Investigation of factors leading to microbial translocation in acute dengue infection

by

Narangoda Liyanage Ajantha Shyamali

Mphil **2019**

Investigation of factors leading to microbial translocation in acute dengue infection

by

Narangoda Liyanage Ajantha Shyamali

Thesis submitted to the University of Sri Jayewardenepura for the award of the Degree of Master of Philosophy in Immunology on 2019

“The work described in this thesis was carried out by me under the supervision of Prof. Gathsaurie Neelika Malavige and Prof. Graham Ogg. A report on this has not been submitted in whole or in part to any university or any other institution for another Degree/Diploma."

Narangoda Liyanage Ajantha Shyamali

25th June 2020

Certification of Supervisors

"We certify that the above statement made by the candidate is true and that this thesis is suitable for submission to the University for the purpose of evaluation."

Prof. Gathsaurie Neelika Malavige

Prof. Graham Ogg

25th June 2020

TABLE OF CONTENTS

Index of figures

Index of tables

Abbreviations

ABC - Avidin-Biotin-Peroxidase complex

ADE - Antibody Dependent Enhancement

ALT - Alanine Transaminase

AST - Aspartate Transaminase

BP - Blood Pressure

C - capsid

cDNA - Complementary DNA

DC - Dendritic Cells

DENV - Dengue Virus

DF - Dengue Fever

DHF - Dengue Hemorrhagic Fever

DNA - Deoxyribonucleic Acid

DSS - Dengue Shock Syndrome

E - Envelope

ELISA - Enzyme-Linked Immunosorbent Assay

FCS - Fetal Calf Serum

FcγR - Fc gamma Receptor

GM-CSF - Granulocyte-macrophage colony stimulating factor

HCT- Hematocrit

HDL - High Density Lipoprotein

HLA - Human Leukocyte Antigen

HRP - Horseradish Peroxidase

I-FABP - intestinal fatty acid binding proteins

IFN-γ - Interferon gamma

IgG - Immunoglobulin G

IgM - Immunoglobulin M

IL - Interleukin

LPS - Lipopolysaccharide

M- Membrane

MCP - monocyte chemoattractant protein

MIF - Macrophage migration Inhibitory Factor

MIP - macrophage inflammatory protein

MMP- matrix metalloproteases

MT- microbial translocation

NAAT - Nucleic acid amplification tests

NF-κB - Nuclear factor-κB

NK - natural killer

NS - Non-structural proteins

OD - Optical Density

PBS - Phosphate Buffered Saline

PCR - Polymerase Chain Reaction

prM - Precursor Membrane

PRNT - plaque reduction neutralization technique

RNA - Ribonucleic Acid

RT PCR - Revers Transcription Polymerase Chain Reaction

sPLA2 - secretory phospholipase A2

TGF - Transforming Growth Factor

TLR -Toll Like Receptor

TMB - Tetramethylbenzidine

TNFα - Tumor Necrosis Factor alpha

UTR - Untranslated Region

VEGF - Vascular Endothelial Growth Factor

WBC - White Blood Cells

WHO - World Health Organization

Acknowledgement

Obtaining a M Phil has been my lifelong dream and taking the first step towards it was made possible by supervisor Prof. Neelika Malavige. Without her guidance and mentoring, this M phil would not have been a possibility. Prof. Malavige's support and dedication to assist me, I feel is my good fortune. Her unwavering guidance, advice and supervision allowed me to learn and master high how to conduct quality medical research, as well, allowed to bring out my potential and overcome weaknesses in expanding the horizon of research in a broader context.

I would like to thank Prof Graham Ogg, renowned scientist in the field of immunology, for his expertise, constant feedback and guidance provided throughout my research.

My sincere gratitude to Mr Laksiri Gomez for his constant support and willingness in educating me during the last three years to acquire the necessary skills to conduct my research. Thank you Dr. Chandima Jeewandara, Dr. Deshni Jayathilake, Dr. Achala Kamaladasa, Ms. Ayesha Wijesinghe, Dr. Samitha Fernando and Dr. Dulharie Wijerathna for being there whenever I needed a helping hand and for sharing your knowledge and experience.

I am thankful to Dr. Ananda Wijewikcrama and the staff members of the National Institute of Infectious Diseases, Sri Lanka for all the support extended in patient recruitment.

Dr. Sameera Mahapatuna, Dr Sanoj Dharmakeerthi, Dr Dilshan Chithrananda and Dr. Yugani Pathegama thank you for helping me in obtaining samples which was an essential part of my research work, and my sincere appreciation for all the patients who willingly consented to participate in this study.

Prof. Kamani Wanigasuriya, if not for your constant motivation and support I would not have started my research studies. I am ever so grateful for your guidance and encouragement not only during the past three years but throughout my career. An extra special thank you goes to my colleagues and the staff of department of medicine for the support they provided during last 3 years.

My gratitude to all the staff members of the Department of Microbiology and the Department of Family Medicine, University of Sri Jayewardenepura, Sri Lanka, for facilitation and support.

I would like to especially acknowledge the National Research Council of Sri Lanka and the Center for Dengue Research, University of Sri Jayewardenepura for providing financial support for the research, without which this project would not have come to fruition.

Last but not least, Amma, Thejana and Aritha thank you for your unconditional love, patience and encouragement not only in the last 3 years but every passing day. You are the strength of my life!

Investigation of factors leading to microbial translocation in acute dengue infection

Narangoda Liyanage Ajantha Shyamali

ABSTRACT

The incidence of dengue has risen dramatically in recent years and it has become one of the major causes of morbidity and mortality in South Asian countries. There is no specific treatment to combat this viral infection yet, although several drugs are currently undergoing clinical trials. Therefore, identifying factors leading to severe illness is of utmost importance for better patient management strategies.

Microbial translocation (MT) is passage of viable and nonviable microbes and microbial products such as endotoxin across an anatomically intact intestinal barrier to mesenteric lymph nodes and from there to other organs. MT has shown to associate with acute dengue and elevated lipopolysaccharide (LPS) levels were found to correlate with disease severity. In this study, the association of presence of LPS with the development of dengue haemorrhagic fever (DHF) was assessed initially, followed by identification of factors contribute to high LPS levels in patients with acute dengue infection.

Along with LPS, levels of other inflammatory markers (interleukein-18, LPS binding protein and C reactive protein) were assessed on daily blood samples in patients with acute dengue infection. LPS levels were found to be higher in patients with DHF compared to those with DF ($p=0.01$) and there was a significant correlation with IL-18 levels (Spearman's $r=0.20$, $p=0.009$). The patients who had elevated serum LPS were significantly ($p=0.025$) more likely to have metabolic diseases (OR=2.9, 95% CI- 1.17 to 7.59)

Although concurrent bacteraemia is uncommon in dengue it is known to associate with severe illness. Procalcitonin (PCT) is an acute phase reactant, which is considered as a marker that can differentiate inflammation due to bacteria from other inflammatory stimuli. Patients with DHF and shock were shown to have elevated serum PCT levels compared to those with DF. Investigating the usefulness of PCT in identifying concurrent bacteraemia in patients with acute dengue was one of the objectives in this study. Assessment of the relationship of PCT, serum LPS and clinical disease severity revealed that PCT was positive (>0.1ng/ml) in 40% patients with DF and 50% patients with DHF.

PCT levels were significantly (p=0.02) higher in patients who had LPS (median-0.1 ng/ml, IQR- 0.03 to 0.27 ng/ml) compared to patients who did not have LPS (median-0.07 ng/ml, IQR 0 to 0.161 ng/ml). However, PCT levels were not significantly (p=0.24) higher in those with DHF (median-0.11 ng/ml, IQR-0 to 0.30 ng/ml) compared to those with DF (median-0.08 ng/ml, IQR-0.02 to 0.19 ng/ml) and elevated PCT was not significantly associated (p=0.22) with DHF (OR 1.481, 95% CI: 0.798 to 2.762). There was a significant correlation (Spearman's r=0.163, p=0.023) between serum LPS and PCT levels.

Microbial translocation in acute dengue infection is more likely to occur in patients with metabolic diseases and this "metabolic endotoxemia" could be a risk factor for development of severe dengue. A positive PCT in acute dengue does not appear to associate with concurrent bacteraemia.

Keywords: Dengue, lipopolysaccharide (LPS) levels, Microbial translocation, Concurrent bacteraemia