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7th July 2017

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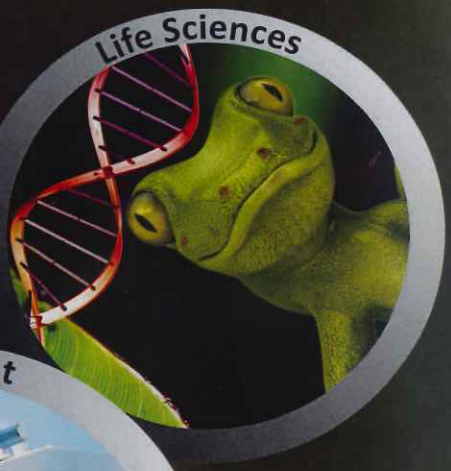

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Institute of Biochemistry, Molecular Biology and Biotechnology

University of Colombo

Our Vision

"To be an International Centre of Excellence In Molecular Life Sciences "

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Programme

8.30 h	Registration
9.00 h	Inauguration
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9.20 h	Address by Professor Lakshman Dissanayake, Vice Chancellor- University of Colombo
9.30 - 10.20 h	Professor Stanley Wijesundera Memorial lecture by Dr Rajiva de Silva, Consultant Immunologist President, Allergy & Immunology Society of Sri Lanka " Primary Immunodeficiency: Cradle to Grave"
10.20 - 10.25 h	Vote of Thanks by Dr Jagathpriya Weerasena
10.25 - 10.45 h	Tea
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12.15 - 12.45 h	Lecture by Industry Partner "Microscopy and Imaging Techniques"
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Message from the Director - IBMBB

Professor Shiroma Handunnetti
Director & Professor in Immunology, IBMBB

It is with great pleasure, I send this message to mark the occasion of the 9th 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 13th Anniversary.

IBMBB is an Institute in Sri Lanka which is dedicated to postgraduate training and research in Molecular Life Sciences, Immunology, Bioinformatics etc. Founder Director, Professor Eric H Karunanayake whose vision has led to the establishment this institute with financial support from Swedish Government. Once the IBMBB was established, SAREC supported research programme together with its MPhil and PhD trainees were relocated to the IBMBB from the Faculty of Medicine, University of Colombo. Since then the MPhil/PhD programme has expanded to have about thirty students conducting their research at the IBMBB, supported by competitive research funding received by academics of the IBMBB. There have been several firsts in Sri Lanka among its MPhils and PhDs including the first molecular genetics study on breast cancer, first molecular study on phytoplasma, first genomic and proteomic study on insulin-like growth factors in relation to fetal growth, first study on the tea genome, first study to genetically characterize traditional and weedy rice. Several MPhil/PhD students who have completed studies at IBMBB are now established and serving as academic staff members of other universities in Sri Lanka, ie., University of Jaffna, Eastern University, University of Kelaniya, University of Sri Jayewardenepura and University of Wayamba. Thus, the IBMBB has made a considerable contribution to capacity building and strengthening of research training for the higher educational sector.

IBMBB commenced two MSc programmes in 2005, one in Molecular Life Sciences and the other in Cellular and Molecular Immunology. A third MSc programme in Bioinformatics was begun in 2012. At IBMBB, the MSc courses have a >80% completion rate and to date 143 students have graduated. Currently, IBMBB has about 80 post-graduate (MSc, MPhil & PhD) students are continuing their studies.

The Annual Scientific Sessions organized by the IBMBB provides a forum for Dissemination of research findings of IBMBB students and postgraduates from other Higher Educational Institutes.

IBMBB has held eight Annual Scientific Sessions starting from 2006 and two International conferences in 2009 and 2014 to mark the 5th and 10th anniversaries.

High point of the 9th Annual Scientific Sessions will be the Professor Stanley Wijesundera Memorial lecture on "Primary Immunodeficiency: Cradle to Grave" to be delivered by Dr Rajiva de Silva, Consultant Immunologist, Medical Research Institute, Colombo. Dr de Silva has made numerous contribution to clinical Immunology in Sri Lanka in various capacities and also been a pioneer, founder member and President of the Allergy and Immunology Society of Sri Lanka. This year there will be seventeen oral presentations and six poster presentations including three from students/researchers from other Higher Educational/Research Institutes. The interactions, links and collaboration studies conducted at IBMBB with other Academic and Research Institutes, Health Sector and Agricultural Sector is clearly evident from the authors and Institutes given in the abstracts. The presentations will be in different areas including research on Molecular Biology, Genetics & Bioinformatics, Immunology & Infectious diseases, Medicinal plants and Biotechnology.

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, Dr Rajiva de Silva for accepting 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 reviewers and IBMBB staff members and also the Chairpersons and Judges of the scientific sessions. I specially thank Dr Jagathpriya Weerasena-Chairperson and Dr Narmada Fernando-Secretary of the Organizing Committee. We thank Mr Sashika and Mr Kanchana for designing the invitation cards and certificates, and coordinating the printing of the abstract book, Ms Anoma for coordinating sponsorships, Ms Thanuja for arrangements of refreshments, Ms Champika, Ms Nadeesha, Ms Sekani, Ms Helani and Ms Thilani for secretarial assistance, Ms Tharini and Ms Nishara for uploading presentations, Ms Ishaka and Ms Shashika, Ms Nilupa and Ms Dakshika for editorial help for the abstract book and Mr Krishan - compere for this event. Finally, I thank all academic, administrative, other IBMBB staff members and students who contributed to organizing this 9th Annual Scientific session.

I wish you all a successful meeting

Oral Communications

Session I – Molecular Biology, Genetics and Bioinformatics

OP01	**10.45 – 11.00 h**	Preliminary study on common human Alu polymorphisms in Sinhalese individuals	Weerasooriya PR, Ranasinghe RACR, Tennekoo KH
OP02	**11.00 – 11.15 h**	*GHRHR* gene variants observed in combination in a cohort of IGHD children in Sri Lanka	Sundralingam T, Tennekoon De Silva KSH, De Silva S, Hewage AS
OP03	**11.15 – 11.30 h**	Usage of Hard, Hierarchical and Fuzzy clustering methods for high dimensional gene expression data analysis	Senadheera SPBM, Weerasir AR, Wijesinghe CR
OP04	**11.30 – 11.45 h**	Preliminary Study on X-linked chronic granulomatous disease in Sri Lanka	Fernando SJA, De Silva NR De Silva AD, Handunnetti S Dassanayake D
OP05	**11.45 – 12.00 h**	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 S Dassanayake D

Session II – Immunology and Infectious Diseases

OP06	**13.30 – 13.45 h**	The *in vitro* effect of pathogenic *Leptospira* on FOXP3 expression in lymphocytes	Dayarathna HMSP, Fernando N, Handunnetti SM, Weerasena OVDSJ
OP07	**13.45 – 14.00 h**	Preliminary study on allergenic pollen records from airborne samples in selected locations of urban and semi-urban areas in Western Province of Sri Lanka	Ishaka GKD, Premawansa S, Prematilake TR, De Silva R Handunnetti SM, Fernando A, Premawansa G
OP08	**14.00 – 14.15h**	Diagnosing a disease of farmers – Leptospirosis	Niloofa R, Premawansa S, Rajapakse S, Handunnetti SM
OP09	**14.15 – 14.30 h**	Identification of allergenic venom components of *Apis dorsata* Fabricius and comparison of those with *Apis mellifera* Linnaeus	Gunasekara DLPE, Handunnetti S, Premawansa S, Witharana EWRA, Dassanayake WMDK , Rathnayake IP, Senevirathne SL, Dias RKS, Premakumara GAS, de Silva R
OP10	**14.30 – 14.45 h**	Optimization of the day of sampling for expression of T cell dependent B cell activation	Ranaweera BVLR, Edward D, Abeysekara AM , Weerasena OVDSJ, Handunnetti SM
OP11	**14.45 – 15.00 h**	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 T, Kanapathippillai K, De Silva AD, Premawansa S

Session III – Medicinal plants and Biotechnology

OP12	**15.00 – 15.15 h**	Inhibition of nitric oxide production in mouse macrophages (RAW264.7) by *Artocarpus nobilis* Thw.	Rukshala BAD, Handunnetti S de Silva ED
OP13	**15.15 – 15.30 h**	Evaluating the anticancer effects of a polyherbal mixture on lung cancer cells (NCI-H292)	Priyadarshani CPVV, Thabre Samarakoon SR, Rajagopala
OP14	**15.30 – 15.45 h**	Identification of *Ralstonia solanacearum* phylotypes causing bacterial wilt of potato in Badulla district of Sri Lanka	Perera AAU, Weerasena OV Dasanayake PN
OP15	**15.45 – 16.00 h**	Studies on *in vitro* anti-inflammatory activities of ethanol extract of bark of *Calophyllum inophyllum* L.	Perera HDSM, Samaraseka Handunnetti S, Weerasena OVDSJ, Jabeer Choudhary MI
OP16	**16.00 – 16.15 h**	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
OP17	**16.15 – 16.30 h**	Phytochemical profile and *in vitro* anti-inflammatory activity of aqueous leaf extract of Sri Lankan variety of *Psidium guajava* L.	Kariawasam KWJC, Pathirana RN, Ratnasoor Handunnetti SM, Abeysekera WPKM

Poster Presentation

PP01	Detection of pathogenic mutations in previously identified Hotspot region/s in *BRCA1* gene in a cohort of Sri Lankan young breast cancer patients	Jayasundara GP, De Silva S, De Silva K
PP02	Assessment of RNS and ROS production by healthy phagocytic cells stimulated with leptospires *in vitro*	Wickramasinghe Arachchi SM, Edward D, Fernando N, Handunnetti SM, Rajapakse S
PP03	Establishment of methods for retrieving *Leptospira* organisms/ antigens in urine and blood	Weerasinghe HS, Fernando N, Rajapakse S, Premawansa S, Handunnetti SM
PP04	Determining performance characteristics of an immunochromatographic *in vitro* diagnostic test for diagnosis of Malaria	Gunasekara WMKT de AW, Handunnetti SM, Premawansa S, Weerasena OVDSJ
PP05	The effect of *Munronia pinnata* on natural killer (NK-92MI) cells	Fernando NA, Handunnetti SM, Hapuaarachchi S
PP06	*In vitro* Butyrylcholinesterase and protease inhibitory activities of selected medicinal plant extracts	Samaradivakara SP, Samarasekara R, Handunnetti SM, Weerasena OVDSJ

Professor Stanley Wijesundera Memorial Lecture

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

Previous Professor Stanley Wijesundera Memorial Lectures were delivered by Pro Rune Liminga, Former Director, International Programme in Chemical Sciences, Univers Uppsala, Sweden; Professor W D Lakshman, Former Vice Chancellor, University of Col Professor Eric Karunanayake, Founder Director, IBMBB & Emeritus Professor of Biochem University of Colombo; Professor Lal Chandrasena, Senior Professor of Biochem University of Kelaniya and Professor E Dilip de Silva, Senior Professor of Organic Che University of Colombo; Professor Kamani Tennekoon, Senior Professor of Molecula Sciences, IBMBB, University of Colombo, Former Director, IBMBB & former Profe Physiology, Faculty of Medicine, University of Colombo; Vidyajyothi Professor Rezvi S Senior Professor of Medicine, Kotelawala Defence University, Sri Lanka and Former Professor of Medicine, Faculty of Medicine, University of Colombo. This year Professor Wijesundera Memorial Lecture will be delivered by Dr Rajiva de Silva, Co Immunologist & President-Allergy and Immunology Society of Sri Lanka.

Professor Stanley Wijesundera Memorial Lecture

Primary Immunodeficiency: Cradle to Grave

Dr Rajiva de Silva, MBBS, MD

Consultant Immunologist, Department of Immunology, Medical Research Institute,
& President, Allergy and Immunology Society of Sri Lanka

Primary immune deficiency diseases (PIDD) are due to genetic defects in the immune system, in contrast to the more common secondary immune deficiencies, such as HIV/AIDS. Patients with PIDD have recurrent infections, or infections with unusual microbes. Primary immune deficiencies may have an incidence of 1:2000 live births in the United States, and onset may be at any age. Thus, it is not uncommon, and is not confined to children, as many clinicians believe. However, mostly present in childhood, and some, particularly those with more severe defects, present in the first few months of life. Some immune deficiencies for example common variable immune deficiency (CVID), present after 2 years of age, and some of them may present in the 7th decade!

The International Union of Immunological Societies (IUIS) has classified PIDD in to 9 groups. Similar to the data from the European Society of Immune Deficiency (ESID), the majority of Sri Lankan patients have antibody deficiency, followed by combined (T and B cell), phagocytic and PIDD with syndromic features. CVID is the most common PIDD in Sri Lanka, as in the West, followed by X-linked agammaglobulinemia. Most patients with PIDD in Sri Lanka are adequately managed, with intra-venous immunoglobulin (IVIG) and prophylactic antibiotics. Unfortunately, those with severe combined immune deficiency (SCID) who needs stem cell transplants succumb to the disease due to the lack of this facility in Sri Lanka. Rare PIDD such as chronic granulomatous disease, autosomal dominant hyper IgE syndrome, leukocyte adhesion deficiency have also been diagnosed, and many are adequately treated. While many PIDD may be diagnosed, with the resources available, the lack of genetic testing is proving to be a major barrier in the diagnosis of many PIDD.

Next Generation sequencing facilities and provision of stem cell transplants may further improve the management of PIDD in Sri Lanka.

Award Winners: 8th IBMBB Annual Scientific Sessions - 27th May 2016

The Best Oral Presentation in Medicinal Plants
was awarded to
Ms Nivethika Sivakumaran
for the paper titled
Anti-proliferative and proapoptotic effects of govaniadine in human breast cancer (MCF-7) c
Sivakumaran N, Samarakoon SR, Adhikari A, Ediriweera MK, Tennekoon KH, Thabrew I, Malavige N

The Best Oral Presentation in Immunology and Infectious Diseases
was jointly awarded to
Mr DL Peshala E Gunasekara
for the paper titled
Comparison of HPLC profiles of venom of *Apis darsata* Fabricius (Giant Asian Honey be
and *Apis mellifera* Linnaeus (Western Honey bee)
Gunasekara DLPE, Handunnetti SM, Premawansa S, Dias RKS, Witharana EWRA, Dasanayake WMDK, Premakumara GAS, De Silva NR

Ms Narmada Fernando
for the paper titled
Effects of pathogenic *Leptospira* on functions and viability of vascular endothelial cells and
epithelial cells
Fernando N, Handunnetti SM, Rajapakse S, de Silva HJ, Premawansa S

Ms Roshan Niloofa
for the paper titled
Diagnostic accuracies of six different immunoassays compared to the gold standard tests re
quantitative PCR and isolation of pathogenic *Leptospira*
Niloofa MJR, Karunanayake L, de Silva HJ, Premawansa S, Rajapakse S, Handunnetti

The Best Oral Presentation in Molecular Biology and Bioinformatics
was awarded to
Ms Vahinipriya Manoharan
for the paper titled
Screening of selected Sri Lankan cancer patients for somatic mutation of *TP53* gene
Vahinipriya M, De Silva S, Karunanayake EH, Tennekoon KH, De Silva K

The Best Poster Presentation
was awarded to
Ms Gayathri Karananidhi
for the paper titled
Mutational analysis of B cell activating factor receptor (BAFF-R) and inducible co-stimulato
patients with common variable immunodeficiency (CVID) in Sri Lanka
Gayathri K, Weeraman CPK, Sathkumara HD, Handunnetti SM, De Silva AD, De Si

Abstract No: OP01

Preliminary study on common human *Alu* polymorphisms in Sinhalese individuals

Weerasooriya PR, Ranasinghe RACR, Tennekoon KH

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

Transposons or jumping genes are classified under repeat sequences and short interspersed elements (SINES) belong to transposons. Humans carry the active member of SINE known as *Alu* elements and 11% of the human genome consists of these sequences. Migration of these elements within the human genome occurs via retrotransposition. Human specific *Alu* Y has been used by many researchers in studies on human evolution and population genetics. Irreversible nature of the insertion, identical by descend and well defined ancestral state are the unique traits of *Alu* polymorphism. Thereby *Alu* polymorphisms act as convenient targets in evolution exploration studies. Origin of Sinhalese ethnicity is still debated. This study was carried out to find out *Alu* polymorphisms (TPA 25, PV92, F13B, APO, APOA1/ACE, D1, A25, B65 loci and Y *Alu* polymorphism (YAP)) in Sinhalese individuals. Blood samples were collected from randomly selected individuals (N=15) Genomic DNA was extracted, quantified and selected *Alu* repeats were amplified by polymerase chain reaction (PCR). PCR products were subjected to agarose gel electrophoresis to visualize presence/absence of *Alu* polymorphisms. A few PCR products were subjected to direct sequencing to confirm the results obtained from agarose gel electrophoresis. Statistical analysis (χ^2 & F) showed that the Sinhalese population studied is in Hardy-Weinberg equilibrium with respect to *Alu* polymorphisms studied. The non-significant F_{ST} values among present study population and selected groups of Indians suggest that there is no significant genetic differentiation among the two populations with regard to *Alu* polymorphism. However further analysis should be carried out using a larger number of samples to obtain valid conclusions.

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

Abstract No: OP02

GHRHR gene variants observed in combination in a cohort of IGHD children in Sri Lanka

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

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[2]Department of Paediatrics, Faculty of Medicine, University of Colombo,
[3]Lady Ridgeway Hospital, Colombo

The implication of the growth hormone (*GH1*) and growth hormone releasing hormone receptor (*GHRHR*) genes in the aetiology of isolated growth hormone deficiency (IGHD) were confirmed in several studies. This attempt is to explore the combined variants observed in *GHRHR* gene in a cohort of IGHD children in Sri Lanka. The genetic variants in coding, 5' and 3' UTR and flanking intronic regions were screened by SSCP/ sequencing technique in 40 IGHD children. Total of 17 single nucleotide variants were observed in the cohort. Ten variants were observed unambiguously in three combinations. In the first combination, two variants (rs4988495 and rs4988496) were observed in two children. Both the variants were observed in heterozygous state in both children. In the second combination, four variants (rs4988499, rs4988501, rs740336 and rs28371563) were observed in eight children. All four variants were observed in heterozygous state in seven children. One child carried the rs4988501 in homozygous state while other three variants were in heterozygous state. In the third combination, four variants (rs2074780, rs35780782, rs2586 and rs28371564) were observed in four children. One child carried all four variants in homozygous state and other three children carried all four variants in heterozygous state. The ten variants include five intronic, two coding and three 3' UTR region variants. The coding region variants include one missense variant and one synonymous variant. When an individual carried a particular combination he/she did not have any of the variants included in the remaining two combinations.

This work was supported by National Science Foundation (RG/2011/BT/03)

Abstract No: OP03

Usage of Hard, Hierarchical and Fuzzy clustering methods for high dimensional gene expression data analysis

Senadheera SPBM, Weerasinghe AR, Wijesinghe CR

University of Colombo School of Computing, University of Colombo

Gene expression data analysis is a major area in biological system interpretation. Since, gene expression data have large number of variables, high dimensional clustering methods are required for analysis. Objectives of the study were understanding effectiveness of different clustering methods in gene expression data analysis based on biological relatedness and study advantages and disadvantages of different clustering strategies in gene expression analysis domain. Data obtained from GSE19830 and brain tumors in TCGA project. R package was used to data filtering. To test the hard clustering, hierarchical clustering and fuzzy clustering, K-means algorithm, HClust and Topic modeling were used respectively. Prior knowledge about the dataset was required to define the number of clusters. Initially, GSE19830 (Brain, Lung, Liver tissue mixture) dataset used for develop clusters. All models performed accurately except K-means. Secondly, Clustering methods were developed with brain tumor dataset consisted 202 samples (Four specified physically categorized tumors). According to hierarchical clustering and Topic modeling, when analyzing similar tissues, gene expression tumor subtypes (clusters) were not aligned with physical categorization. Finally, 28 cancer genes were filtered and reproduced the topic model for the brain tumor dataset. In order to understand the biological relevance, Reactome web tool and PCViz tools were used. Reactome results supported Topics developed from Topic modeling. According to the results, in high dimensional data analysis Topic modeling was promising approach for gene expression based clustering but K-means was inappropriate. Tissue specificity and mixed proportion have high impact in gene expression data clustering.

This work was supported by University Grants Commission 2016

Abstract No: OP04

Preliminary study on X-linked chronic granulomatous disease in Sri Lanka

Fernando SJA[1], De Silva AD[2], Handunnetti SM[1], Dassanayake D[3], De Silva R[3]

[1]Institute of Biochemistry, Molecular Biology & Biotechnology, University of Colombo,
[2]Genetech Research Institute, [3]Department of Immunology, Medical Research Institute

Chronic granulomatous disease (CGD) is a rare primary immunodeficiency of the phagocytic cells, which results in absent or diminished levels of microbicidal reactive oxygen species (ROS). The disease occurs due to germline mutations in the genes encoding the five subunits of NADPH oxidase complex. CGD is categorized in to two main subtypes, viz. X-linked and autosomal recessive, based on the mode of inheritance. The present study is the pilot study to understand the clinical and genetic aspects of CGD in Sri Lanka. Clinical records of twelve CGD patients were analyzed and compared with similar studies done in other countries and regions to identify patterns in demographics, clinical manifestations and infectious agents. The onset of age at diagnosis was early (mean 1.6 years after birth) compared to other studies; 4.5 years in India and 6.1 years in Europe. Pulmonary manifestations were the most common (91.6%), followed by skin/subcutaneous infections (75%) and lymphadenopathy (58.3%). Majority of the patients (83%) were treated for tuberculosis at some point in their lives. The death rate of local patients (41%) is higher than in India (35%) and Europe (20%). Residual ROS levels were quantified by liquid NBT assay in five patients out of which four recorded less than 20% compared to controls. Mutation analysis of *CYBB* gene was carried out in five possible X-linked CGD patients using bi-directional sequencing. Two patients had a single nucleotide deletion in exon 10 while another had a deletion in exon 11. Furthermore, a nonsense mutation was identified in exon 3 in one patient. In the last patient a novel splice site mutation was identified which resulted in the deletion of entire exon 2. In conclusion, the overall clinical presentation and manifestations in the local cohort appear to be comparable with regional and global trends. All five X-linked CGD patients studied showed mutations in *CYBB* gene.

This work was supported by the IBMBB & Medical Research Institute (06/2016) and constitutes a part of the MSc studies of SJAF

Abstract No: OP05

Preliminary study on identification of autosomal recessive p47*phox* mutations in patients with chronic granulomatous disease in Sri Lanka

Faiz NM[1], de Silva R[2], Handunnetti SM[1], Dassanayake D[2], de Silva AD[3]

[1]Institute of Biochemistry, Molecular Biology & Biotechnology, University of Colombo,
[2]Department of Immunology, Medical Research Institute, [3]Genetech Research Institute

CGD is a rare primary immunodeficiency disorder associated with recurrent infections due to the incapability of phagocytes to produce superoxide anions. Mutations in *NCF1* gene encoding the p47*phox* subunit of NADPH oxidase lead to the most common form of autosomal recessive CGD. This study aimed to analyze the predominant GT deletion in the *NCF1* gene in order to identify the A47° CGD cases in Sri Lanka. Although study population included 12 patients who have been clinically diagnosed and laboratory confirmed with CGD, only 07 samples were analyzed due to 04 patients have been already deceased at the time of sample collection and one defaulted visit. Liquid NBT assay performed in all 07 patients showed phorbolmyristate acetate (PMA) induced superoxide production was less than that of healthy controls. The percentage increase of residual reactive oxygen species (ROS) by all patients with PMA stimulation was less than 20% compared to the healthy controls, with the exception of one patient. Mutation analysis in exon 2 of *NCF1* gene was performed in five patients as two patients were presumed with X-linked CGD based on the reported clinical and laboratory data; and the test confirmed 02 patients with homozygous GT deletion. Furthermore, Western blot analysis performed in all seven patients confirmed the absence of p47*phox* protein in the two patients with identified *NCF1* mutation. In conclusion, autosomal recessive p47*phox* mutation was identified and confirmed only in two patients within the study group.

This work was supported by the IBMBB & Medical Research Institute (05/2016) and constitutes a part of the MSc studies of NMF

Abstract No: OP06

The *in vitro* effect of pathogenic *Leptospira* on FOXP3 expression in lymphocytes

Dayarathna HMSP, Fernando N, Handunnetti SM, Weerasena OVDSJ

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

Leptospirosis is a widespread zoonosis and one of the leading communicable diseases in Sri Lanka. Knowledge on lymphocyte involvement in the disease is limited and no investigations have been carried out to test the involvement of T regulatory cells (T_{REG} cells) during the disease. Objective of this study was to investigate the effects of pathogenic *Leptospira* in inducing apoptosis and/or T_{REG} cells in a lymphocyte population *in vitro*. *Leptospira interrogans* serovar Pyrogenes cultures were maintained in EMJH media with 5% rabbit sera at room temperature. Lymphocytes were isolated from healthy volunteers (n=12) of 25-35 years of age using histopaque and cells were stabilized for overnight in 10% RPMI at 5% CO_2 at 37 °C. Then the cells were incubated with *L. interrogans* serovar Pyrogenes at different multiplicity of infections (MOIs) ranged from 5×10^{-5}– 5 for 3, 6, 12 and 24 hours separately. The ratio between lymphocytes to *Leptospira* organisms were maintained according to the analogous leptospiremic ratio in human patients (10^2 to 10^7 *Leptospira* organisms/mL of blood). Upon the completion of each incubation period, percentage viability of lymphocytes was assessed by Tryphan blue exclusion method. Apoptosis and FOXP3 (main transcription factor of T_{REG} cells) expression were determined using DNA fragmentation assay and reverse transcription PCR on FOXP3 respectively. The results showed that the viable lymphocyte count was decreased with the increasing *Leptospira* density ($p<0.05$) after 12 and 24 hour incubations. In contrast, after 24 hours, viable lymphocyte count was significantly increased over the control at the 10^7 *Leptospira* burden. RT PCR results indicated that, although FOXP3 expressing T_{REG} cells are present in healthy state that might have been suppressed at the 10^7/mL *Leptospira* burden. Therefore, the pathogenic *Leptospira* interaction with lymphocytes at 0.05-5.0 MOI for 12 and 24 hours showed a down regulation in FOXP3 expression *in vitro*.

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

Abstract No: OP07

Preliminary study on allergenic pollen records from airborne samples in selected locations of urban and semi-urban areas in Western Province of Sri Lanka

Ishaka GKD[1], Premawansa G[2], Fernando A[3], De Silva NR[4], Handunnetti SM[1], Premathilake TR[5], Premawansa S[6]

[1]Institute of Biochemistry, Molecular biology and Biotechnology, University of Colombo, [2]Chest Clinic, Colombo North Teaching Hospital, [3]Central Chest Clinic, National Hospital of Sri Lanka, [4]Department of Immunology, Medical Research Institute, [5]Post Graduate Institute of Archaeology, University of Kelaniya, [6]Faculty of Science, University of Colombo

There is a great lack of information to correlate clinical data with palynological data regarding pollen allergies in Sri Lanka. Hence basic research for identification and characterization of prevalent pollen allergens is an emerging need. In the current study four (4) in-house pollen traps were placed in selected urban (Maradana and Narahenpita) and semi-urban (Ja-Ela) areas of Western province. Pollen traps were processed and analyzed to identify the abundance of different pollen grains in lower atmosphere and their allergenicity according to literature. Immuno-chemical analysis was performed for the characterization of pollen allergens. Potential allergenic pollen varieties including the families *Amaranthaceae, Asteraceae, Casuarinaceae, Cyperaceae, Fabaceae, Palmae* and *Poaceae* were found in this study to be transmitted in lower atmosphere of selected locations. Among them *Helianthus annuus sp. 2, Tridax procumbens* were reported with high amount of protein content wherein *Poaceae sp. 2* was reported with least amount of protein content which appear to have a link with the morphological complexity. Separated pollen antigens of *H. annuus sp. 2* and *T. procumbens* were reacted with sera from allergic rhinitis patients. No allergenic bands were identified from the selected pollen extracts. Presence of high number of pollen protein specific IgG bands were identified in patient sera compared to healthy controls. Among them eight (8) major antigens with molecular weights of 62, 58, 55, 45, 42, 39, 27 and 14 kDa were recognized in *H. annuus sp. 2* while four (4) major antigens with mol. weights of 112, 71, 35 and 8 kDa were recognized in *T. procumbens.*

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

Abstract No: OP08

Diagnosing a disease of farmers – Leptospirosis

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Leptospira interrogans is the causative agent of leptospirosis and excreted in urine of small animals. Farmers who work in this paddy field and marshy land are infected. Leptospirosis produces wide disease spectrum from mild flu like illness to severe forms such as Weil's syndrome and pulmonary haemorrahge, which may lead to death. Early confirmation of disease is vital for prompt treatment and management that could prevent the infection leading to organ failure and death. Clinical diagnosis is distracted due to the mimicking of other tropical febrile illnesses. According to WHO, the serological reference test microscopic agglutination test (MAT) could produce positive results only after 8 days of illness and/or require paired sample testing which making it unsuitable for early diagnosis. Therefore, we have evaluated two other tests i.e. IgM detectable ELISA and a rapid-test (Immunochromatography - ICT) in 888 clinical leptospirosis patients. Rapid test (ICT) showed higher positivity (45.8%) compared to the other assays. Bayesian Latent Class Modeling analysis showed that IgM-ELISA and rapid-test had high sensitivities (84.5% and 87.4%), while acute MAT had the lowest (77.4%). Paired MAT and IgM-ELISA showed high specificity (97.6% and 97.5%) than the rapid-test (82.9%). Pathogenic *Leptospira* has more than 200 serovars. Each serovar seems to be specific towards different geographical area. Different serovars have also said to be responsible severity and affecting different organs. Thus identifying the presence of different serovars along with the diagnosis is an advantage to the clinicians providing predictable information regarding the infective agent. Therefore, an In-House ELISA was developed to detect IgM antibodies against the antigens from five most prevalent *L. interoggans* serovars in Sri Lanka. The serovar-specific IgM-ELISA (using pooled antigens of pathogenic serovars) showed a sensitivity of 80.2% and a specificity of 89% against MAT. This assay may be a promise in future for identifying the infective serovars together with the early diagnosis of disease.

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Abstract No: OP9

Identification of allergenic venom components of *Apis dorsata* Fabricius and comparison of those with *Apis mellifera* Linnaeus

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Apis dorsata (Giant Asian Honeybee) is the commonest cause of anaphylaxis due to insect stings in Sri Lanka. Diagnostic and therapeutic reagents, however, are not available for this species. High Performance Liquid Chromatography (HPLC) profiles of venom of *A. dorsata* and *Apis mellifera* (Western Honeybee) were compared. Immunoblot assay was performed to test IgE reactivity to both venom using sera from 30 patients with anaphylaxis following *A. dorsata* stings. Venom of *A. dorsata* and *A. mellifera* showed similar HPLC profiles. Three IgE reactive bands of 39, 16 and 15 kDa were observed in venom of both species. Commercial preparations of PLA_2 of *A. mellifera* venom had immune reactive bands of 15 and 16 kDa (doublet) when tested with patients' sera, however, apamin and melittin of *A. mellifera* venom had no observable bands. Twenty-nine (96.7%) and 26 (86.7%) out of 30 sera had IgE reactivity to the PLA_2 doublet of *A. dorsata* and *A. mellifera* venom respectively. Hyaluronidase (39kDa) also was identified in both *A. dorsata* and *A. mellifera* venom. Twelve (40%) sera, all of whom from severe anaphylaxis patients, had IgE reactivity to hyaluronidase of venom of both species. In conclusion, the presence of 2 allergenic components PLA_2 (15 and 16 kDa doublet) and hyaluronidase (39 kDa) in *A. dorsata* venom was identified and cross reactivity of these 2 components in venom of *A. dorsata* and *A. mellifera* with patients' sera was confirmed. This similarity in the venom of *A. dorsata* and *A. mellifera* and their IgE cross reactivity suggests the possibility of using commercial preparations of *A. mellifera* venom as a diagnostic test and immunotherapy for *A. dorsata* venom allergy.

This work was supported by National Science Foundation Grant No-2015/HS/02 and Medical Research Institute Grant No-46/2013.

Abstract No: OP10

Optimization of the day of sampling for expression of T cell dependent B cell activation

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Link Samahan is a commercial product which is commonly used for prophylaxis against cold and cold related symptoms in Sri Lanka. The aim of this pilot study was to find out the most appropriate day to collect rat peritoneal cells (RPC) to determine the expression of T cell dependent B cell activation. Water and sugar were used as two controls as Link Samahan (LS) contains sugar as excipient. Three groups of rats used for the assay were immunized with BSA on day 1 and 15 followed by the oral treatment with LS, sugar and water respectively from day 1- 5 and again from day 15 - 19. RPC obtained on day 0 and 14 prior to the 1st and 2nd BSA immunizations respectively were used as controls. RPC collected on day 2, 4, 6, 8, 16, 18, 20 and 22 were cultured *in vitro* overnight and tested for the expression of co-stimulatory molecules. Total RPC count in all three groups, increased significantly by day 2 and 16 (after 1st and 2nd immunizations respectively) compared to day 0 ($p<0.05$). In contrast, in the LS treated group percentage of B cells expressing CD86, CD40 as well as T cells expressing CD3, CD40L (as detected by immunofluorescence assay) increased significantly by day 2 (16% - B cells; 34% - T cells) and increased to the highest level by day 6 (27% - B cells; 68% - T cells) compared to the two controls ($p<0.05$). In the two control groups, the highest percentage of B (20%) and T (40%) cells were reached by day 6 whereas the LS treated group reached these levels by day 2. Same patterns of B and T cells increase were observed after 2nd immunization. In conclusion, this study showed that day 2 and 16 are the most suitable days to obtain RPC to determine the gene expression in B and T cells. However, quantitative RT-PCR needs to be performed to determine the gene expression level to confirm these findings. Further, these preliminary results reflect the immunostimulatory effect of Link Samahan as it had induced an early and higher percentage of lymphocytes expressing co-stimulatory molecules.

This study is a collaborative research project, supported by Link Natural Products (Pvt) Limited and IBMBB, University of Colombo

Abstract No: OP11

Low total serum nitrite and nitrate levels on admission in dengue patients as prognostic markers for developing dengue hemorrhagic fever

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Potential use of total nitrite plus nitrate (NOx) and nitrite (NO_2^-) separately as surrogate markers for serum nitric oxide in severe dengue and their longitudinal changes along with the progression of infection was studied here to determine their prognostic value. Confirmed dengue fever (DF, n=180) and dengue hemorrhagic fever (DHF, n=80) patients were selected from Colombo North Teaching Hospital, Ragama. Out of confirmed 180 DF patients, 80 were selected matched to age-gender-area and days of fever on admission in DHF patients. Blood samples were collected on admission (A), critical (C), discharge (D) and convalescence (CON) stages. Age-gender-area matched healthy individuals were also recruited (n=77). Griess and Modified Griess assays were carried out using de-proteinized sera to detect the NO_2^- and NOx levels respectively. Serum NOx in DHFA was significantly lower compared to DFA ($p<0.001$). HC had the lowest NOx and NO_2^- levels which were significantly low compared to all patient categories tested ($p<0.001$). DHF patients admitted on fever day 3 (DHFA-3) and day 4 (DHF-4) had lower NOx compared to DF ($p<0.05$). Serum NO_2^- was significantly low in DHFA-3 ($p<0.001$) compared with that of DF. Cut off values were determined for NOx and NO_2^- for DHFA-3 over DF-3 (n=24) using receiver operating characteristic curve analysis and obtained 4.46 μM cut-off with 93.3% sensitivity and 100% specificity for NOx. Higher NOx in DFA may reflect the protective anti-viral effect of NO which may be one of the reasons resulting mild dengue fever whereas low NOx in DHFA with the lack of such potential protective effect may be related to the disease severity. Serum NOx on day 3 of fever could be used as a potential prognostic marker for DHF patients admitted at early stage (day 3 of fever) of the disease which could facilitate the prompt clinical management of severe dengue infection.

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Abstract No: OP12

Inhibition of nitric oxide production in mouse macrophages RAW264.7 by *Artocarpus nobilis* Thw.

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The discovery of plant derived drugs that can be used for the treatment of allergic and inflammatory diseases is important for human health. We have previously showed the inhibitory activity of mast cell degranulation by methanol/dichloromethane extract (MDE) of root of *Artocarpus nobilis*. The objective of this study is to investigate the inhibitory activity of nitric oxide (NO) production by assessing MDE and sequential solvent extracts with hexane, chloroform, ethyl acetate and methanol of *A. nobilis* root using lipopolysaccharide (LPS)-stimulated (1 μg/mL) mouse macrophages RAW264.7. NO inhibitory activities of the respective solvent extracts were determined within non-toxic dilution range on LPS induced RAW cells. Briefly, RAW264.7 cells were cultured using Dulbecco's modified Eagle's medium (DMEM) and 10% fetal bovine serum (FBS) in 96-well plates. The cells were treated with MDE for 30 min followed by 1 μg/mL of LPS treatment for 24 h. The nitrite levels in the culture supernatant were measured by the Griess method. NMMA (1mM) was used as the positive control and showed 89% inhibition of NO production. MTT assay was carried out to determine the non-toxic dilution range. IC_{50} of MDE for NO inhibitory activity was 360 μg/mL. Next the dried powdered root was sequentially extracted into hexane, chloroform, ethyl acetate and methanol and each extract was tested for the NO inhibitory activity. Both chloroform and ethyl acetate fractions showed high NO inhibitory activity (IC_{50} values were 62.5 and 37 μg/mL respectively). Further chromatographic purification by silica gel of ethyl acetate fraction led to the isolation of bioactive fraction (single spot by TLC; ethyl acatate: hexane; 4:1, R_f; 0.62) which was active at IC_{50} 17 μg/mL. The ethyl acetate fraction of *A. nobilis* is effective in inhibiting NO production, hence may have potent anti-inflammatory activity.

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Abstract No: OP13

Evaluating the anticancer effects of a polyherbal mixture on lung cancer cells (NCI-H292)

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A number of *in vitro* and *in vivo* studies have revealed that a decoction prepared from a polyherbal mixture comprised of *Nigella sativa* (seeds), *Hemidesmus indicus* (roots) and *Smilax glabra* (rhizomes) possesses anti-hepatocarcinogenic properties. The present study was carried out to determine whether the organic solvent based extracts (that are widely used in pharmaceutical industry) of the churna form of the above polyherbal mixture can mediate anti-proliferative effects on human lung cancer cells (NCI-H292). Initially, a set of organic solvent based extracts were prepared from the polyherbal mixture. Thin Layer Chromatographic analysis (TLC) was performed to characterize these extracts. Based on the cytotoxic effect as evaluated by Sulphorhodamine B assay, the "ethyl acetate only" extract was selected for further analysis. The cytotoxic activity of this extract was further analyzed by colony formation assay; apoptosis induced morphological changes by light and fluorescent microscopy; apoptosis related gene expressions (*Bax, Survivin, p53, Hsp 70* and *Hsp90*) via real time PCR; and free radical scavenging activity by DPPH assay. The study indicated that the selected extract can mediate cytotoxity to NCI-H292 cells in a time and dose dependent manner. A moderate free radical scavenging activity and colony formation inhibition effects were observed. The expression levels of *Bax* and *p53* were significantly ($P<0.05$) increased while those of *Survivin, Hsp70* and *Hsp90* were significantly ($P<0.05$) decreased by 25 µg/mL and 50 µg/mL dosages of the same extract. The overall result suggests that the "ethyl acetate only" extract has the potential to be developed as a nutraceutical against lung cancer.

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Abstract No: OP14

Identification of *Ralstonia solanacearum* phylotypes causing bacterial wilt of potato in Badulla district of Sri Lanka

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Bacterial wilt is considered as one of the most destructive diseases of potato caused by *Ralstonia solanacearum* (E. F. Smith). More recently, a hierarchical classification scheme was proposed to distinguish the genetic variation within *R. solanacearum* species complex which is subdivided into four phylotypes: phylotype I from Asia; phylotype II from the America; phylotype III from Africa and surrounding islands; and phylotype IV from Indonesia, Japan, the Philippines, Korea and Australia. Thus, this study was aimed to identify phylotypes of *R. solanacearum*. Samples were collected from the potato-growing areas in Badulla district of Sri Lanka namely, Bandarawela, Boralanda, Koslanda, Passara and Welimada. A total of 32 bacterial isolates of *R. solanacearum* was isolated using triphenyl tetrazolium chloride agar medium. Genomic DNA extracted from those bacterial isolates was subjected to PCR with Rsol_*fliC* primers to confirm the identity of *R. solanacearum* and then subjected to the phylotype-specific multiplex PCR (Pmx-PCR) with *R. solanacearum* species-specific primers, 759/760, in combination with phylotype-specific primers, Nmult:21:1F, Nmult:21:2F, Nmult:23:AF, Nmult:22:InF and Nmult:22:RR. The Pmx-PCR revealed that 29 isolates (91%) belonged to the Asian phylotype I and the remaining 3 isolates (9%) belonged to the American phylotype II which were found only from Welimada area. These results indicate that the major causal agents of bacterial wilt of potato in Badulla district belonged to *R. solanacearum* phylotype I and in addition, *R. solanacearum* phylotype II were also responsible for the disease.

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Abstract No: OP15

Studies on *in vitro* anti-inflammatory activities of ethanol extract of bark of *Calophyllum inophyllum* L.

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Inflammation is one of the key factors in the progression of many human diseases. The extracts of *Calophyllum inophyllum* L. (Family name: Clusiaceae; Sinhala name: Domba; Tamil name: Dommakottai) have long been traditionally used for treating inflammatory diseases. In the present study, anti-inflammatory properties of total ethanol extract of bark of *C. inophyllum* was determined in terms of inhibitory activities of Arachidonate-5-lipoxygenase (A5-LOX), hyaluronidase, xanthine oxidase, nitric oxide (NO) production in lipopolysaccharide (LPS) activated RAW 264.7 macrophages and oxidative burst response on human whole blood and polymorphoneutrophils (PMN). Cytotoxicity was determined using MTT assay. The extract showed moderate, dose dependent A5-LOX inhibitory (IC_{50}: 74.82±1.35 µg/mL; Baicalein:1.76±0.15 µg/mL) and high, dose dependent oxidative burst inhibitory activities on human whole blood (IC_{50}: 18.60±0.50 µg/mL; Ibuprofen:11.20±1.90 µg/mL) and PMN (IC_{50}: 6.87±0.88µg/mL; Ibuprofen: 2.50±0.60 µg/mL), moderate xanthine oxidase inhibitory (38.95±1.28%, 250 µg/mL; Allopurinol: 99.26±0.72%), NO production inhibitory (31.12±0.78%; 500 µg/mL; L-NMMA: 97.10±0.56%; Cell viability: 88.89±0.82%) and no hyaluronidase inhibitory activity ($p<0.5$). The present study may support the traditional uses of *C. innophyllum* in the treatment of various inflammatory diseases. This is the first report on *in vitro* anti-inflammatory activities of ethanol extract of bark of *C. innophyllum,* indicating its potential as an anti-inflammatory agent.

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Abstract No: OP16

Isolation of native antagonistic fungi against *Rigidoporus microporus*, the causative fungus of White Root Disease from different rubber growing soils

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Rubber (*Hevea brasiliensis*) is a prominent low input plantation crop with considerable significance to the Sri Lankan economy. *Rigidoporus microporus* (Polyporales, Basidiomycota) syn. is the most destructive root pathogen of rubber plantations which causes huge economic losses. Hence an attempt was taken to control of white root disease using biological methods to reduce the usage of harmful chemicals. Soil samples were collected at rubber plantations of Polonnaruwa, Kalutara, Rathnapura, Monaragala and Ampara districts. Colony forming unit counts of collected soil samples were taken using dilution plate technique. Soil pH and moisture contents were measured using standard methods. The fungal species were isolated and their antagonistic effects were tested against *Rigidoporus microporus* using dual plate culture test. The highest soil pH value was recorded in Polonnaruwa district (6.49) while the lowest value was recorded in Kalutara district *Pueraria* growing rubber land (4.65). The highest moisture content was recorded in Kalutara district (19.81%) while the lowest value was recorded in Polonnaruwa district (6.35%). The dual plate culture test indicated that twenty three fungi out of forty had more than 65% inhibition against *Rigidoporus microporus*. Combination of antagonistic fungi can be used to control the *R. microporus* effectively.

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Abstract No: PP01

Detection of pathogenic mutations in hotspot regions in exon 11 of *BRCA1* gene in a cohort of Sri Lankan young familial breast cancer patients

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Breast cancer is the most frequent death causing malignancy in women around the world. Germline mutations in breast cancer susceptibility genes *BRCA1* and *BRCA2* genes cause 20%-40% of familial breast cancer. *BRCA1* mutation carriers have a 57%-65% lifetime risk of developing breast cancer. Exon 11 encodes nearly 60% of *BRCA1* and harbours the most significant mutations in breast cancer patients. Previous studies conducted on Sri Lankan breast cancer patients, irrespective of age, have reported several pathogenic mutations in exon 11. The present study was a preliminary step taken to detect pathogenic mutations in young Sri Lankan familial breast cancer patients and at risk individuals with a strong family history of breast cancer. *BRCA1* sequence variants were studied in a total of 18 individuals less than 45 years of age with a family history of breast cancer. Collected blood samples were subjected to genomic DNA extraction followed by PCR, using sequence specific primers to amplify the selected hotspot regions of exon 11. DNA sequencing was carried out for amplified products and generated sequences were analysed using several bioinformatics software. Seven sequence variants were observed including one pathogenic variant (c.3857G>A), two missense variants with uncertain significance (c.823G>A, c.2612T>C), one missense variant with no clinical significance (c.3548A>G) and three silent variants (c.2082C>T, c.2088T>C, c.2311C>T). Out of those, two were novel and five were reported sequence variants. Two of the sequence variants identified were common high frequent polymorphisms that appeared in both patients and healthy controls. The prevalence of pathogenic *BRCA1* mutations identified in this study was 6.7% (1/15).

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

Abstract No: PP02

Assessment of RNS and ROS production by healthy phagocytic cells stimulated with leptospires *in vitro*

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Leptospirosis is a zoonotic disease caused by pathogenic spirochetes of the genus *Leptospira*. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are anti-microbial molecules generated in phagocytic cells during the respiratory burst. Objective of this study was to evaluate the effect of *in vitro* stimulation of healthy phagocytic cells with live pathogenic and saprophytic *Leptospira* to produce RNS and ROS. Total leukocytes ($4x10^5$ leukocytes/well) purified from healthy volunteers (n=12) was subjected to *in vitro* stimulation by *L. biflexa* (saprophytic) and *L. interrogans* (pathogenic) live organisms. Their ability to produce nitrite and superoxide anions was assessed using Griess assay and quantitative NBT assay respectively. The mean nitrite and superoxide levels produced by *L. biflexa*-stimulated and *L. interrogans*-stimulated phagocytic cells were significantly higher compared to unstimulated cells ($p<0.05$). *L. interrogans* had induced significantly high production of nitrite and superoxide anions compared to *L. biflexa* ($p<0.05$). Expression of NF-κB levels was assessed in high (n=3) and low (n=3) nitrite producing phagocytic cell donors using RT-PCR. The intensity of NF-κB bands observed were as follows, *L. interrogans*-stimulated > *L. biflexa*-stimulated > unstimulated cells. This indicates that the expression of NF-κB was comparable with the nitrite levels of the *in vitro* stimulated phagocytic cells. Further, the increased expression of NF-κB may reflect the enhanced signal transduction in phagocytic cells leading to production of high RNS levels through increased expression of inducible nitric oxide synthase enzyme. The findings of this study may indicate that the innate immune response (RNS and ROS production) by phagocytes interacted with pathogenic leptospires is elevated than that by saprophytic leptospires *in vitro*.

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

Abstract No: PP03

Establishment of methods for retrieving *Leptospira* organisms/ antigens in urine and blood

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Most of the laboratory tests are targeted on a significant or a rising serum antibody titer. However, detectable antibody titers mostly appear after 7-10 days of the infection and it is an indirect method of diagnosis. *Leptospira* antigens are known to be present in blood and urine during the acute phase and after 7 days of illness respectively and can be detected using specific antibody reagents. Therefore, detection of *Leptospira* antigens can provide an early diagnosis rather than the detection of antibodies. Aims of this study were to optimize methods for retrieval of *Leptospira* antigens from blood and urine, asses the stability of the recovered antigens by using SDS-PAGE and to detect the immunoreactive antigens from the retrieved antigens by Western blotting. Pathogenic *L. interrogans* and saprophytic *L. biflexa* culture adapted species were used in this study. Results showed that approximately 20% of *Leptospira* can be retrieved from blood whereas it is more than 20% from urine. However, it was observed that higher number of live motile *Leptospira* were present after recovery from blood than urine. Proteins at 25, 32, 45, 48, 54, 82, and 104 kDa were found to be stable after the retrieval from blood whereas proteins at 32, 37, 39, 45, 58, 82, and 104 kDa were found to be stable after the retrieval from urine. Antigens at 32, 48, 62, 76, and 82 kDa were found to be immunoreactive from the retrieved proteins. 32 kDa antigen was detected only after the recovery from blood but not after recovery from urine. It is likely to be LipL32 which is expressed only in pathogenic serovars. 82 kDa protein was found to be stable and immunoreactive after recovery from both blood and urine. Therefore, 32 kDa protein may be useful in detecting antigens during acute phase whereas 82 kDa protein may be useful in detecting antigens in both leptospiremic and leptospiruric phases.

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

Abstract No: PP04

Determining performance characteristics of an Immunochromatographic *in vitro* diagnostic test for diagnosis of *Plasmodium falciparum* malaria

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With the renewed global interest in elimination of malaria in recent years, and the concept of universal access for malaria diagnostic testing of the World Health Organization, Immununochromatographic *in vitro* diagnostic tests (IVDs) are being increasingly used for parasitological surveillance. Since operational characteristic of IVDs are known to vary under different disease endemic conditions, the Carestart™ malaria histidine-rich protein 2/ *Plasmodium* lactate dehydrogenase (HRP2/pLDH; Pf/PAN) Combo Test, an IVD test kit used in Sri Lanka was evaluated under the zero endogenous malaria setting. From 245 suspected malaria patients referred to Anti-Malaria Campaign Headquarters, IVD and nPCR (reference test) were done using capillary whole blood. Sensitivity, specificity and predictive values of IVDs were compared with matched nPCR. Malaria negative patients by nPCR were considered as negative controls. Considering the pan specific (pLDH) antigen to detect *P. falciparum*, sensitivity and specificity of IVD was 67.61% (CI=55.45-78.24%) and 99.43% (CI=96.84-99.99%) respectively. Positive and negative predictive values were 97.96% (CI=87.17-99.71%) and 88.27% (CI=84.31-91.33%) respectively. Positive and negative likelihood ratio were 117.63 (16.55- 835.94) and 0.33 (0.23-0.46) respectively. Sensitivity of IVD based on HRP2 and both antigens combined was 100% (CI=94.94-100.00%) while specificity was 94.83% (CI=90.41-97.61%). Positive and negative predictive values were 88.75% (CI=80.68-93.71%) and 100.00% respectively. Positive and negative likelihood ratio were 19.33 (CI=10.23-36.53) and 0 respectively. This study shows that inclusion of HRP2 with pan specific pLDH antigen in IVD increased the accuracy of the IVD as well as provides a more sensitive test for diagnosis of *P. falciparum* infections.

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Abstract No: PP05

The effect of *Munronia pinnata* on natural killer cells

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Immunomodulatory effect of *M. pinnata* on peripheral blood nature killer (NK) cells (NK-92MI cell line) was assessed in this study. To study *M. pinnata* induced cytotoxicity to NK cells, HepG2 cells; a hepatocellular carcinoma cell line was used as target cells. Aqueous extract of *M. pinnata* was prepared according to the conventional method used by traditional practitioners in Sri Lanka. Non-toxic concentration of *M. pinnata* to treat NK cells (30 min) was determined by Trypan blue exclusion method and 100 µg/mL was chosen as the appropriate concentration to treat NK cells. Direct immunofluorescence assay showed the expression of NKp46 receptor on NK cells indicating that NK cells were activated by this concentration. *M. pinnata* aqueous extract induced cytotoxicity of NK cells on HepG2 cells was evaluated using Sulforhodamine B assay after incubating HepG2 cells for 24h with treated NK cells. The survival of HepG2 cells after 24h interaction with treated NK cells was 50.60±1.33% whereas with untreated NK cells was 78.20 ±1.34% ($p<0.05$). Similar, but lower decrease in survival of HepG2 cells was observed after 12h interaction (89.20±1.22%; $p<0.05$). However, 500 µg/mL was required to reach the same cytotoxic effect on HepG2 cells as a direct effect by *M. pinnata* aqueous extract (without NK cells) after 24h exposure. Positive control, Thymoquinone also showed a dose-dependent cytotoxicity on HepG2 cells with 50% inhibition recorded after 3h treatment with 10 µg/mL of Thymoquinone. HepG2 treated with *M. pinnata* extract displayed cell shrinkage with irregular forms and the cell numbers were lower than that of controls. Marked morphological changes were observed with longer treatment times and higher concentrations (500 and 1000 µg/mL), which corresponded to the observations of the cytotoxicity assay. In conclusion, this preliminary study showed immunomodulatory effect of *M. pinnata* on NK cells and the level of cytotoxic effect shown by direct exposure of HepG2 cells was achieved by treating NK cells with lower concentrations of *M. pinnata* extract.

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Abstract No: PP06

In vitro butyrylcholinesterase and protease inhibitory activities of selected medicinal plant extracts

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Plasma cholinesterase enzyme; butyrylcholinesterase (BChE) is reported to contribute for the pathogenesis of Alzheimer's Disease (AD). AD is also characterized by the amyloid plaque formation in the brain due to the activity of serine proteases. The aim of this study was to evaluate and determine BChE inhibitory (BChEi) and protease inhibitory activity of the ethanolic extracts of *Toona ciliata* Roem.-Leaves (TCL) and Bark (TLB), *Annona muricata* L.-Bark (AMB), *Caesalpinia bonducela* L.-Bark (CBB), *Biophytum sensitivum* Linn.-Whole plant (BSW) and *Baeleria prionitis* Linn.-Leaves (BPL). The plants were selected with the intention of augmenting their already known bioactivities with ChE and protease inhibitory activities. BChEi activity and the protease inhibitory activity against α-chymotrypsin and elastase were measured according to modified Ellman and Cannell methods, respectively. The ethanolic extract of CBB showed the highest BChEi activity (IC_{50} 5.12±0.25 µg/mL) in comparison to Galanthamine, a clinical inhibitor, with IC_{50} 3.99±0.25 µg/mL. The BChEi IC_{50} values (µg/mL) of, TCL, TCB, AMB, BSW and BPL were 11.02±1.17, 29.19±4.16, 69.17±3.90, 149.58±11.03 and 1947.68±248.47, respectively. Elastase inhibitory activity was highest for AMB (97.88±5.61 µg/mL) and TCB (115.88±5.69 µg/mL) compared with that of Qurecetin (IC_{50} 221.69±5.52 µg/mL). At 500 µg/mL, < 20 % inhibition was obtained for TCL and BSW and no inhibition was found for CBB and BPL. Low % inhibition ranging from 2.97–57.03 was exhibited by the plant extracts towards the α-chymotrypsin except for AMB, which gave an IC_{50} of 672.55±19.41 µg/mL, in comparison to the standard Chymostatin (IC_{50}. 5.93±0.10). This is the first report of BChE, elastase and α-chymotrypsin inhibitory activities of ethanol extracts of the aforementioned plants and further bioassay guided isolation and identification of active principles could result in finding selective enzyme inhibitors for AD management.

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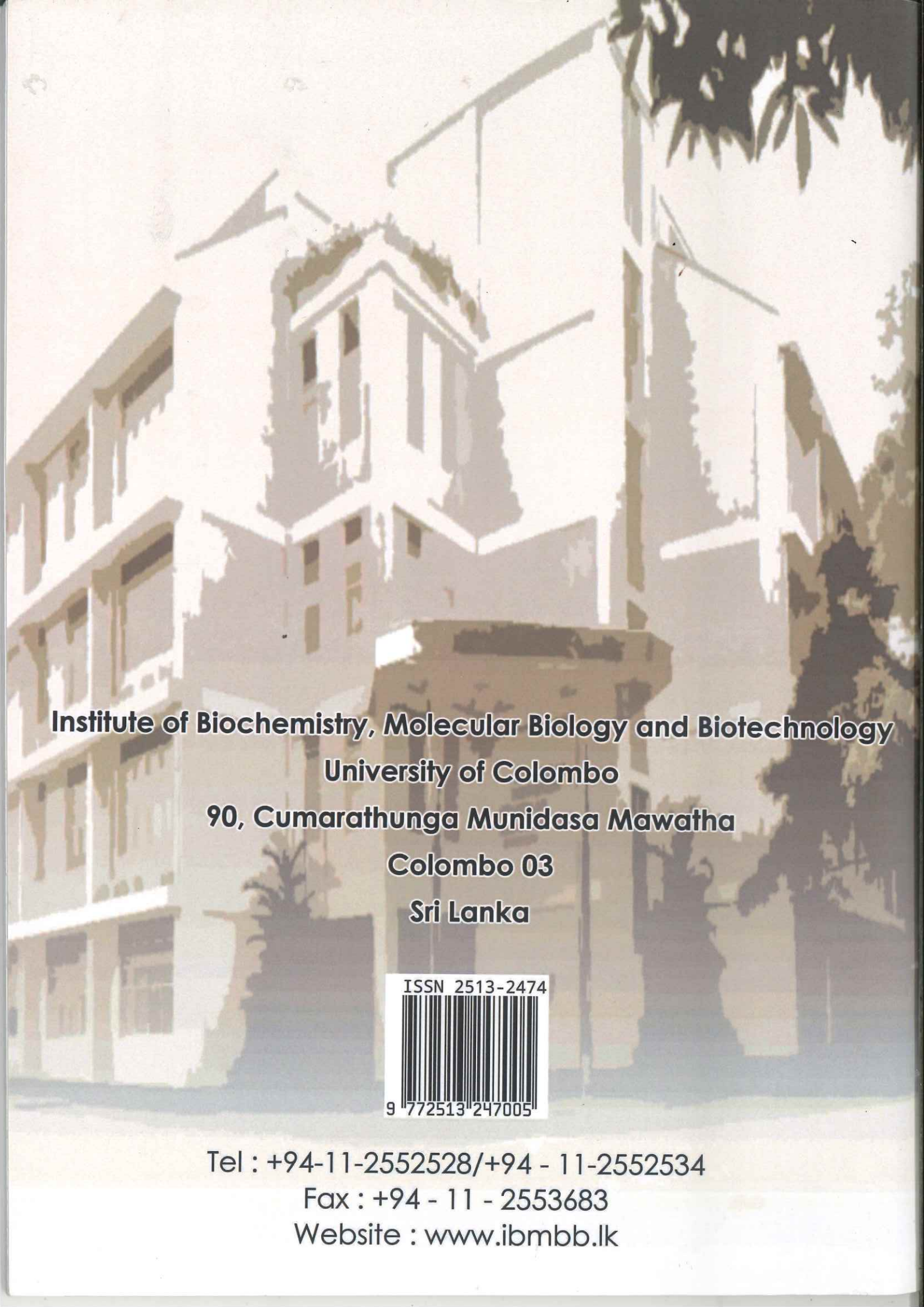

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