Institute of Biochemistry, Molecular Biology and Biotechnology

University of Colombo

Proceedings of the 10th Annual Scientific Sessions of the IBMBB

21st November 2018

Contents

Programme

08.30 h	Registration
09.00 h	Inauguration
09.05 h	Lighting of the Traditional oil Lamp
09.10 h	Welcome Address by Director, IBMBB
09.20 h	Address by Vice Chancellor, University of Colombo
09.30 - 10.20 h	Professor Stanley Wijesundera Memorial Lecture by *Vidyajyothi* Professor Harendra de Silva on **"Youth Violence"**
10.20 - 10.30 h	Address by Wijesundera Family Member
10.30 - 10.35 h	Vote of Thanks
10.35 - 11.00 h	Tea
11.00 - 11.30 h	Guest Lecture by Dr Mary Elizabeth McMillan on **"Comparing genetic and psychological factors in the development of anxiety and depression in prostate cancer patients"**
11.30 - 12.45 h	Free paper session I on "**Molecular Biology and Genetics**"
12.45 - 13.30 h	Lunch break
13.30 - 15.00 h	Free paper session II on "**Immunology and Infectious Diseases**"
15.00 - 16.15 h	Free paper session III on "**Medicinal plants, Biotechnology and Bioinformatics**"
16.15 - 16.30 h	Tea
16.30 - 17.00 h	Award Ceremony

Message from the Director, IBMBB

Professor Shiroma Handunnetti
Director, IBMBB & Professor in Immunology

It is a great pleasure to send this message to mark the occasion of the 10th Annual Scientific Sessions of the Institute of Biochemistry, Molecular Biology and Biotechnology (IBMBB), University of Colombo which is being held as the IBMBB completes its 14th Anniversary.

IBMBB is an Institute in Sri Lanka which is dedicated to postgraduate training and research in Molecular Life Sciences, Molecular and Cellular Immunology, Bioinformatics and allied filed. Our Founder Director, Professor Eric H Karunanayake's vision has led to the establishment this institute with financial support from Swedish Government in year 2004. Since then the MPhil and PhD degree programme has been offered by the IBMBB which are financially supported by national and international competitive research funding received by academics of the IBMBB. There have been several significant firsts in Sri Lanka among its MPhils and PhDs including the first (i) molecular genetics study on breast cancer, (ii) molecular study on phytoplasma, (iii) molecular study on genetics of primary immunodeficiency, (iv) tea genome, (v) genetic characterization of traditional and weedy rice (vi) proteomic study on bee venom. To date IBMBB has facilitated 67 MPhil and PhD students have completed their studies at IBMBB. These research studies have established and been conducted through numerous collaborations with academics and researchers in other faculties and Institutes of University of Colombo, other universities in Sri Lanka and Research Institutes affiliated to Ministry of Health and Science and Technology and private Institutions. Through these research and MPhil & PhD degrees, IBMBB has made a significant contribution to capacity building and strengthening of research training for the higher educational sector.

With the commencement of the Masters degrees at IBMBB in (i) Molecular Life Sciences in 2005, (ii) Cellular and Molecular Immunology in 2005 and (iii) Bioinformatics in 2012, to date IBMBB had a total of 178 registered Masters students with a total output of 123 graduates. Currently, IBMBB has about 100 registered post-graduate (MSc, MPhil and PhD) students who are continuing their studies.

The IBMBB Annual Scientific Sessions is the forum for Dissemination of research findings of IBMBB students plus it also includes research from postgraduates from other Higher Educational Institutes. Annual sessions are held also as event to mark the IBMBB anniversary. It is a pleasure to state that these sessions had been held continuously since 2006 to date except on year in which IBMBB had the International conferences in 2009 and 2014.

Professor Stanley Wijesundera Memorial Lecture had been/is one of the key events of the IBMBB Annual Scientific sessions. This year the memorial lecture will be delivered by *Vidyajyothi* Professor Harendra de Silva on "Youth violence". Professor Harendra de Silva is an Emeritus Professor of Paediatrics at the University of Colombo. He is an eminent researcher and Honorary Fellow of the Royal College of Paediatrics and Child Health who has been a pioneer in establishment of National Child Protection Authority. We are also pleased to have the Guest lecture this year delivered by Dr Mary McMillan from University of New England, Australia on "Genetic and psychological factors in the development of anxiety and depression in prostate cancer patients". Since 2016, IBMBB had initiated the award of cash prizes for the Best Presenters of each scientific session with much appreciated sponsorship from the Wijesundera family.

This year it was a late but a firm decision to hold the IBMBB Annual Scientific sessions in November with a total of 16 oral and 09 poster presentations. The presentations in the three sessions will be in research on Molecular Biology & Genetics, Immunology & Infectious diseases, Medicinal plants, Biotechnology & Bioinformatics. The authors and affiliations indicate the wide research collaborations that IBMBB has at present.

I take this opportunity to thank Professor Lakshman Dissanayake, Vice Chancellor, University of Colombo for his continued support, inspiration and specially, for gracing the inauguration and Awards ceremony, Wijesundera family for continued support for the Memorial Lecture and sponsoring the awards for best presenters, *Vidyajyothi* Professor Harendra de Silva for delivering the Professor Stanley Wijesundera Memorial Lecture, all our collaborators for facilitating research studies, all those from other Higher Educational Institutes presenting their research at our Scientific Sessions, sponsors for their generous support and, the students and staff of the IBMBB for their dedication and hard work. A special thanks to the reviewers, both external and IBMBB staff members and also the Chairpersons and Judges of the scientific sessions. I specially thank Prof Nimal Punyasiri - Chairperson and Dr Narmada Fernando and Ms Dilini Ishaka - Joint Secretaries of the Organizing Committee. I thank **all members of the Organizing Committee** for the continuous support, including Deputy Bursar-Ms Manjula Kahawita and Sen. Asst. Registrar - Mr Janaka Gunasekera. I am also very thankful to Prof Tara D Silva, Acting Director, IBMBB for initiating this event and her support.

I wish you all a successful meeting.

Organizing Committee

Main Committee

Prof Shiroma Handunnetti - Advisor
Prof Nimal Punyasiri - Chairperson
Dr Narmada Fernando - Joint Secretary
Ms Dilini Ishaka - Joint Secretary
Dr Sisira Pathirana - Member
Dr Jagathpriya Weerasena - Member
Dr Sumadee De Silva - Member
Ms Manjula Kahawita - Member
Mr Janaka Gunasekara - Member
Ms Sumali Champika - Member
Mr Sashika Niranjan - Member

Editorial Committee

Prof Shiroma Handunnetti
Prof Nimal Punyasiri
Dr Sisira Pathirana
Dr Narmada Fernando
Ms Dilini Ishaka
Dr Nilupa Gunaratna
Ms Dakshika Gangani

Inauguration Committee

Ms Manjula Kahawita
Mr Janaka Gunasekara
Ms Anoma Jayasoma
Ms Sumali Champika
Ms Nishara Batagoda
Ms Tharini Somarathna
Ms Sekani Premadasa
Ms Chameera Helani
Mr Nawarathna Yapa Bandara
Mr Prasad Fernando
Ms Nadeema Chandrapathma

Logistics Committee

Ms Manjula Kahawita
Mr Janaka Gunasekara
Ms Jayani Kariyawasam
Ms Varuni Rathnayaka
Ms Anoma Jayasoma
Ms Priyangani Dabare
Ms Sekani Premadasa
Mr Sashika Niranjan
Ms Lakdini Nikesha
Ms Dilmi Irushika
Ms Hiruni Gamachchige
Ms Thilani Hettiarachchi
Mr Dhanushka Bandara

Oral Communications

Session I – Molecular Biology and Genetics

Session Chairpersons: Prof Sunil Premawansa and Prof Ranil Dassanayake

OP01	**11.30-11.45 h**	Analysis of mitochondrial and Y-chromosome haplogroups among Sinhalese individuals with different *Alu* polymorphisms	Fernando AS, Weerasooriya PR, Ranasinghe R, Tennekoon KH
OP02	**11.45-12.00 h**	Mitochondrial DNA haplogroups and D-loop hypervariable region I mutations in sporadic breast cancer	Kotelawala JT, Tennekoon KH, Ranasinghe R, Rodrigo HACIK De Silva GKS
OP03	**12.00-12.15 h**	Association of *TP53* mutations and p53 protein expression in a cohort of Sri Lankan head and neck cancer patients	Manoharan V, Karunanayake EH, Tennekoon KH, De Silva S, Angunawela P, De Silva K, Lunec J
OP04	**12.15-12.30 h**	*CYP21A2* gene mutations in a cohort of children with the salt wasting form of Congenital Adrenal Hyperplasia	Praveenan S, Hewage AS, Tennekoon KH, de Silva KSH
OP05	**12.30-12.45 h**	Characterization and identification of the mechanism behind formation of a novel gross deletion at *GHRHR* gene locus leading to isolated growth hormone deficiency	Sundaralingam T, Tennekoon KH de Silva KSH, De Silva S, Hewage AS, Ranasinghe R

Session II – Immunology and Infectious Diseases

Session Chairpersons: Dr Enoka Corea and Prof Sharmini Gunawardena

OP06	**13.30-13.45 h**	Direct degranulation of rat mast cells (RBL-2H3) and increase in generation of superoxide anion (O2-) induced by aqueous extract of *Acacia* pollen and *Apis dorsata* venom	Ishaka GKD, Gangani PD, Rukshala BAD Gunasekara DLPE, Fernando N Handunnetti SM
OP07	**13.45-14.00 h**	Use of allergenic cross-reactivity between *Apis dorsata* Fabricius (Giant Asian Honeybee) and *Apis mellifera* Linnaeus (Western Honeybee) for diagnosis of *A. dorsata* venom allergy	Gunasekara DLPE, Handunnetti SM, Premawansa S, Witharana EWRA, Dasanayake WMDK, Ratnayake IP, Seneviratne SL, Dias RKS, Premakumara GAS, De Silva R
OP08	**14.00-14.15 h**	Comparison of hyaluronidase from venom of *Apis dorsata* Fabricius and *Vespa affinis* Linnaeus	Gunasekara DLPE, Handunnetti SM, Premawansa S, Dasanayake WMDK, Ratnayake IP, Seneviratne SL, Dias RKS, Premakumara GAS, De Silva R
OP09	**14.15-14.30 h**	High levels of Angiopoietin 2 and Angiopoietin 2/1 at critical stage of DHF patients and their association with clinical and biochemical parameters	Mapalagamage MS, Handunnetti SM, Wickremasinghe R, Premawansa G, Thillainathan S, Fernando T, Khanapathypillai K, De Silva AD, Premawansa S
OP10	**14.30-14.45 h**	IgM and IgG response against a specific protein antigen in rats orally fed with a herbal formulation	Ranaweera BVLR, Abeysekera A, Weerasena OVDSJ , Handunnetti SM
OP11	**14.45-15.00 h**	*In vitro* study on the effect of antimicrobial agents on inflammatory cytokine response in leptospirosis	Sam G, Fernando N , Rajapakse S, Handunnetti SM

Session III – Medicinal plants, Biotechnology and Bioinformatics

Session Chairpersons: Prof Tara D Silva and Dr Kumudu Fernando

OP12	**15.00-15.15 h**	Development of a Colourimetric Reverse Transcription Loop-mediated Isothermal Amplification assay for the rapid detection of different Serotypes of the Dengue virus	Ariyaratne MHJD, Nguyen A De Silva AD, Fernando N Weerasena OVDSJ, Handunnetti SM
OP13	**15.15-15.30 h**	Development of In silico primer designing tool to design species specific primers by using DNA barcode regions of land plants	Fernando MATM, Weerasena OVDSJ
OP14	**15.30-15.45 h**	Studies on in *vitro* anti-inflammatory potential and phytoconstituents of ethanol bark extract of *Flacourtia indica*	Perera HDSM, Samarasekera R, Handunnetti SM, Weerasena OVDSJ , Jabeen A, Choudhary MI
OP15	**15.45-16.00 h**	Production of toxic metabolite by native antagonistic fungi, against *Rigidoporus microporus*, the causative fungi of White Root Disease of rubber (*Hevea brasiliensis*)	Poorna Balasooriya, Fernando THPS Weerasena OVDSJ
OP16	**16.00-16.15 h**	Protein network building for malignant peripheral nerve sheath tumor via genetic SNP mutations	Senadheera SPBM, Weerasinghe AR, Wijesinghe CR

Poster Presentation

Session Chairpersons: Prof Nilanthi Dassanayake and Dr Inoka C Perera

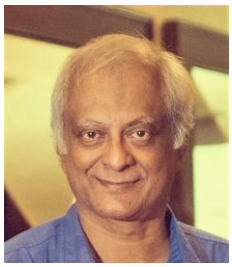
PP01	Preliminary study on *CTLA-4* gene mutations in Inflammatory Bowel Disease in Sri Lanka	Hewagama AHS, Pathirana SL, Handunnetti SM, Seneviratne S
PP02	Expression and purification of recombinant anti-diabetic drug target, peroxisome proliferator activated receptor gamma (Pparɣ)	De Silva MCJ, YS Wijayasinghe
PP03	Immuno-proteomic characterization of *Vespa affinis* (Lesser Banded Hornet) venom and its cross-reactivity with the venom of *Vespula vulgaris*	Gunasekara DLPE, Handunnetti SM, Premawansa S, Dasanayake WMDK Karunatilake C, Ratnayake IP, Seneviratne SL, Dias RKS Premakumara GAS, De Silva R
PP04	Preliminary study on pollen records during South-West monsoon in selected locations in Sri Lanka and direct effect of pollen on basophil degranulation	Guruge BMA, Ishaka GKD, Handunnetti SM, de Silva R, Premawansa S, Premathilake TR
PP05	Comparison of a two-step and a novel single step method for neutrophil isolation	Lakmali AKD , Fernando N, Handunnetti SM, Premawansa S
PP06	Development of barcodes for medicinal plant "Babila" (*Sida* species) in Sri Lanka	Rajphriyadharshini R, Weerasena OVDSJ, Tennakoon TMSG, Gunaratna LNR, Suraweera CD
PP07	Optimization of isolation of BK viral DNA from renal transplant patients and *VP1* gene-based genotyping	Ratnayake AKDVY, Fernando N, Gajanayake T, Handunnetti SM, Jayamaha CJS
PP08	Reactivity of population samples from Colombo District for anti-leptospiral antibodies in Microscopic Agglutination Test	Balaji K, Weeratunga PN, Ramchandani KC, Wijerathne PBP, Sam G, Fernando N, Handunnetti SM, Fernando SD, Rajapakse S
PP09	Serological prevalence and determination of cutoff levels of Leptospirosis IgM and IgG in relation to Patoc I strain of *Leptospira biflexa* in a cohort of individuals from a high prevalence region in Sri Lanka	Balaji K, Weeratunga PN, Ramchandani KC, Wijerathne PBP, Sam G, Fernando N, Handunnetti SM, Fernando SD, Rajapakse S

Professor Stanley Wijesundera Memorial Lecture

Introduction

Professor Stanley Wijesundera Memorial Lecture is an annual event of the IBMBB held to commemorate late Professor Stanley Wijesundera, who served as Professor of Biochemistry and as First Vice Chancellor, University of Colombo from 1978-1987. Professor Wijesundera was a visionary leader during whose tenure, infrastructure development and academic progress in the University of Colombo was at its peak. He played a key role in the development of the discipline of Molecular Biology in the University of Colombo.

Previous Professor Stanley Wijesundera Memorial Lectures were delivered by Professor Rune Liminga, Former Director, International Programme in Chemical Sciences, University of Uppsala, Sweden; Professor W D Lakshman, Former Vice Chancellor, University of Colombo; Professor Eric Karunanayake, Founder Director, IBMBB & Emeritus Professor of Biochemistry, University of Colombo; Professor Lal Chandrasena, Senior Professor of Biochemistry, University of Kelaniya and Professor E Dilip de Silva, Senior Professor of Organic Chemistry, University of Colombo; Professor Kamani Tennekoon, Senior Professor of Molecular Life Sciences, IBMBB, University of Colombo, Former Director, IBMBB & former Professor of Physiology, Faculty of Medicine, University of Colombo; *Vidyajyothi* Professor Rezvi Sheriff, Senior Professor of Medicine, Kotelawala Defence University, Sri Lanka and Former Senior Professor of Medicine, Faculty of Medicine, University of Colombo; Dr Rajiva de Silva, Consultant Immunologist & President-Allergy and Immunology Society of Sri Lanka. This year Professor Stanley Wijesundera Memorial Lecture will be delivered by *Vidyajyothi* Professor Harendra de Silva, Emeritus Professor of Paediatrics at the University of Colombo, Honorary Fellow of the Royal College of Paediatrics and Child Health.



Professor Stanley Wijesundera Memorial Lecture

On Youth Violence by

Vidyajyothi Prof Harendra de Silva

MBBS, DCH, MSc(Birm), FRCP(Lond & Edin), FRCPCH(UK), FSLCP, FCPS (Pak)

Youth Violence is a Global and a burning Issue! Everyday we see in the media acts of violence especially involving youth in acts of Physical, Emotional and Sexual violence including self-harm. At a rate of Males 58.8 /100,000, Sri Lanka is heading the list for suicide.

Adolescence is complicated by: little lateral thought, parental and societal pressures, Anti-authority feelings, Peer pressure, Volatile, Labile and hot emotions and Increased risk taking, among others. Norms violence gets pre-programmed minds of children and adolescents. It depends on domestic, peer, societal and media violence. We have shown Sexual Abuse to be widespread in Sri Lanka. Sixty four percent of abusers had been abused during childhood thus illustrating "today's abused become tomorrow's Violent abusers". Another study showed adverse experience in childhood including physical, emotional and sexual abuse has a very strong association with adolescent physical, & emotional violence, deviant sexual activity, and alcohol abuse. Yet another study by us showed, suicidal contemplation and deliberate self-harm to be related to exposure to violence.

Foreign Invaders especially the British, in their quest for survival in the midst of multitudes of resistance, legalized, Justified and established Corporal Punishment (CP) of children. This was virtually to create a population that could be 'disciplined'. The status quo of idolization and justification of CP continues till today. We found: 80% and 72.5% of students faced at least one episode of CP and emotional abuse in the past term respectively (2017).

Children and young people who play violent video games, even for short periods, are more likely to behave aggressively in the real world. Dopamine is known to modulate the effects. It is also associated with aggression. Internet Gaming Disorder (IGD) listed as a "Condition for Further Study" in the *DSM-5* (2013). Alcohol, Cocaine, Marijuana, Heroin, ICE Crystal Meth, all cause increase in Dopamine in the limbic system, and deprivation leads to reduction of Dopamine and

violence related to addiction. All these aspects lead to Stress, Mood changes, Agitation & Violence.

Left Hemisphere Processes language, and logical thinking. The Limbic System is the Emotional "lower" and more primitive area which acts on instinct. Emotionally violent swear words trigger the "fight or flight" response in humans, and does not need the Left Hemisphere. It stimulates the flow of adrenalin/dopamine and endorphins, and can relieve pain and stress. In sex addicts the dopamine - the pleasure-reward system - is activated during sex, similar to drugs, alcohol, or gambling.

The United Nations, its Security Council and the UN CRC or laws based on the guidelines has had a minimal impact on the incidence of Childhood or Youth Violence? The *1995 amendment (22) to the Penal Code of Sri Lanka section 308 a;* on cruelty to children has not had a single successful prosecution to date. Emperor Ashoka brought Buddhism to Sri Lanka and Sri Lanka remains a Majority Buddhist Country. Why have we not been able to achieve Non-Violence in spite of non-violence of religion, Top down laws or programs! The natural physiological instinct originating in the limbic system of survival to "fight or flight" has an uncontrollable is more powerful than the notion of 'Non - Violence' which originates from the Left Hemisphere!

The International arms industry by the powerful Nations cannot be devolved since most bearing arms will be children and Youth. The Money and Power associated with proxy wars, and the conflict of interest by all the Security Council members will never bring Youth Violence to an end nor Peace to the world.

Guest Lecture on

"Comparing genetic and psychological factors in the development of anxiety and depression in prostate cancer patients"

by Dr Mary Elizabeth McMillan, *BSc (Hons), PhD*

Depression is a common comorbidity in patients diagnosed with cancer. Approximately 18% of prostate cancer (PCa) patients become clinically depressed after diagnosis and treatment. There is a strong need to identify and treat depression in these patients, however there is currently no method by which high-risk patients might be identified at intake. This study examined psychological and genetic factors as potential predictors of anxiety, depression, and chronic stress in PCa patients.

Ninety-five PCa patients completed psychological inventories for anxiety, depression, and psychological resilience (PR) and also gave a saliva sample for cortisol and a mouthwash sample for genetic testing for the presence of the BDNF Val66Met polymorphism, the 5-HTTLPR ss/ll polymorphism, and telomere length.

Patients with high PR scores had significantly lower anxiety and depression, but showed no significant differences in their salivary cortisol. Carriers of the Met allele of the BDNF Val66Met polymorphism had significantly higher salivary cortisol concentrations, thus it may prove valuable to screen PCa patients for it upon intake, as hypercortisolaemia has been associated with an elevated risk of anxiety and depression. Contradictory to overall meta-analytic findings, there was no significant difference in the depression scores of ss vs ll carriers of the 5-HTTLPR polymorphism. The ss genotype may negate the protective effects of PR, or PR may protect the depression-prone ss carrier after major stress. Telomere length was significantly correlated with a major indicator of depression, irritability.

Together, these findings have implications for the early identification of prostate cancer patients at risk of developing depression, and challenges the idea that provision of general PR training is sufficient to buffer against depression.

Award Winners: 9th Annual Scientific Sessions, IBMBB - 07th July 2017

The Best Oral Presentation in Molecular Biology and Bioinformatics
was awarded to
Ms Mifra Faiz
for the paper titled
Preliminary study on identification of autosomal recessive p47*phox* mutations in patients with chronic granulomatous disease in Sri Lanka
Mifra FN, de Silva AD, de Silva NR, Handunnetti SM, Dassanayake D

The Best Oral Presentation in Immunology and Infectious Diseases
was awarded to
Ms Maheshi Mapalagamage
for the paper titled
Low total serum nitrite and nitrate levels on admission in dengue patients as prognostic markers for developing dengue hemorrhagic fever
Mapalagamage M, Handunnetti SM, Premawansa G, Thillainathan S, Fernando GTG, Kanapathippillai K, De Silva AD, Premawansa S

The Best Oral Presentation in Medicinal Plants
was jointly awarded to
Ms Ayesha Perera
for the paper titled
Identification of *Ralstonia solanacearum* phylotypes causing bacterial wilt of potato in Badulla district of Sri Lanka
Perera AAU, Weerasena OVDSJ, Dasanayake PN

Mr Poorna Balasooriya
for the paper titled
Isolation of native antagonistic fungi against *Rigidoporus microporus*, the causative fungus of White Root Disease form different rubber growing soils
Balasooriya PW, Fernando THPS, Weerasena OVDSJ

The Best Poster Presentation
was awarded to
Ms Gethmini Jayasundara
for the paper titled
Detection of pathogenic mutations in Hotspot regions in exon 11 of *BRCA1* gene in a cohort of Sri Lankan young familial breast cancer patients
Jayasundara GP, De Silva S, De Silva K

Abstract No: OP01

Analysis of mitochondrial and Y-chromosome haplogroups among Sinhalese individuals with different *Alu* polymorphisms

Fernando AS[1], Weerasooriya PR[1], Ranasinghe R[1], Tennekoon KH[1]

[1]Institute of Biochemistry, Molecular Biology and Biotechnology, University of Colombo

Sri Lanka has a total population of over 20 million of which majority (74.9%) is Sinhalese. Until now, the origin of Sinhalese is not scientifically proven. Thus, it is of vital importance to study the genetic constitution of Sinhalese and to obtain a more comprehensive knowledge. The present study focuses on the maternal and paternal inheritance of Sinhalese by studying the single nucleotide polymorphisms (SNPs) present in mitochondria and Y chromosomes. Five male samples were randomly selected from a pool of samples with different *Alu* variation patterns. Polymerase chain reaction (PCR) conditions were optimized for annealing temperature and Magnesium chloride concentration. PCR were performed on the control region of mitochondria using primers M23 and M24 and three SNPs in Y chromosome (LM20, HM69 and RIbM412). PCR products were confirmed using agarose gel electrophoresis and further purified using commercially available columns. The concentrations of the purified PCR products were determined prior to sequencing reactions followed by capillary electrophoresis. Resulted sequences were initially analyzed via BioEdit software. BLAST and Ensembl web browsers were used to further confirm the SNPs obtained for the Y chromosome. Mitochondrial sequences were compared against revised Cambridge Reference Sequence (rCRS) and detected SNPs were reported. Thereafter, respective SNPs were categorized into specific haplotypes and haplogroups. Haplotypes were further assigned into appropriate global haplogroups using Haplogrep and EMPOP (web applications) and manually through PhyloTree Build 17. In conclusion, five samples were classified into five distinct mitochondrial haplogroups (A: M18'38; B: H6; C: R81 + 16093; D: M10a1 + 16129 and E: U2a). Y chromosome SNP LM20 was not observed in all five samples nevertheless, RIb412 was present in all five samples. HM69 was observed only in samples D and E. Results obtained illustrate possible maternal and paternal lineages of Sinhalese with Indian, European and South East Asian populations.

Keywords: Sinhalese, single nucleotide polymorphism, Mitochondria, Y chromosome, Haplogroups

This work was supported by IBMBB and constitutes a part of MSc studies of ASF.

Abstract No: OP02

Mitochondrial DNA haplogroups and D-loop hypervariable region I mutations in sporadic breast cancer

Kotelawala JT[1], Tennekoon KH[1], Ranasinghe R[1], Rodrigo HACIK[1], De Silva GKS[2]

[1] Institute of Biochemistry, Molecular Biology and Biotechnology, University of Colombo,
[2]National Cancer Institute, Maharagama

Breast cancer is the most common female cancer, globally, as well as in Sri Lanka accounting for nearly 25% of diagnosed cancers. Several mitochondrial DNA (mtDNA) mutations are reported to be associated with breast cancer, but there is currently no data for the Sri Lankan population. In this study, thirty patients with sporadic primary breast cancer and their age, BMI and menopausal status matched controls were studied. Macro-haplogroups M and N were determined using coding region SNP based PCR-RFLP. A 900 bp region covering the hypervariable region I of mtDNA was PCR-amplified, sequenced and analysed. The results indicated that 18 patients and 17 controls belonged to the M macro-haplogroup and the remainder to the N macro-haplogroup. In total 72 and 45 mutations were identified in the patients and controls respectively, with 38 and 15 of them occurring exclusively in each group. Prevalence of mutations previously reported to be associated with breast cancer, namely T16189C, T16519C, T16311C and C16207T were not significantly different between patients and controls in the present study sample. Exclusive mutations seen in 23 patients were distributed as follows: 05 (N=3), 04 (N=2), 03 (N=3), 02 (N=7) and 01 mutation (N=8). Some patients had more than one exclusive mutation with the highest number co-existing being five. GraphPad Prism was used for all statistical analysis. Mitochondrial DNA D-loop mutations previously unreported to be associated with breast cancer observed in the present study require further analysis especially since several such mutations co-exist in some patients. Prevalence of HVI mutations reported to be associated with breast cancer or M and N macro-haplogroup status did not significantly differ between patients and controls in present study.

Keywords: Breast Cancer; mitochondrial DNA; haplogroups; hypervariable region I; Sri Lanka

This work was supported by National Science Foundation, Sri-Lanka (NSF/SCH/2016/04).

Abstract No: OP03

Association of *TP53* mutations and p53 protein expression in a cohort of Sri Lankan head and neck cancer patients

Manoharan V[1], Karunanayake EH[1], Tennekoon KH[1], De Silva S[1], Angunawela P[2], De Silva K[3], Lunec J[4]

[1]Institute of Biochemistry, Molecular Biology and Biotechnology, University of Colombo, [2]Department of Pathology, Faculty of Medicine, University of Colombo, [3]National Cancer Institute, Maharagama, [4]Northern Institute for Cancer Research, Newcastle University

Mutations in the *TP53* tumour suppressor gene disrupts function of its protein product, p53, and leads to carcinogenesis. Pathogenic nucleotide variants of *TP53* and p53 protein expression have not been analysed together in Sri Lankan cancer patients. Our current study aimed to analyse the nucleotide variants of *TP53* and protein expression in head and neck cancer patients. DNA extracted from tumour tissue (N=32) was subjected to Sanger sequencing and bioinformatics analysis was carried out to identify pathogenic nucleotide variants of the *TP53* gene. Tissue sections from a subset (N=24) were subjected to immunohistochemistry to analyse p53 protein expression. Ten patients (31.25%) had pathogenic *TP53* variants, including 2 frameshift and 2 nonsense variants leading to truncated protein and 7 missense variants altering single amino acids. Two patients had likely pathogenic variants and another two patients had likely benign variants. All samples with protein truncating variants, except one that co-existed with a missense variant, showed complete absence of p53 protein expression. All missense variant samples showed strong expression of p53. Wild type *TP53* was also associated with immuno-negativity. Missense mutations led to strong immunoreactivity, as mutant protein accumulates in the cell. However, protein truncating mutations could not be differentiated from the wild type *TP53* gene as both showed immuno-negativity, perhaps due to nonsense mediated mRNA decay in the former and rapid degradation in the latter. Thus protein expression analysis alone appears to be of limited value in assessing the functional status of p53.

Keywords: Head and neck cancer, TP53, Genetic analysis, Immunohistochemistry

This work was supported by National Research Council (NRC 15-33) & Commonwealth Scholarship Commission (LKCN-2015-100).

Abstract No: OP04

CYP21A2 gene mutations in a cohort of children with the salt wasting form of Congenital Adrenal Hyperplasia

Praveenan S[1], Hewage AS[1], Tennekoon KH[1], de Silva KSH[2]

[1]Institute of Biochemistry, Molecular Biology and Biotechnology, University of Colombo.
[2]Department of Paediatrics, Faculty of Medicine, University of Colombo.

Steroid hydroxylase deficiency due to mutations in *CYP21A2* gene is the most frequent cause of Congenital Adrenal Hyperplasia (CAH). However, mutation spectrum in Sri Lankan CAH patients has not been adequately investigated. We have characterized disease causing mutations in the *CYP21A2* gene in 30 patients with salt wasting CAH, the commonest form of the disease. Allele Specific Polymerase Chain Reaction was carried out using mutation site specific primers for eight mutations (P30L, I2G, 8bp deletion, I172N, E6 cluster, V281L, Q318X and R356W) reported as frequently occurring in other populations. DNA extracted from peripheral venous blood was PCR amplified using normal and mutation specific primers. PCR products were visualized through agarose gel electrophoresis. Homozygous and heterozygous mutations and the wild type were inferred based on the PCR amplification results. Mutations were identified in 54 chromosomes out of the 60 chromosomes tested. Fourteen patients had homozygous mutations. The following Allele frequencies were observed for each mutation P30L-10%, I2G- 40%, 8bp-18.33%, I172N-3.33%, E6 cluster- 5%, Q318X-40% and R356W-3.33%. V281L mutation was not observed in the study cohort. The frequencies of mutations did not differ substantially from what is reported in other ethnic groups. However the 8bp deletion occurred at a higher frequency. Genetic cause for the salt wasting phenotype has been resolved in 46.6 % of the patients in our cohort. Parental testing is needed to confirm whether some of the patients with heterozygous mutations are compound heterozygotes as compound heterozygosity has been reported to cause enzyme deficiency.

Keywords: Steroid hydroxylase, Allele specific PCR, CAH, Cohort, Salt wasting

This work was supported by IBMBB and constitutes a part of MSc studies of PS.

Abstract No: OP05

Characterization and identification of the mechanism behind formation of a novel gross deletion at *GHRHR* gene locus leading to isolated growth hormone deficiency

Sundralingam T[1], Tennekoon KH[1] , de Silva KSH[2,3], De Silva S[1], Hewage AS[1], Ranasinghe R[1]

[1]Institute of Biochemistry, Molecular Biology and Biotechnology, University of Colombo, [2]Department of Paediatrics, Faculty of Medicine, University of Colombo, [3]Lady Ridgeway Hospital, Colombo

Defects in the growth hormone releasing hormone receptor gene (*GHRHR)* have been identified as the etiology of isolated growth hormone deficiency in numerous studies. The present report describes characterization, consequences and possible mechanism behind the formation of a novel gross deletion of *GHRHR*. The homozygous novel gross deletion (c.-3166_58-2057del) was identified at 5' end of *GHRHR* gene using MLPA assay, short range PCR amplifications and sequencing. The gross deletion includes last 3118 bp of upstream intergenic region of *GHRHR*, complete exon 1, first 2620 bp of intron 1 and a 32 bp microhomology segment. The deletion was screened in the parents via long range PCR amplification. Mother was heterozygous for the deletion and father (who shares *Alu* polymorphisms with the proband) did not carry the deletion. Hence the proband appears to have obtained one allele from the mother and the other allele could have arisen de-novo. The pathogenic effect of the deletion was confirmed by identification of important elements and motifs that fall within the deleted region. Break points of the deletion were identified within *Alu* elements predicted by CENSOR *in-silico* tool. On analysis with nBMST search tool, accumulation of non B-DNA structures were identified within 360 bp upstream from 32 bp microhomology in intron 1. Hence the region could act as a common fragile site, leading to genomic instability. This deletion has not yet been reported in any other unrelated individuals. Thus it may have occurred via *Alu* specific microhomology mediated non-recurrent genomic rearrangement, which could be either microhomology-mediated end joining (MMEJ) or single event of Fork stalling and template switching (FoSTeS) or microhomology mediated break induced replication (MMBIR). Homozygous state of the deletion was identified to be the cause of isolated growth hormone deficiency in the proband.

Keywords: *GHRHR*; gross gene deletion; microhomology; non-recurrent genomic rearrangement

This work was partially supported by National Science Foundation, Sri Lanka (grant number: RG/2011/BT/03).

Abstract No: OP06

Direct degranulation of rat mast cells (RBL-2H3) and increase in generation of superoxide anion (O_2^-) induced by aqueous extract of *Acacia* pollen and *Apis dorsata* venom

Ishaka GKD, Gangani PD, Rukshala BAD, Gunasekara DLPE, Fernando N, Handunnetti SM

Institute of Biochemistry, Molecular Biology and Biotechnology, University of Colombo

Mast cells play a central role in inflammatory allergic responses, mainly due to IgE-dependent and to a lesser extent due to IgE-independent mechanisms. The objective of this study was to determine the ability of two known allergens, ie, *Acacia spp.* pollen and *Apis dorsata* venom to induce direct activation of mast cells (IgE-independent mechanism). RBL-2H3 cells (RBL) were cultured in DMEM containing 10% FBS with 5% CO_2 at 37 ºC. Cells were tested using Sulfordamine B (SRB) assay in triplicates to determine the non-toxic concentration of aqueous extract of *Acacia spp.* pollen and *Apis dorsata* venom compared to negative control (0.05 M PBS) and positive controls, phorbol myristate acetate (PMA) and compound 48/80 at 500 ng/ml and 750 ng/ml. Compound 48/80, at 750 ng/ml resulted in a viability less than 75% and was excluded. 100 and 50 µg/ml concentrations of aqueous extract of *Acacia* pollen and *A. dorsata* venom were used to assess for O_2^- production by quantitative NBT assay and direct degranulation by toludine blue staining method. Compared to the unstimulated cells, RBL treated with 100 µg/ml *Acacia* pollen exhibited a 19-fold increase (19 pM vs 1 pM) in intracellular O_2^- production while *A. dorsata* venom resulted in a 48-fold (48 pM) and 37-fold (37 pM) increase by 100 and 50 µg/ml respectively. RBL treated with 100 µg/ml *Acacia sp* extract induced 70.8% direct degranulation of mast cells while RBL treated with 100 and 50 µg/ml of *A. dorsata* venom induced 95.9% and 87.3% direct degranulation respectively. These findings indicate that aqueous extract of *Acacia spp.* pollen and *Apis dorsata* venom could activate mast cells in an IgE-indipendent manner while increasing the generation O_2^- which may reflect their potential in enhancing the allergy.

Keywords: Rat mast cells, IgE-independent, direct degranulation, superoxide anion, allergy

This work was supported by IBMBB.

Abstract No: OP07

Use of allergenic cross-reactivity between *Apis dorsata* Fabricius (Giant Asian Honeybee) and *Apis mellifera* Linnaeus (Western Honeybee) for diagnosis of *A. dorsata* venom allergy

Gunasekara DLPE[1], Handunnetti SM[1], Premawansa S[2], Witharana EWRA[3], Dasanayake WMDK[4], Ratnayake IP[5], Suranjith L Seneviratne[6,7], Dias RKS[8], Premakumara GAS[9], De Silva R[4]

[1]Institute of Biochemistry, Molecular Biology and Biotechnology, University of Colombo, [2]Department of Zoology and Environment Sciences, Faculty of Science, University of Colombo, [3]Base Hospital, Deniyaya, [4]Department of Immunology, Medical Research Institute, Colombo 08, [5]District Hospital, Bandarawela, [6]Royal Free Hospital, UK, [7]Department of Surgery, Faculty of Medicine, University of Colombo, [8]Department of Zoology and Environmental Management, Faculty of Science, University of Kelaniya, [9]Herbal Technology Section, Industrial Technology Institute, Colombo

Allergy to *Apis dorsata* (Giant Asian Honeybee) venom is the commonest insect allergy in Sri Lanka and in South East Asia. However, laboratory diagnosis is not possible as the pure venom for this insect is not commercially available. We hypothesized that the commercially available venom of the Western honeybee, *Apis mellifera,* would be similar to that of Giant Asian Honeybee, and could be used instead. Comparison between *A. dorsata* and *A mellifera* venom and reactivity of the two with IgE of patients allergic to *A. dorsata* venom was detected using Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE), immunoblots and High-Performance Liquid Chromatography (HPLC). Diagnostic value of component resolved diagnosis (CRD) with commercially available recombinant allergens of Phospholipase A_2 (rApi m 1) and Icarapin (rApi m 10) was determined using an immunocap system. Venom of both species had similar banding patterns in SDS-PAGE and both species had identical peaks in HPLC profiles. Of 30 patients who developed anaphylaxis to *A. dorsata* venom, 25 (83%) had positive serum IgE (sIgE) to commercially available crude venom of *A. mellifera*, 21 (70%) to rApi m 1 and 23 (76.6%) to rApi m 10. The sensitivity increased up to 90% when both rApi m 1 and rApi m 10 were used. We suggest that it is possible to use commercially available *A. mellifera* venom to diagnose the patients allergic to *A. dorsata* venom allergy. Also, immunocap sensitivity can be increased by using both Api m 1 and Api m 10.

Keywords: *Apis dorsata*, recombinant allergens, *Apis mellifera*, Icarapin, component resolved diagnosis

This work was supported by National Science Foundation (Grant No-NSF/HS/02/2015) & Medical Research Institute (Grant No-MRI/46/2014).

Abstract No: OP08

Comparison of hyaluronidase from venom of *Apis dorsata* Fabricius and *Vespa affinis* Linnaeus

Gunasekara DLPE[1], Handunnetti SM[1], Premawansa S[2], Dasanayake WMDK[4], Ratnayake IP[5], Seneviratne SL[6,7], Dias RKS[8], Premakumara GAS[9], and De Silva R[4]

[1]IBMBB, University of Colombo, [2]Department of Zoology and Environment Sciences, Faculty of Science, University of Colombo, [4]Department of Immunology, Medical Research Institute, Colombo, Sri Lanka, [5]District Hospital, Bandarawela [6]Royal Free Hospital, UK, [7]Department of Surgery, Faculty of Medicine, University of Colombo, [8]Department of Zoology and Environmental Management, Faculty of Science, University of Kelaniya, [9]Herbal Technology Section, Industrial Technology Institute, Colombo

Hyaluronidase is a common component of both *Apis dorsata* and *Vespa affinis* venom. IgE cross-reactivity of hyaluronidase may hamper the specific diagnosis of the patients who developed allergies to *A. dorsata* and *V. affinis* venom, which is important in venom immunotherapy. In this study, hyaluronidase of the venom from two species were assessed using biochemical and immunochemical methods. Venom of both *A. dorsata* and *V. affinis* were collected using electrical stimulation. Hyaluronidase enzyme activity was assessed using turbidimetric assay. Fractions which had hyaluronidase enzyme activity were collected separately from venom using high performance liquid chromatography (HPLC). Purity of the collected fractions were evaluated by sodium dodecyl sulphate gel electrophoresis (SDS-PAGE). Sera from 60 patients (n=30 for each venom, obtained from patients who had allergic reactions to *A. dorsata* or *V. affinis* venom) were used to determine IgE reactivity to hyaluronidase by immunoblots. To detect the IgE cross-reactivity to hyaluronidase, immunoblots were cross examined with sera from either *A. dorsata* venom allergic patients or *V. affinis* venom allergic patients. Molecular weights of the fraction which had hyaluronidase activity were determined as 39 kDa and 45 kDa for *A. dorsata* and *V. affinis* venom respectively. Of the patients allergic to venom of *A. dorsata*, 40% (12/30) had IgE to hyaluronidase of *A. dorsata* venom whereas in *V. affinis* venom allergic patients, the IgE reactivity to its hyaluronidase was 96.7% (29/30). Likewise, 53.3% (16/30) of patients who had allergy to *A. dorsata* venom, had IgE to hyaluronidase of *V. affinis* venom whereas of 18 % (18/30) patients who had allergy to *V. affinis* venom, had IgE to hyaluronidase of *A. dorsata* venom. These results indicate possible IgE cross-reactivity of hyaluronidase between *A. dorsata* and *V. affinis* venom and would be considered in diagnosis of bee and hornet venom allergy.

Keywords: *Vespa affinis*, *Apis dorsata*, immunoblots, hyaluronidase

This work was supported by National Science Foundation (Grant No-NSF/HS/02/2015) & Medical Research Institute (Grant No-MRI/46/2014).

Abstract No: OP09

High levels of Angiopoietin 2 and Angiopoietin 2/1 at critical stage of DHF patients and their association with clinical and biochemical parameters

Maheshi Mapalagamage[1], Shiroma M. Handunnetti[1], Rajitha Wickremasinghe[2], Gayani Premawansa[3], Sharmila Thillainathan[3], Tharanga Fernando[3], Karunayokini Khanapathypillai[3], Aruna Dharshan De Silva[4], Sunil Premawansa[5]

[1]Institute of Biochemistry Molecular Biology and Biotechnology, University of Colombo, [2]Faculty of Medicine, University of Kelaniya, [3]Colombo North Teaching Hospital, Ragama, [4]Genetech Research Institute, [5]Department of Zoology and Environment Science, Faculty of Science, University of Colombo

Increased vascular permeability is thought to be a main feature of severe dengue infection, resulting plasma leakage which leads to the serious complications of dengue. Serum Angiopoietin-1 (Ang-1) and Angiopoietin-2 (Ang-2) produced by endothelial cells are known to associate with endothelial stability in dengue infection. Ang-1 and Ang-2 were studied in different stages of dengue patients, using commercially available kits. From confirmed uncomplicated dengue fever (DF) patients, samples were taken on admission (DFA, n=36) and discharge (DFD, n=18) and from confirmed dengue hemorrhagic fever patients (DHF) three set of samples were used; on admission (DHFA, n=36), at critical stage (DHFC, n=26) and on discharge (DHFD, n=18). Age-gender matched healthy individuals were also recruited as controls (HC, n=24). Results showed that low levels of Ang-1 and high levels of Ang-2 were associated with disease status in DHF and not in DF. DHFC had recorded the significantly higher Ang-2 levels compared to DHFA ($p=0.028$) as well as significantly lower Ang-1 levels compared to DHFA, DHFD and HC ($p<0.050$). Specifically, serum Ang-2/Ang-1 levels in DHFC were recorded as the highest compared to all other study categories tested ($p<0.001$). Moreover, significant positive correlation was observed between serum Ang-1 and platelet count in the DHFC patient category ($r=0.674$, $p<0.001$, Pearson correlation). Thrombocytopenia in DHFC may lead to less serum Ang-1 which could harbor more receptors for the binding of Ang-2 thus facilitating the endothelial dysfunction. Having a cut-off value of 1.02 with 82.6% sensitivity and 80.4% specificity in this study showed a potential use of serum Ang-2/Ang-1 as a biomarker for DHF critical stage.

Keywords: Angiopoietin-1, Angiopoietin-2, Dengue hemorrhagic fever, Biomarker

This work was supported by National Science Foundation (NSF Grant No: RG/2014/HS/04).

Abstract No: OP10

IgM and IgG response against a specific protein antigen in rats orally fed with a herbal formulation

Ranaweera BVLR[1], Edward D[1], Abeysekera AM[2], Weerasena OVDSJ[1], Handunnetti SM[1]

[1]Institute of Biochemistry, Molecular Biology and Biotechnology, University of Colombo,
[2]Link Natural Products (Pvt) Limited

Link Samahan (LS) is a commercial polyherbal formulation which is commonly used for prophylaxis against cold and cold related symptoms in Sri Lanka. This study was designed to evaluate the effect of LS on IgM and IgG production in rats, against bovine serum albumin (BSA). Three groups of rats were immunized with BSA on day 1 and 15 followed by the oral treatment on day 1- 5 and again on day 15 – 19 with LS. Water and sugar treated groups were used as two controls as LS contains sugar as excipient. Rat sera were collected on day 0 and weekly thereafter from day 7 to 35. Rat anti-BSA IgM and IgG antibodies were determined by an in-house BSA-ELISA established previously using OD_{450} at 1 in 100 serum dilution and antibody titers as indicators of antibody levels measured. LS treated group showed a significantly higher level of IgM (OD_{450} = 0.610±0.09; titer - 356) on day 7 compared to the two control groups, ie, water (OD_{450} = 0.186±0.03; titer - 112) and sugar (OD_{450} = 0.245±0.04; titer - 126) ($p<0.05$). The highest IgG level (OD_{450} = 1.135±0.09; titer- 5702) in LS treated group was observed on day 21 compared to that of the controls, ie, water (OD_{450} = 0.297±0.12; titer - 800) and sugar (OD_{450} = 0.465±0.11; titer - 1008) ($p<0.05$). These findings suggest that LS may act as an immunostimulant/ adjuvant to induce IgM and IgG production against a specific antigen with antibody production in both primary and secondary immune response. Higher IgG titer compared to that of IgM observed in LS treated group compared to controls suggests that LS may be involved in inducing efficient class switching from IgM to IgG and also in boosting the IgG production by B lymphocytes.

Keywords: Link Samahan, BSA, IgM, IgG, IgM and IgG titer, ELISA, Immunostimulant

This work was supported by Link Natural Products (Pvt) Limited & IBMBB.

Abstract No: OP11

In vitro study on the effect of antimicrobial agents on inflammatory cytokine response in Leptospirosis

Sam G[1], Fernando N[1], Rajapakse S[2], Handunnetti SM[1]

[1]Institute of Biochemistry, Molecular Biology and Biotechnology, University of Colombo

[2] Post Graduate Institute of Medicine

Antimicrobial agents despite being active against a wide range of micro-organisms are known to influence physiological response to infections, through interaction with host cell functions. Ceftriaxone (CRO), doxycycline (DOX) and penicillin G (PEN), three common drugs used in the treatment of leptospirosis were investigated for their anti-inflammatory activity, independent of their antimicrobial function under *in vitro* conditions. Effect of these antimicrobials on the mRNA expression of inflammatory cytokines IL-6, IL-8 and the anti-inflammatory cytokine IL-10 by peripheral blood mononuclear cells (PBMCs) were determined at different time intervals, by means of reverse transcription – polymerase chain reaction (RT-PCR). PBMC responses were measured after *in vitro* stimulation with *Leptospira interrogans* serovar Pyrogenes sonicates and under concomitant setting with the simultaneous addition of antimicrobials and the stimulant. The selected concentrations of antimicrobials, 275 µg/mL of CRO, 25 µg/mL of DOX, and 150 U/ml of PEN, exhibited excellent (>90%) *in vitro* activity against live *L. interrogans* and were not toxic to the PBMCs. *L. interrogans* serovar Pyrogenes sonicates were effective in inducing the prompt expression of IL-6 and IL-8 by 1 h. The concomitant setting revealed a definite inhibition of IL-6 and IL-8 mRNA expression by DOX at both 8 and 24 h, analogous inhibition was not evident with CRO nor PEN whose total mRNA expression was similar to those of unstimulated PBMCs. The incessant expression of control protein Glyceraldehyde 3-Phosphate dehydrogenase (GAPDH) at all incubation times assessed elucidates the specific inhibitory potential of DOX. This study provides *in vitro* evidence that DOX can cause prolonged inhibition of mRNA expression of pro-inflammatory cytokines IL-6 and IL-8 in leptospirosis and hence its potential, in attenuating the detrimental inflammatory cytokine response during later stages in leptospirosis.

Keywords: antimicrobials, leptospirosis, cytokines, inflammatory response

This work was supported by IBMBB and constitutes a part of MSc studies of SG.

Abstract No: OP12

Development of a Colourimetric Reverse Transcription Loop-mediated Isothermal Amplification assay for the rapid detection of different Serotypes of the Dengue virus

Ariyaratne MHJD[1,2], Weerasena OVDSJ[1,] De Silva AD[2], Fernando N[1], Handunnetti SM[1], Nguyen AV[3]

[1]Institute of Biochemistry, Molecular Biology and Biotechnology, University of Colombo, [2]KDU-CARE/Biomedical Lab-2, Department of Para clinical Sciences, Faculty of Medicine, General Sir John Kotelawala Defense University, Ratmalana, [3]The Department of Biological Sciences and Geology, The City University of New York – Queensborough, New York, USA

Dengue viruses are responsible for causing a significant burden on healthcare in Sri Lanka and around the world. In Sri Lanka, the mainstay of diagnosis is mainly clinical, backed up with the detection of antibody specific to nonstructural protein 1 (NS1) antigen. ELISA antibody detection and/or PCR are available, albeit expensive. However, none of the commercially available tests in Sri Lanka offer a serotype specific diagnosis. Emerging evidence suggests the possibility of variation of clinical presentation and severity with different serotypes. Thus, developing a serotype specific, less expensive, easier to use dengue assay would be helpful in aiding clinical diagnosis and epidemiological surveillance in the field. The Dengue loop mediated isothermal amplification (LAMP) assay was developed for testing four different dengue serotypes; Dengue 1,2,3 and 4. As a pilot study, four different serotypes of viruses were grown in a Vero cell line and the viruses were harvested at day 5 of post infection. Viral RNA was confirmed by nested PCR amplification and gel electrophoresis. A modified version of LAMP based on colourimetry was developed using hydroxyl naphthol blue (HNB) dye which changes colour with Mg^{2+}depletion as hydrolysis of incorporated nucleotides generating available phosphates binding to Mg^{2+}. Previously developed HNB-based LAMP assays for Dengue had utilized 24 primers for 4 serotypes. Our study demonstrated that utilizing nine primers is sufficient to cover all 4 serotypes. The optimum temperature for amplification was found to be 65°C and 5 mM of Mg^{2+}concentration for both amplification and the distinct colour change from violet to sky-blue using HNB. qPCR based quantification indicated that the colorimetric LAMP assay could detect the virus down to $1X10^2$ copies. The colourimetric RT-LAMP assay for dengue serotype identification offers a cheaper, easy to perform alternative to dengue PCR which was 10-fold more sensitive than PCR, and that can be implemented in laboratories with limited resources or in field settings. However the assay should be validated by using samples from patients and mosquitoes from the field.

Keywords: Dengue, Isothermal amplification, Colourimetric-assay

This work was supported by IBMBB and constitutes a part of MSc studies of AMHJD.

Abstract No: OP13

Development of primer designing tool to design species specific primers by using DNA Barcode sequences

Fernando MATM[1], Weerasena OVDSJ[2]

[1]Department of Plant Sciences, University of Colombo
[2]Institute of Biochemistry, Molecular Biology and Biotechnology, University of Colombo

Identification of plants/ plant materials by conventional taxonomy based on morphological characters is impossible where whole plant is not available. DNA barcodes have been used in identification of plant species in processed plant materials especially adulterated or substituted in herbal medicinal formulations. However, DNA barcoding is also inapplicable when materials are in mixtures such as in powdered or liquid formulations, processed foods, animal droppings etc. Polymerase Chain Reaction (PCR) is one of the useful applications in identification of plant materials in mixtures, if species specific PCR primers are available. Therefore the objective of this study is to develop a method to design species specific primers *in silico* using barcoding DNA sequences as templates. Pywinauto library was used to automate the Clustal W aligning process of sequences, Python based codes were scripted to identify variations between two sequences and generating pair of primers by including the sequence variation at 3' end of the primers. Separate codes were generated to analyse length, GC content and melting temperature of designed primers. The above codes were included into a GUI using JavaFX framework and Cascading Style Sheets (CSS) and developed a primer designer software. Barcoding sequences from land plants were selected and BLASTn search was carried out over the GenBank database to obtain the sequence with highest similarity to each sequence. Sequence of interest and its highest hit were used as inputs for Primer Designer software. The software generated possible primer pairs and listed with their physicochemical properties. The best primers were further analyzed by Primer-BLAST to obtain species specific primers. Based on the results, Primer Designer software tool, could be used to design species specific primers to uniquely recognize plant species. Work is currently in progress to validate the designed primers by wet lab PCR experiments.

Key words: Primer designing, DNA barcodes

Abstract No: OP14

Studies on *in vitro* anti-inflammatory potential and phytoconstituents of ethanol bark extract of *Flacourtia indica*

Perera HDSM[1], Samarasekera R[1], Handunnetti SM[2], Weerasena OVDSJ[2], Jabeen A[3], Choudhary MI[3,4]

[1]Industrial Technology Institute (ITI), Colombo 07, [2]Institute of Biochemistry, Molecular Biology and Biotechnology, University of Colombo, [3]Dr. Panjwani Center for Molecular Medicine and Drug Research, International Center for Chemical and Biological Sciences, University of Karachi, Pakistan, [3,4]H. E. J. Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Pakistan

Flacourtia indica (Salicaceae) is a multi-functional medicinal plant that has been traditionally used to treat a number of diseases including inflammatory diseases. The objective of the present study is to determine the *in vitro* anti-inflammatory potential of ethanol bark extract of *F indica* and identify its phytoconstituents. The air-dried and powdered bark was extracted with ethanol using a cold extraction technique. The anti-inflammatory potential of ethanol bark extract (EBE) of *F. indica* was determined measuring oxidative burst inhibition in human whole blood (HWB) and isolated polymorphoneutrophils (PMNs) using luminol-enhanced chemiluminescence assay and nitric oxide production inhibitory assay on raw 264.7 macrophage cells. The EBE of *F. indica* was chemically characterized using High Performance Liquid Chromatography (HPLC) and Gas Chromatography-Mass Spectrometry (GC-MS). Results were statistically analysed at 95% confidence interval. The extract showed a significant, dose-dependent oxidative burst inhibition on HWB (IC_{50}: 47.64 ±2.32 µg/mL) and PMNs (IC_{50}:5.02±0.38 µg/mL), which was found to be comparable with the reference standard Ibuprofen (IC_{50}: 5.12±0.45 µg/mL) and moderate NO production inhibition (38.07±0.93%) at 500 µg/mL with a cell viability of 87.3±0.50%. The GC-MS analysis of EBE revealed the presence of six compounds including linoleic acid ethyl ester and hexadecanoic acid, ethyl ester as major compounds (> 2% peak area). The HPLC analysis of EBE of *F. indica* revealed the presence of phenolic compounds. The EBE of *F. indica* is identified as a source of anti-inflammatory agents having marked oxidative burst inhibitory properties, which may be attributed to the presence of fatty acid derivatives and phenolic compounds.

Keywords: *Flacourtia indica*, Anti-inflammatory, Gas chromatography-mass spectrometry, high performance liquid chromatography

This work was supported by National Research Council (NRC-12-100).

Abstract No: OP15

Production of toxic metabolite by native antagonistic fungi, against *Rigidoporus microporus*, the causative fungi of White Root Disease of rubber (*Hevea brasiliensis*)

Balasooriya PW [1, 2], Fernando THPS [1], Weerasena OVDSJ [2]

[1]Department of Plant Pathology and Microbiology, Rubber Research Institute of Agalawatta,
[2]Institute of Biochemistry, Molecular Biology and Biotechnology, University of Colombo

White Root Disease is the most destructive root disease in rubber growing lands in Sri Lanka. *Rigidoporus microporus* is the causative pathogen of the disease. Although the chemical methods have been developed to control the pathogen, they have many limitations due to the health hazards and environmental pollution. Therefore, as an environmental friendly solution, antagonistic fungi for *R. microporus* were isolated and their ability to secrete secondary metabolites to control the pathogen was tested. Soil samples were collected from Kalutara, Ampara and Vavuniya districts and different fungal colonies were isolated using dilution plate technique. Antagonism against *R. microporus* was tested using dual plate culture test method and three best antagonistic fungi which showed over 70% inhibitions were selected. Selected fungi were identified as *Trichoderma* spp. Antagonists were separately grown on Potato Dextrose Broth (PDB) media in presence and absence of pathogen (*R. microporus*) to produce toxic metabolites. Culture filtrates were harvested at 12^{th} and 18^{th} days and autoclaved at 121^0C for 15min. The effectiveness of the metabolites in fungal filtrates against the pathogen was tested using Poison Food Technique (PFT). Filtrate was tested at three different concentrations (10%, 20% and 30%). According to the results, in both 12^{th} and 18^{th} days the highest toxic metabolite production was showed in pathogen absence flasks. The highest inhibition (86.06%) was shown by the *Trichodrma* spp isolated from Kalutara district at 30% concentration in day 18. Comparison of pathogen present and absent culture media showed, the toxic production was higher in pathogen absent media compare to present media. Heat stability of the secondary metabolites could be an extra benefit to mass production. Use of combination of these toxic metabolites may increase the effectiveness against *R. microporus*.

Keywords: *Rigidoporus microporus*, toxic metabolites, *Trichoderma spp*, Antagonism, *Hevea brasiliensis*

This work was supported by National Science Foundation (NSF/RG/2016/AG/01)..

Abstract No: OP16

Protein Network Building for Malignant Peripheral Nerve Sheath Tumor via Genetic SNP Mutations

Senadheera SPBM, Weerasinghe AR, Wijesinghe CR

University of Colombo School of Computing

Cancer is a genetic disease which begins by accumulating mutations in an organ. Cancergenesis can be explored by analysing genomic mutations and protein changes. Our objectives of this study are data conversion to common platform, data annotation and create biological meaningful network for Malignant Peripheral Nerve Sheath Tumors. We have analysed 15 Malignant Peripheral Nerve Sheath Tumors in order to developed protein network. Dataset: MSKCC, Nat Genet 2014 was used for the study. R packages, UniProt conversion tool, Ensemble Genome conversion tool, VEP tool, Cytoscape, Genemania and Vizbi tools were used fot the analysis. Data annotation and biological interaction identification had performed via using IntAct, Reactome, IMEx and ChEMBL databases. Gene clusters and Protein clusters were created based on MCODE algorithm. Finally, information was interpreted based on biological literature evidences. While exploring the mutations we design a workflow to analyse single nucleotide mutations. Data conversion methods rely on genome build, annotation database version and genome position. Best method for genomic mapping is chromosome position mapping. Molecular complexes generated based on MSKCC protein mutations has interaction between PIK3CA and Guanine nucleotide exchange factor VAV3 proteins. Protein which are altered in the dataset had 59.64% physical interaction biological evidence. Since cancers are having diversified mutations, it difficult to achieve direct protein level interactions. However, there can be intermediate protein which connect the hidden biological facts. As future work, we will try to merge other cancer datasets for this workflow. Moreover, more visualizing techniques needs to be integrated for the workflow.

Keywords: Cancer, SNP mutation, protein network, MCODE

This work was supported by University Grants Commission 2017.

Abstract No: PP01

Preliminary study on *CTLA-4* gene mutations in inflammatory bowel disease in Sri Lanka

Hewagama AHS[1], SeneviratneSL[1], Handunnetti SM[2], Pathirana SL[3]

[1]Institute of Biochemistry, Molecular Biology and Biotechnology, University of Colombo, [2]Royal Free Hospital, Department of Parasitology, Faculty of Medicine, University of Colombo

Ulcerative Colitis (UC) and Crohn's Disease (CD) are two main forms of inflammatory bowel disease (IBD). Genetic factors and altered immune regulation were associated as causative factors. The human cytotoxic T lymphocyte associated antigen 4 (CTLA-4) is a negative T-cell regulator associated with many autoimmune diseases and aim of this study was to investigate the association of *CTLA-4* polymorphisms with disease manifestations in Sri Lankan IBD patients. Clinical data and blood samples from 24 IBD patients and 6 healthy controls were obtained from the Gastroenterology Clinic of the National Hospital of Sri Lanka (over 5 months). DNA was extracted using the DNA Blood Mini Kit. The coding sequence of *CTLA-4* was amplified using primers for exon 1, 2, 3, 4 and products visualized on 2% agarose gel electrophoresis. Purified PCR products were subjected to bi-directional sequencing PCR and automated Sanger sequencing to detect possible polymorphisms in the *CTLA-4* against reference sequence (NG_011502.1) from NCBI. The occurrence of known polymorphism, +49A/G in exon 1 was higher in IBD patients compared to controls, suggesting it's has a predisposing role rather than disease causing. Homozygous GG genotype frequency (16.7%) and G allele frequency (31.2%) were high in IBD patients compared to healthy controls (0% and 16.6% respectively). A allele frequency was higher in UC (75%) than CD (62.5%). The AA genotype (80%) and A allele (90%) were more frequent in UC females than their males (57.1% and 64.3% respectively) and male or female in CD (AA- 44.4%, A allele- 66.7%, AA- 33.4% A allele- 50% respectively) while GG genotype (33.3%) and G allele (50%) were frequent in CD females than their males (11.1% and 33.4% respectively) and males (GG- 28.6%, G allele- 35.7%) or females (GG- 0% G allele- 10%) of UC. Genotype AA and A allele, are positively associated with IBD mild disease who were negative for extra-intestinal and auto-immune manifestations. Disease affected location and severity were significantly correlated with UC genotype ($p<0.05$).Stronger associations may be found with a larger sample size.

Keywords: Inflammatory bowel disease, *CTLA-4* gene, Sri Lanka

This work was supported by the IBMBB and constituted a part of MSc studies of HAHS.

Abstract No: PP02

Expression and purification of recombinant anti-diabetic drug target, peroxisome proliferator activated receptor gamma (Pparɣ)

De Silva MCJ[1], Wijayasinghe YS[2]

[1]Institute of Biochemistry, Molecular Biology and Biotechnology, University of Colombo
[2]Department of Biochemistry, Faculty of Medicine, University of Kelaniya

Thiazolidinediones are effective anti-diabetic drugs that elicit the hypoglycemic effect through activating the nuclear receptor Pparγ. Pparγ is still a focus in the search of novel therapies with fewer side effects to treat type II diabetes mellitus. Objective of the present study is to establish an optimized protocol for *in vitro* production of the recombinant Pparγ protein, which could later be used in natural product-based anti-diabetic drug discovery research. Open reading frame of the mouse Pparγ (containing amino acids 102-505; Pparγ 102) has been cloned into pET28 plasmid vector with a N terminal His_6 tag. It was transformed into Rosetta 2 (DE3) *E. coli* and successful transformants were induced with isopropyl β-D-thiogalactoside (IPTG). A combination of metal affinity and cation exchange chromatography was performed to purify the protein. Pparγ 102 construct poorly expressed at temperatures above 20 °C even with IPTG levels as low as 0.1×10^{-3} M. However, it reasonably expressed in the soluble form with 0.5×10^{-3} M IPTG at 16 °C. His tagged Pparγ102 was purified first using Ni^{2+} affinity chromatography and the protein was eluted around 250×10^{-3} M imidazole. Subsequently, purification with cation exchange chromatography eluted the protein around 500×10^{-3} M NaCl with a reasonable purity as detected on SDS-PAGE. In conclusion, our results evidence the possibility of producing large quantities of pure recombinant Pparγ 102 protein using a cost effective and convenient expression system.

Keywords: Diabetes mellitus, Pparγ, protein expression, protein purification

This work was supported by IBMBB & University of Kelaniya (Research grant RP/03/SR/04/02/01/2016) and constitutes part of the MSc studies of MCJD.

Abstract No: PP03

Immuno-proteomic characterization of *Vespa affinis* (Lesser Banded Hornet) venom and its cross-reactivity with the venom of *Vespula vulgaris*

Gunasekara DLPE[1], Handunnetti SM[1], Premawansa S[2], Dasanayake WMDK[3], Karunatilake C[3], Ratnayake IP[4], Seneviratne SL[5,6], Dias RKS[7], Premakumara GAS[8], and De Silva R[3]

[1]Institute of Biochemistry, Molecular Biology and Biotechnology, University of Colombo, [2]Department of Zoology and Environment Sciences, Faculty of Science, University of Colombo, [3]Department of Immunology, Medical Research Institute, Colombo, Sri Lanka, [4]District Hospital, Bandarawela, [5]Royal Free Hospital, UK, [6]Department of Surgery, Faculty of Medicine, University of Colombo, [7]Department of Zoology and Environmental Management, Faculty of Science, University of Kelaniya, [8]Herbal Technology Section, Industrial Technology Institute, Colombo

Allergy to *Vespa. Affinis* L. (*English: Lesser Banded Hornet, Sinhalese: Debara and Tamil: Kuḷavikaḷai*) (family: Vespidae) stings is common in rural areas in Sri Lanka. However, the unavailability of diagnostic or therapeutic preparations of *V. affinis* venom impede its laboratory diagnosis and immunotherapy. This study was aimed to assess i) allergenic proteins in *V. affinis* venom using HPLC, SDS-PAGE and immunoblots with 30 sera from patients who developed *V. affinis* sting allergy and ii) usefulness of Phadia ImmunoCAPs with *V. vulgaris* L. crude venom and two recombinant components of *V. vulgaris* venom, rVes v1 and rVes v5, in diagnosis of *V. affinis* sting allergy. Of the eight bands visualized in SDS-PAGE, five allergens (100, 80, 45, 34 and 24 kDa) were recognized by IgE in patients' sera. Reactivity of allergens of 34 and 24 kDa with 30 sera tested was 96.7% (29/30) while the reactivity for 45 kDa was 93.3% and reactivity for 100 and 80 kDa ones was 90%. Conversely, IgE cross-reactivity of these sera were low with ImmunoCAPs comprising *V. vulgaris* crude venom (43.3%; 13/30) and rVes v 1 (3.3%; 1/30), although a higher level of cross-reactivity was evident with rVes v 5 (73.3%; 22/30). Healthy control sera did not show any IgE reactivity with either *V. affinis* or *V. vulgaris* venom/components. In conclusion, this study has shown the presence of five allergens in *V. affinis* venom which could be useful in component resolved diagnostics for *V. affinis* sting allergy in future. The low level of IgE cross-reactivity with *V. vulgaris* venom/components, except rVes v 5 which showed a considerable IgE cross-reactivity, indicate that the use of *V. vulgaris* crude venom/components for diagnosis of *V. affinis* venom allergy may be limited. Evaluation of *in vivo* diagnosis; Skin prick testing with crude and individual venom components of *V. affinis*, would be the next step forward.

Keywords: *Vespa affinis*, *Vespula vulgaris*, IgE, Cross-reactivity, HPLC

This work was supported by National Science Foundation (Grant No-NSF/HS/02/2015) and Medical Research Institute (Grant No-MRI/46/2014).

Abstract No: PP04

Preliminary study on pollen records during South-West monsoon in selected locations in Sri Lanka and direct effect of pollen on basophil degranulation

Guruge BMA[1], Ishaka GKD[1], Handunetti SM[1], de Silva R[2], Premawansa S[3], Premathilake TRD[4]

[1]Institute of Biochemistry, Molecular Biology and Biotechnology, University of Colombo, [2]Department of Immunology, Medical Research Institute, Colombo, [3]Department of Zoology and Environment Sciences, Faculty of Science, University of Colombo, [4]Postgraduate Institute of Archaeology, University of Kelaniya

Pollen grains cause allergy in humans with seasonality and its prevalence is on rise imposing significant health burden. Proteins and glycoproteins that assist in reproduction on pollen wall (exine) will trigger allergic reactions in sensitized people. Information on allergenic pollen taxa have not been reported due to lack of investigations in Sri Lanka. The current study aims to determine airborne 'allergenic' pollen taxa and their seasonality in selected locations. Three in-house pollen traps were placed in three locations on a weekly basis, at three sites in urban, semi urban and rural areas in Gampaha district, during South West monsoon (June-September, 2017). Pollen traps were processed and analyzed to identify the abundance of pollen taxa in the lower atmosphere. Total airborne pollen counts showed a variation with time, in semi-urban and rural areas, but not in the urban area. The weekly total pollen count increased during the second week of June, peaked during July and gradually decreased by September to reach the lowest pollen counts. The weekly total pollen count did not show a significant correlation (r=0.410, p=0.345) with temperature and showed a negative correlation (r=-0.435, P=0.512) with rainfall. Pollen taxa which mainly contributed for the seasonal variation of airborne pollen count were *Acacia auriculiformis, Cryptolepis buchanani*, *Panicum maximum, Oryza sativa* and *Cyperus aromaticus*. Fresh exine protein extracts showed five major pollen coat proteins which ranged in between 10-45 kDa by SDS-PAGE. Crude exine protein extracts (500 µg/ml) of all test plants were incubated for 30 minutes with rat basophils (RBL-2H3) and the Sulforhodamine B assay showed 90-95% cell viability. To the same protein concentration in basophil degranulation assay (n=4), the highest degranulation percentage (30.29±6.44 %) was shown by *P. maximum* and the lowest (14.69±5.37%) was shown by *O. sativa* (paddy) and others ranged in between. Study results shows the contribution of IgE independent basophil degranulation to trigger allergy. Studying with increased number of pollen traps for longer periods would improve these experiments.

Keywords: pollen, allergenic, seasonality, proteins, direct degranulation

This work was supported by the IBMBB and constitutes a part of MSc studies of GBMA.

Abstract No: PP05

Comparison of a two-step and a novel single step method for neutrophil isolation

Lakmali AKD[1], Fernando N[1], Handunnetti SM[1], Premawansa S[2]

[1]Institute of Biochemistry, Molecular Biology and Biotechnology, University of Colombo,
[2]Department of Zoology and Environmental Sciences, University of Colombo

Most of the bioassays involving neutrophils require their isolation effectively at a high purity. In our study we optimized a novel method for neutrophil isolation and yield, purity, viability, the appearance of the neutrophils and time consumption for isolation with a conventional method. Neutrophils were isolated using two methods i) a two-step method with total leukocyte isolated with 3.5% (w/v) Dextran sedimentation followed by Percoll gradient (conventional method) ii) a single-step method comprising of 6% (w/v) Dextran sedimentation with osmotic lysis. Neutrophil viability and yield were higher in the single step 6% Dextran sedimentation (97.9±0.47% and 48.6±4.89% respectively, n=6) than the two-step method (92.3±2.2 and 46.0±4.16 respectively). Purities of the samples were 76% and 72.3% for two methods respectively. Neutrophils isolated using the single step method appeared to be less stressed and activated and the processing time was 50% less than the two-step method. Collectively, the single-step 6% Dextran sedimentation method can be suggested as a better method for neutrophil-involving experiments.

Keywords: neutrophils, Dextran sedimentation, two-step method, single-step method

This work was supported by the IBMBB and constituted a part of MSc studies of AKDL.

Abstract No: PP06

Development of barcodes for medicinal plant "Babila" (*Sida* species) in Sri Lanka

Rajphriyadharshini R[1], Weerasena OVDSJ[1], Tennakoon TMSG[2], Gunaratna LNR[1], Suraweera CD[2]

[1]Institute of Biochemistry, Molecular Biology and Biotechnology, University of Colombo
[2] Research Laboratory, Link Natural Products (Pvt) Ltd

Babila (*Sida* spp) is known for its medicinal value as it has enormous applications in Ayurvedic system of medicine. Several *Sida* species are found in Sri Lanka and difficult to identify correct species used in traditional medicine. Substitution and adulteration by other plants with similar morphology are more frequently found in raw materials supply chain in the country. Moreover it is difficult to identify required *Sida* species by using chromatographic, macroscopic and microscopic methods when available in formulations as processed or half processed form. Thus this obstacle can now be overcome by using DNA barcoding. Additionally, due to its economical and biological significance, developing DNA barcodes may contribute to the trade control and conservation of *Sida* species. General objective of this study is to develop barcodes for identification of *Sida* species in Sri Lanka. As DNA barcodes, three chloroplast DNA regions *i.e.* matK. rbcL, psbA-trnH and the Internal Transcribed Spacer (ITS) of nuclear ribosomal DNA were selected in this study. The genomic DNA was extracted from three different authentic *Sida* spp., barcoding regions were amplified by PCR and bidirectionally sequenced. The barcode sequences obtained were confirmed by searching over the GenBank database using Basic Local Alignment Search Tool and the sequences were deposited in GenBank database. Phylogentic trees were constructed by available sequence of three different *Sida* spp. used in this study, using MEGA V 7. The results suggested that DNA barcodes have the potential to differentiate different *Sida* species. Based on the species specific sequence divergence, the ITS-2 and rbcL were found to be the most suitable regions to distinguish *Sida* species out of the four barcode regions used. The current study strongly suggests that the raw materials of herbal medicines need to be properly authenticated before use, and DNA barcoding has been found to be a suitable method in authentication of half processed form of the material.

Keywords: DNA Barcodes, *Sida,* Adulteration

This work was supported by the IBMBB & Link Natural Products (Pvt) Ltd and constituted a part of MSc studies of RR.

Abstract No: PP07

Optimization of isolation of BK viral DNA from renal transplant patients and *VP1* gene based genotyping

Ratnayake AKDVY[1], Fernando N[1], Gajanayake T[2], Handunnetti SM[1], Jayamaha CJS[3]

[1]Institute of Biochemistry, Molecular Biology and Biotechnology, University of Colombo,
[2]Faculty of Science, Horizon Campus,[3]Department of Virology, Medical Research Institute

BK virus (BKV) is a cause of a common viral infection during post-transplantation period that could be progressed into allograft nephropathy. Active replication of BKV in urothelial cells cause releasing of BKV into urine which can be monitored by molecular assays. BKV has four main genotypes; I, II, III, and IV. BKV genotype II and III are rare, whereas genotype I shows ubiquitous distribution. However, persisting BKV genotypes and subtypes among Sri Lankan renal transplant patients have not been studied. Therefore, the objective of this preliminary study was to investigate the prevailing BKV genotypes and subtypes in a cohort of renal transplant patients in Sri Lanka. Urine samples received at Medical Research Institutefor routine testing which had high viral load based on Real Time PCR results were obtained. Prior to viral DNA extraction, urine processing was optimized by increasing the volume of urine used, centrifugation speed and time. Optimum criteria were determined as 12-15 ml, 2800*g* and 15 minutes respectively. Viral *DNA was extracted from* sedimented urine *using QIAGEN DNA Mini-kit.* Conventional PCR was performed for the amplification of subtyping region of *VP1* gene of BKV and sequencing data were subjected to clustal-W alignment in BioEdit and MEGA6 software for the identification of the genotype and for subtyping followed by phylogenetic analysis respectively. Out of 41 patient samples, 25 were positive and their BKV loads varied from 1×10^3 to 3×10^8 copies/ml. BKV genotyping showed genotype I in 18 (72%) and genotype II in 7 (28%) of post-renal transplant patients. Genotype III and IV were not detected. BKV subtypes of Ia, Ib-1, Ib-11 and Ic were identified with the frequencies of 8/18 (44.4%), 5/18 (27.7%), 2/18 (11.11%) and 3/18 (16.67%) respectively. Patients who were infected with BKV subtype Ib-11 had significantly higher mean BKV load ($8.5\times10^7\pm1.03\times10^8$) compared to other subtypes of genotype I ($p<0.05$). Findings of this preliminary study showed prevalence of BKV genotype 1 and II among post-renal transplant patients in Sri Lanka. Prevalence of genotype I is consistent with other previous studies whereas the prevalence of genotype II which is rare and emphasize further studies on BKV genotypes prevalent in Sri Lanka.

Keywords: BK virus, renal transplantation, Sri Lanka, *VP1* gene, genotyping, subtyping

This work was supported by the IBMBB and constitutes a part of MSc studies of AKDVYR.

Abstract No: PP08

Reactivity of population samples from Colombo District for anti - leptospiral antibodies in Microscopic Agglutination Test

Balaji K[1], Weeratunga PN[2], Ramchandani KC[1], Wijerathne PBP[1], Sam G[2], Fernando N[1], Handunnetti SM[1], Fernando SD[c], Rajapakse S[2]

[1]Institute of Biochemistry, Molecular Biology and Biotechnology, University of Colombo [2]Department of Clinical Medicine, Faculty of Medicine, University of Colombo, [3]Department of Parasitology, Faculty of Medicine, University of Colombo

Leptospirosis is a tropical disease with significant morbidity and mortality impact. There is paucity of data on Sero – prevalence, epidemiology and distribution of non – pathogenic serovars and pathogenic serovars in community based populations in Sri Lanka**.** This study included a community - based sample of individuals in the Colombo district in Sri Lanka. Out of 566 primary cluster Grama Nildhari (GN) divisions, 27 GN divisions were selected, 30 individuals (15 males and 15 females) were selected by simple random sampling. Microscopic agglutination test (MAT) was carried out at the Medical Research Institute using a panel of 17 *Leptospira* strains. A MAT titre ≥ 1/40 was considered positive as this is a population cohort. Descriptive statistics were derived for MAT positivity and associations were examined using chi square analysis. The total sample size of the population was 810. A total of 430/810 (53.5%) were MAT positive for the non-pathogenic serovar *L. biflexa*. Of the pathogenic strains evaluated, the commonest noted serovars were Ratnapura (9.9%), Bankinang (9.6%) and Australis (9.3%). The serovar Bankinang showed the highest cumulative positivity, being the commonest pathogenic strain in 14/27 GN divisions analyzed. MAT positivity for the strains Australis, Bankinang, Bataviae, Canicola, Ratnapura,Pyrogenes, Pomona and Hebdomadis demonstrated significant association with the male gender ($p<0.05$). GIS analysis provided information for mapping the above individuals for proximity to water sources. Buffer zones were generated at 100, 250 and 500 m from water sources. MAT positivity for serovar Australis was significantly associated with residence within 250m from a water source ($p = 0.008$), A similar positive association was noted for the serovar Pomona in individuals residing within 500m from a water source ($p = 0.032$). Knowledge scores of < 5/10 (denoted as poor knowledge regarding leptospirosis) were associated with seropositivity for the pathogenic serovar Australis ($p=0.037$), Bankinang ($p=0.037$) and Icterohemorrhagicae ($p=0.010$). The epidemiological patterns noted above may have significance in determining adequate preventive strategies and surveillance methods for pathogenic serovars in the analyzed region.

Keywords: *Leptospira,* Serovar, Microscopic agglutination test, community, surveillance

*This work was supported by University of Colombo Research Grants (*AP/3/2/2014/RG/14).

Abstract No: PP09

Serological prevalence and determination of cutoff levels of Leptospirosis IgM and IgG in relation to Patoc I strain of *Leptospira biflexa* in a cohort of individuals from a high prevalence region in Sri Lanka

Balaji K[1], Weeratunga PN[2], Ramchandani KC[1], Wijerathne PBP[1], Sam G[2], Fernando N[1].

Handunnetti SM[1], Fernando SD[3], Rajapakse S[2]

[1] Institute of Biochemistry, Molecular Biology and Biotechnology, University of Colombo, [2]Department of Clinical Medicine, Faculty of Medicine, University of Colombo, [3] Department of Parasitology, Faculty of Medicine, University of Colombo

Leptospirosis is a tropical disease with significant morbidity and mortality impact. The gold standard for confirmation of the disease is the Microscopic Agglutination Test (MAT). Enzyme-linked Immunosorbant Assay (ELISA) is suitable alternative for diagnosis and community screening. The seroprevalence and performance of the IgM and IgG ELISA, along with optimal cutoff points for Leptospirosis has not been determined in high prevalence, community - based setting in Sri Lanka. Out of 566 primary cluster GN divisions, 27 GN divisions were selected, among which 30 individuals were selected by simple random sampling for serological analysis. Two Indirect in house ELISAs were used to measure IgM and IgG antibodies with *Leptospira biflexa.* Patoc 1 strain as the antigen. Absorbance was read at 450 nm by an ELISA plate reader. MAT was carried out at the Medical Research Institute with the *Leptospira biflexa* Patoc 1 strain. A MAT of 1/40 or a higher dilution is considered as the reference standard. ROC curves were generated for MAT positivity versus IgM and IgG. The characteristics of the generated ROC curves are as follows. Cutoff points were not calculated as the ROC curves demonstrated poor correlation between MAT and ELISA for this population. For IgM vs MAT; Area under curve = 0.501 (95% Confidence interval = 0.460 – 0.540, Standard Error 0.020, p = 0.976). For IgG vs MAT Area under curve = 0.533 (95% Confidence interval = 0.495 – 0.570, Standard Error 0.019, p = 0.086). The generated ROC curves demonstrate poor relationship between MAT positivity and Leptospirosis IgM and IgG ELISA for non – pathogenic serovars in a community - based cohort in the Colombo district of Sri Lanka

Keywords: *Leptospira*, IgM, IgG, MAT, ELISA

*This work was supported by University of Colombo Research Grants (*AP/3/2/2014/RG/14).

Notes: