG-3'*); R17-500, reverse (5'- *CTT GCC ATT GCC CAT CAG GTT G* -3') and TZ 15, forward; (5'- *TTC TCA ATT CGG TAA GGG C* -3') TZ 16, reverse (5' - *ATA TTG ACC AGT GCT ATT TC* - 3'). Total volume of the primary and nested reaction solutions were 25 ìl each. The reaction mixture included, 5X PCR buffer 5 ìl, Taq DNA polymerase (5X Colourless Go Taq, 5 units / ìl solution) 0.25 ìl, 2.5mM dNTP - 2 ìl, forward and reverse primers (2.5 ìM solution) 2.5 ìl each, Magnesium chloride (25 mM solution) - 3 ìl, template DNA - 1 ìl and sterile distilled water 7.75 ìl. In the nPCR, 2.5 µl of 1:8 diluted primary PCR products was used as a template DNA. PCR products purification and DNA sequencing were carried out in the Institute of Biochemistry, Molecular Biology and Biotechnology, University of Colombo, Sri Lanka. DNA sequence similarity searching was performed using the NCBI BLAST programme (http://www.ncbi.nlm.gov/blast/). Multiple sequence alignments were done using CLUSTAL W and/or BIOEDIT (Version: 7.0.5.3). Phylogenetic analysis was done using maximum likelihood method in BIOEDIT (Version: 7.0.5.3).

Two hundred and ten patients were clinically diagnosed as having rickettsial infection and 134 (63. 8%) of them were serologically positive with higher titre values (IgG titre >1/256 and IgM titre >1/32). PCRs were done to amplify the rickettsial 17 kDa antigen gene fragment using six skin biopsy samples. We have successfully amplified the 247 bp region of the 17 kDa spotted fever group specific protein antigen. The edited sequence lengths of six samples (R166, R352, R401, R407, R413 and R453) were 234, 246, 225, 248, 229 and 236 bp respectively. Nucleotide substitutions were observed at the position 44, 91 and 202. Of the six samples, three (R352, R401 and R407) were phylogenetically clustered in the spotted fever group of rickettsiae. Out of these three, two isolates (R352 and R407) were clustered in a group with *R. conorii sub sp. conorii, R. rickettsii and R. parkeri* while the other one (401) was clustered with *R. honei*. Interestingly, other isolates (R166, R413 and R453) were clustered as a separate group (figure 1).

+Rickettsia typhi
14
+Rickettsia typhi
Typhus Group

+R352
11
+R407
10
+ Rickettsia conorii subsp. Conorii
+ Rickettsia parkeri
8
+ Rickettsia rickettsii
6
+ Rickettsia philipii
5
+Rickettsia sp.
7
+Rickettsia sp.
13
3
+R401
+Rickettsia honei
12
+ Rickettsia sp.
Spotted Fever Group
+R453
1
+R166
R413

Fig 1: Mid rooted phylogenetic tree inferred by maximum-likelihood method

The study clearly showed the existence of Spotted fever group of rickettsial infections in the Central Province of Sri Lanka. Furthermore, the existence of a separate cluster denotes a possibility of having a different species of *Rickettsiae* in the region. Further studies are planned to determine the species at strain level based on the 18S rDNA gene.

REFERENCES

1. Richards AL. Worldwide detection and identification of new and old rickettsiae and rickettsial diseases. FEMS Immunol Med Microbiol. 2012; 64 (1),.107-10 pp.
2. Kularatne SA, Edirisingha JS, Gawarammana IB, Urakami H, Chenchittikul M, Kaiho I. Emerging rickettsial infections in Sri Lanka: the pattern in the hilly Central Province. Trop Med Int Health. 2003; 8 (9),.803-11 pp.
3. Liyanapathirana VC, Thevanesam V. Seroepidemiology of rickettsioses in Sri Lanka: a patient based study. BMC Infect Dis. 2011; 25; 11, .328 p.