Institute of Biochemistry, Molecular Biology and Biotechnology

University of Colombo

Proceedings of the 11th Annual Scientific Sessions of the IBMBB

12th July 2019

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 Prof Shiroma Handunnetti, Director, IBMBB
09.20 h	Address by Senior Professor Chandrika Wijeyaratne, Vice Chancellor, University of Colombo
09.30 - 10.20 h	Professor Stanely Wijesundera Memorial Lecture by Senior Professor Sagarika Ekanayake on 'Eat rice to be healthy: Variety, Processing and Preparation'
10.20 - 10.30 h	Address by a Wijesundera Family member
10.30 - 10.35 h	Launch inaugural newsletter of the IBMBB
10.35 - 10.40 h	Vote of Thanks
10.40 - 11.15 h	Tea
11.15 -12.30 h	Free paper session I - "Cancer genetics and Bioinformatics"
12.30 - 13.30 h	Lunch break & Poster Session
13.30- 15.15 h	Free paper session II - "Immunology and Infectious Diseases"
15.15 - 16.15 h	Free paper session III - "Molecular Biology and Genetics"
16.15 - 16.45 h	Tea
16.45 - 17.15 h	Award Ceremony

Message from the Director, IBMBB

Professor Shiroma Handunnetti
Director, IBMBB & Professor of Immunology

2019 marks the 15th anniversary of the Institute of Biochemistry, Molecular Biology and Biotechnology (IBMBB), University of Colombo. Hence it is with great pleasure that I send this message for the 11th Annual Scientific Sessions of the IBMBB, University of Colombo which is the main event to commemorate its 15th Anniversary.

IBMBB was established as a postgraduate institute and has a mandate for research and training in Molecular Life Sciences, Molecular and Cellular Immunology, Bioinformatics and allied fields. Our Founder Director, Professor Eric H Karunanayake's vision has led to the establishment of 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 molecular genetics study on breast cancer, molecular study on phytoplasma, molecular study on genetics of primary immunodeficiency, tea genome, genetic characterization of traditional and weedy rice proteomic study on bee venom, to mention a few. To date IBMBB has facilitated 20 MPhil and PhD candidates to complete their studies at IBMBB. These research studies have been conducted through numerous collaborations established with academics and researchers in other faculties and Institutes of the University of Colombo, other universities in Sri Lanka, Research Institutes affiliated to Ministry of Health, Ministry of Science and Technology and private Institutions. Through these research and MPhil & PhD programmes, IBMBB has made a significant contribution to capacity building and strengthening for the higher educational and other sectors in the country.

2019 is a special year in the IBMBB calendar since we commenced with the student intakes for all three degrees programmes, Molecular Life Sciences, Cellular and Molecular Immunology and Bioinformatics under revised curricula. The new curricular are now converted to a credit based system and aligned with the Sri Lanka Qualifications Framework (SLQF). Hence, they offer Master of Science degree (SLQF level 10 – with 60 Credits including a major research component) and Masters degrees (SLQF level 9 – with 30 Credits by course work) and also a Post graduate Diploma (SLQF level 8-25 Credits) as a fall

back option. This year we have 35 students in the 2019 Intake and altogether with MPhil and PhD students, IBMBB has a total of 87 students studying for their postgraduate degrees.

The IBMBB Annual Scientific Sessions facilitates the dissemination of research findings of the IBMBB students and scientists from other Higher Educational Institutes. It is a pleasure to state that these sessions have been held continuously since 2006 to date excluding the years on which IBMBB held the International conferences in 2009 and 2014.

Professor Stanley Wijesundera Memorial Lecture is one of the key events of the IBMBB Annual Scientific sessions. This year the memorial lecture will be delivered by Senior Professor Sagarika Ekanayake, Professor in Department of Biochemistry, Faculty of Medical Sciences, University of Sri Jayewardenepura. Prof Ekanayake is an eminent researcher, an expert of nutrition, Traditional food and medicine & health. In 2015 she has won the prestigious Prof M US Sultanbawa award for Research in Chemistry in 2015. We are also pleased to have Prof Ekanayake to deliver this years Professor Stanley Wijesundera Memorial Lecture titled "Eat Rise to be Healthy: variety, Processing and Preparation".

2019 Annual Scientific Sessions have a total of 21 papers including 16 oral and 05 poster presentations. The presentations will be in three sessions namely Cancer Genetics and Bioinformatics, Immunology & Infectious diseases and Molecular Biology & Genetics. The authors and affiliations indicate the wide research collaborations that IBMBB has at present. Since 2016, IBMBB initiated the award of cash prizes for the Best Presenters of each scientific session which is a much-appreciated sponsorship from the Wijesundera family.

I take this opportunity to thank Senior Professor Chandrika Wijeyaratne, Vice Chancellor, University of Colombo for her support, inspiration and especially, for gracing the inauguration and Awards ceremony, Wijesundera family for continued support for the Memorial Lecture, gracing the event and sponsoring the awards for best presenters, Senior Professor Sagarika Ekanayake 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 Dr Sisira Pathirana - Chairperson, Dr Nadeesha Lewke Bandara, Secretary, Dr Nilupa Gunaratne, Assistant Secretary of the Organizing Committee. I thank **all members of the Organizing Committee** for their continuous support and ideas, including Deputy Bursar-Ms Manjula Kahawita, Sen. Asst. Registrar - Mr Janaka Gunasekera and Assistant Registrar, Ms Harshani Jayaweera.

I wish you all a very successful Scientific Sessions.

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Oral Communications

Session I –Cancer Genetics and Bioinformatics

Session Chairpersons: Dr. Nalinda Silva and Dr. OVDSJ Weerasena

OP01	**11.15-11.30 h**	Mitochondrial DNA control region variations and breast cancer risk in South Asian populations: a comparative analysis with Sinhalese population	Kotelawala JT, Tennekoon KH, Ranasinghe R, Rodrigo HACIK, De Silva GKS
OP02	**11.30-11.45 h**	Genomic data annotation workflow for SNP mutations via bioinformatics tools	Senadheera SPBM, Weerasinghe AR, Wijesinghe CR, Pablo Porras, Birgit Medal
OP03	**11.45-12.00 h**	Detection of somatic mutations of *KRAS*, *BRAF* and *PIK3CA* in a cohort of patients with colorectal carcinoma in Sri Lanka	Imthikab AIA, De Silva S, De Silva K
OP04	**12.00-12.15 h**	Comparison between cDNA and DNA sequencing in detection of *TP53* variants in head and neck, breast and colorectal cancer	Manoharan V, Karunanayake EH, Tennekoon KH, De Silva S, Angunawela P, De Silva K, Lunec J
OP05	**12.15-12.30 h**	Human nervous system cancer mutation analysis from protein sequences and structures	Senadheera SPBM, Weerasinghe AR, Wijesinghe CR, Pablo Porras, Birgit Medal

Session II – Immunology and Infectious Diseases

Session Chairpersons: Dr. Inoka C Perera and Dr. Darshan De Silva

OP06	**13.30-13.45 h**	Expression of co-stimulatory molecules involved in T-cell dependent B-cell activation in rats orally treated with a polyherbal formulation	Ranaweera BVLR, Abeysekera AM, Weerasena OVDSJ, Handunnetti SM
OP07	**13.45-14.00 h**	*In vitro* study of IgE independent basophil degranulation due to selected food items	Wijerathna DMCK, Handunnetti SM, Fernando N, Premawansa S
OP08	**14.00-14.15 h**	Comparison of IgG and IgM responses in rats immunized with two types of antigen preparations of *Leptospira biflexa* serovar Patoc	Gangani PD, Ishaka GKD, Fernando Karunanayake L, Rajapakse S, Premawansa S, Handunnetti SM
OP09	**14.15-14.30 h**	*In vitro* study on the involvements of Angiopoietin-1 and Angiopoietin-2 in severe dengue patient sera leading to endothelial dysfunction and fluid leakage	Dasanayaka HMNN, Mapalagamage M, Premawansa G, Handunnetti SM, Premawansa S
OP10	**14.30-14.45 h**	Diagnosis of *Vespa affinis* venom allergy: use of immunochemical methods	Gunasekara DLPE, Handunnetti SM, Premawansa S, Dasanayake WMDK, Ratnayake IP, Dias RKS, Premakumara GAS, Seneviratne SL and De Silva R
OP11	**14.45-15.00 h**	Effects of ion pump and ion channel modulators on activation and function THP-1 derived macrophages	Mitini-Nkhoma SC, Pathirana PPSL, Fernando TRGN, Handunnetti SM, Ishaka D
OP12	**15.00-15.15 h**	*In vitro* effects of methanol-dichloromethane extract of *Vernonia zeylanica* and its solvent fractions on human, matured KU 812-derived basophils	Ezechukwu HC**,** Rukshala BAD, Dilini I, Fernando TRGN, Handunnetti SM

Session III – Molecular Biology and Genetics

Session Chairpersons: Prof. P Nilanthi Dassanayake and Prof. TL Shamala Tirimanne

OP13	**15.15-15.30 h**	Preliminary study on bacterial load of *Leptospira* among severe and mild leptospirosis patients	Chandrapathma WPNS, Weerasena OVDSJ, Rajapakse S, Premawansa S, Karunanayake L, Handunnetti SM
OP14	**15.30-15.45 h**	Development of a triplex Reverse Transcription-Real Time Polymerase Chain Reaction for simultaneous detection of dengue, Japanese encephalitis and chikungunya viruses	Perera HIT, Muzeniek T, Targosz A, Yapa W, Premawansa S, Premawansa G, Handunnetti S, Weerasena OVDSJ, Kohl C, Nitsche A
OP15	**15.45-16.00 h**	Isolation and molecular identification of ethanol producing microbes in fermented decoctions (Arishta)	Heendeniya SN, Weerasena OVDSJ, Gunaratna LNR, Tennakoon TMSG
OP16	**16.00-16.15 h**	Identification of *Mimusops elengi* through DNA barcoding	Wanniarachchi WWMS, Siridewa K, Weerasena OVDSJ, Gunaratna LNR, Tennakoon TMSG

Poster Presentation

Session Chairpersons: Dr Rinukshi Wimalasekera and Dr. T Nilakshi Samaranayake

PP01	*PNPLA3* I148M and *TM6SF2* E167K single nucleotide polymorphisms: Development of a genotype assay and characterization in a cohort of tamoxifen treated breast cancer patients	Dayananda KRTLK, Hewage AS, Tennekoon KH, Ariyarathne MAY, Wickramage I
PP02	Development of a Y chromosomal SNP array with the use of SNaPshot minisequencing technique	Welikala AHJ, Ranasinghe R, Tennekoon KH
PP03	Comparison of DNA extraction protocols for tooth samples	Fernando AS, Ranasinghe R, Tennakoon KH
PP04	Ion pump and ion channel modulators as antimycobacterial agents	Mitini-Nkhoma SC, Pathirana PPSL, Fernando TRGN, Ishaka D, Handunnetti SM
PP05	A preliminary study on the roles of oxidative stress and anti-oxidant therapy in resistant hypertension patients in Sri Lanka	Ranasinghe RAHN, Fernando N, Handunnetti S, Weeratunga P, Galappatthy P, Constantine GR

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; *Vidyajyothi* 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 Defense 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; *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.

This year Professor Stanley Wijesundera Memorial Lecture will be delivered by Senior Professor Sagarika Ekanayake, Professor in Department of Biochemistry, Faculty of Medical Sciences, University of Sri Jayewardenepura.

Professor Stanley Wijesundera Memorial Lecture

On

Eat Rice to be Healthy: Variety, Processing and Preparation by Senior Prof. Sagarika Ekanayake

BSc (Hon), M.Phil (USJ), PhD (Sweden), C Chem, F I Chem C.

Rice (*Oryza sativa* .L) is the world's second largely produced cereal and is the staple food in most of the Asian countries including Sri Lanka. A positive association between consumption of highly refined white rice and diabetes has been found and as in other Asian countries, in Sri Lanka an increased prevalence of non-communicable diseases such as diabetes, cancer and cardiovascular diseases is reported. Food containing high amount of carbohydrates generate a stronger postprandial glucose response which ultimately leads to insulin resistance and obesity related non communicable diseases. Thus both the quality and the quantity of carbohydrate in foods we consume need to be considered when being incorporated in to the meal. Glycaemic index (GI) reflects the quality of the carbohydrates in starchy food and the quantity which is calculated based on the glycaemic index and the amount of carbohydrate in a portion ingested gives the glycaemic load (GL). Both indices reflect the glycaemic response of a particular food and are classified as high, medium and low.

When considering rice, the major contributor to carbohydrate and energy in a Sri Lankan diet novel and traditional varieties indicated differences in glycaemic responses. Commonly available raw (*kekulu*) rice irrespective of the variety (red, white, Sri Lankan white or red basmati) produced high glycaemic (GI- 73-81) responses except for *keeri* samba which produced a medium GI (66), but all provided a high glycaemic load (GL) when accompanied only with either *kirihodi* or *Pol sambol*. However, the glycaemic load for a normal portion size of red basmati was lower when compared to the white variety studied due to increased fibre. Among the commonly consumed raw rice varieties a better metabolic response was obtained with *Samba* rice. When the GI of Pakistan and Indian white basmati rice were studied, Indian basmati elicited low (54) GI with higher fibre content and Pakistani basmati elicited medium GI (64).

Many traditional rice varieties when consumed alone *(kirihodi)* elicited medium or low GI in contrast to the varieties tested above when used after milling without subjecting to polishing. Out of the sixteen varieties studied four varieties were white in colour. Glycaemic indices (GI) of analyzed varieties ranged between 49-67 and were categorized as either low or medium GI rice. GI value was independent of the colour of the pericarp but depended on fiber and resistant starch contents of rice.

Effect of processing on rice was studied with differently processed rice. Among the rice, parboiled *nadu* rice elicited the lower GI (low GI: 40) compared to *kekulu* (raw) rice. A lower peaking was seen even when given with only *pol sambol*. Effect of processing on traditional rice clearly demonstrated the same when *Godaheenati, Batapola el, Dik wee, Dahanala, Unakola samba* and *Hangimuththan* rice varieties were differently processed as raw under milled, raw polished (4% polishing) and parboiled (under milled). The GIs of raw under milled rice were (54-69) either low or medium irrespective of pericarp colour. When subjected to polishing an increase in GI was observed for all varieties (69- 74) with high digestible carbohydrate content. However, parboiling the same varieties decreased the GI to low GI range (43-48). Digestible starch contents of all the differently processed varieties were different when an edible portion was considered with an increase in moisture and fibre in parboiled and under milled varieties.

How rice is cooked, incorporated in a meal and accompaniments added also influences the glycemic response. When raw red rice is consumed in the typical Sri Lankan way with many different accompaniments (curries) the GI decreased significantly by 39% compared to raw red rice only meal and the mixed meal was categorized as low GI (47). Glycaemic load and the glucose peak also reduced compared to the rice given only with *kirihodi* or *pol sambol*. Glycaemic load of a normal edible portion was also less as two thirds of a portion was adequate due to addition of other accompaniments. Increased addition of vegetables containing high dietary fibre, contributes to lowering the GI to a certain extent. However, beyond 16 g dietary fibre /portion failed to elicit a significant effect on the GI.

When rice was used to make milk rice, white raw (72) and red basmati (70), both elicited high GI and high GL. However, addition of green gram to white raw rice (1:3) reduced the GI to medium (65) and further addition of green gram (3:1 rice) elicited low GI (55). Likewise, eight green leafy porridges made with a variety of leaves and red raw rice elicit low GI (< 55) with the added advantage of beneficial phyto-nutrients. The lowering of GI was attributed to the high moisture content. When the method of cooking in rice cooker and microwave was compared the GI values of Indian and Pakistani basmati rice cooked in microwave (GI; 43 and 56) showed a percentage reduction in GI values of 20.4% and 12.5% when compared to GI of rice cooked in the rice cooker respectively.

A rice meal with accompaniments irrespective of the variety would induce a lower glycaemic response. Traditional rice when compared to other varieties even when consumed with few accompaniments leads to lower glycaemic response. However, parboiled rice irrespective of variety elicits even further reduction in glycemic response and would be a healthier choice. Thus, when selecting rice, variety, processing rice is subjected to and how we prepare rice at home for consumption if properly managed will be the most nutrition meal one can consume.

Award Winners: 10th Annual Scientific Sessions, IBMBB – 21st November 2018

The Best Oral Presentation in Molecular Biology and Bioinformatics
was awarded to
Ms. Sundaralingam T
for the paper titled
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

The Best Oral Presentation in Immunology and Infectious Diseases
was jointly awarded to
Gunasekera DLPE
for the paper titled
Comparison of hyaluronidase from venom of Apis dorsata Fabricius and Vespa affinis Linnaeus
Gunasekera DLPE, Handunnetti SM, Premawansa S, Dasanayake WMDK Ratnayake IP, Seneviratne SL, Dias RKS, Premakumara GAS, De Silva R

Mapalagamage MS
for the paper titled
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.

The Best Oral Presentation in Medicinal plants, Biotechnology and Bioinformatics
was awarded to
Ariyaratne HMHJD
for the paper titled
Development of a Colourimetric Reverse Transcription Loop-mediated Isothermal Amplification Assay for the rapid detection of different Serotypes of dengue virus
Ariyaratne HMHJD, Weerasena OVDSJ, De Silva AD, Fernando N, Handunnetti SM, Nguyen AV

The Best Poster Presentation
was awarded to
Ratnayake AKDVY
for the paper titled
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

Erratum

1) Abstract No PP01 of the 10th Annual Scientific sessions of the IBMBB

The authors list should be corrected as follows:

Hewagama AHS[1], Handunnetti SM[1], Pathirana SL[1], Nawarathna NMM[2], De Zoysa IM[3], Riza R[3], Seneviratne SL[1,3,4]

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

[2] Department of Gastroenterology, National Hospital of Sri Lanka

[3] Department of Surgery, Faculty of Medicine, University of Colombo

[4] Institute of Immunity and transplantation, Royal free Hospital, UK

Abstract No: OP01

Mitochondrial DNA control region variations and breast cancer risk in South Asian populations: a comparative analysis with Sinhalese population

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 one of the leading cancers, globally as well as in Sri Lanka, where it accounts for 25.2% of diagnosed cancers in females. A growing body of research points out to the association of mitochondrial DNA mutations with sporadic breast cancer. Many studies also indicate the potential of using mitochondrial DNA as a predictive biomarker for analysis of breast cancer risk. Studies on mitochondrial DNA in breast cancer have been conducted on South Asian populations such as Bangladesh and South India. These studies identified the following mutations: 16189 T>C, 16290 C>T, 16293 A deletion and T310 C insertion, to be significantly associated with breast cancer in their populations. In the present study 63 patients, confirmed to be diagnosed with breast cancer, and their matched controls (age, BMI and menopausal status), who had no reported family or personal history of cancer, were studied. All the patients and the controls belonged to the Sinhalese ethnicity. The complete mitochondrial DNA control region was sequenced and the data analysed in comparison with the revised Cambridge Reference Sequence. Occurrence of the above reported mutations in our study cohort was as follows:16189 T>C was observed in 17 controls and 11 patients. 16290 C>T was not observed in patients but was present in one control. Deletion of A at 16293 was not observed in our study cohort, however a 16293 A>AG transition was seen in one patient. Furthermore, T310 C insertion was observed in 6 controls and 3 patients. The mitochondrial DNA mutations reported to be associated with breast cancer in other South Asian populations failed to show a significant correlation with breast cancer in our study cohort. Thus it can be concluded that these mutations may not be associated with a risk of developing breast cancer in the Sinhalese population. Further studies are being carried out at present to assess possible association within two other Sri Lankan ethnicities.

Keywords: Mitochondrial DNA; Breast Cancer; Control Region; South Asia; Sinhalese

This work was supported by the National Science Foundation of Sri Lanka (Grant no.NSF/SCH/2016/04).

Abstract No: OP02

Genomic data annotation workflow for SNP mutations via bioinformatics tools

Senadheera SPBM [1], Weerasinghe AR [1], Wijesinghe CR [1], Porras P [2], Medal B[2]
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[2]European Bioinformatics Institute.

Cancer is a genetic disease which cells gain abnormal growth and have potential to invade other normal tissues. Carcinogenesis can be explored by analysing genomic mutations and protein changes. Data annotation to predict the effect of the mutations is a critical point in mutation analysis. Our objectives of this study are to build common platform for data conversion to common platform and data annotation for human nervous system cancer mutations. We have used 15 datasets in order to develop the workflow. R packages, UniProt conversion tool, Ensemble Genome conversion tool and VEP tool were used for the analysis. Data annotation were performed via using DBSNP, COSMIC, Clinvar and Uniprot databases. Mutation protein effect prediction was performed with SIFT and PolyPhen. Data filtration and SNP mutation analysis were completed with R packages and MS Excel. Firstly, we performed data purification step which rely on genome build, annotation database version and genome position. According to the results, Best method for genomic mapping is chromosome position mapping. Secondly, we annotated mutations with genomic data. We filtered protein mutated genomic level mutations. Protein position identification and data annotation was the final step of the workflow. IDH1 (IDH1 protein 132 R>H) was the highest mutated position of the datasets. We have identified genomic level mismatches in previous published data and tool errors occurred in UNIPROT. Research output provided a feedback to correct the existing tool drawbacks. We designed a reusable workflow for large mutation datasets analysis via using human nervous system cancer mutation data. As future work, we will try to merge other cancer datasets for this workflow.

Keywords: Cancer, SNP mutations, Annotation,

This work was supported by the UGC Grant 2018 and EBI Intern program.

Abstract No: OP03

Detection of somatic mutations of *KRAS, BRAF* and *PIK3CA* in a cohort of patients with colorectal carcinoma in Sri Lanka

Imthikab AIA[1], De Silva S[1], De Silva K[2]
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Colorectal cancer (CRC) is one of the most common cancers worldwide. Continuous activation of genes such as *KRAS, BRAF and PIK3CA* is known to be involved in colorectal carcinogenesis. Detection of mutations in hotspots of these genes helps in prognosis and treatment. *KRAS* and *BRAF* mutations are established predictive biomarkers for anti-epidermal growth factor receptor (EGFR) treatment. Studies suggested that *PIK3CA* mutations play a role in prognosis of CRC. For CRC, mutations in *KRAS* exon 2 (codons 12/13), *BRAF* exon 15 (codon 600) and *PIK3CA* exon 20(codon 1047) are most common with frequencies of 40%, 10–15% and 10–20% respectively. Less common *KRAS* mutations are found in codons 61 and 146. In the current study *KRAS* codons 12, 13, 61 and 146, *BRAF* codon 600 and *PIK3CA* codon 1047 were analyzed to detect possible mutations. DNA was extracted from fresh CRC tissue samples (N=16) collected from patients at the Maharagama Cancer hospital. Specific primers designed for targeting hotspot mutations were used for PCR amplification. DNA sequencing was done and the results were analyzed using several bioinformatics tools. *KRAS* mutations were present in 25% of the patients. 3 of them had *KRAS* codon 12 mutations; c.34G>C, c.35G>T and c.35G>A while one had *KRAS* codon 61 mutation, c.183A>T. All of these mutations are already established activating *KRAS* mutations which are pathogenic and deleterious. None of the patients had *KRAS* codons 13, 146, *BRAF* codon 600 or *PIK3CA* codon 1047 hotspot mutations. It was observed that 25 % of the patients had the silent variant, c.3075C>T (p.Thr1025Thr) in the *PIK3CA* gene which was reported in other studies on cancer. More data is required for the association of this variation with CRC. In conclusion, a larger number of patients need to be studied to document the prevalence of the hotspot mutations in CRC in Sri Lankans.

Keywords: Colorectal cancer, *KRAS, BRAF, PIK3CA*, detection.

This work was supported by MSc Research Fund, IBMBB, University of Colombo and National Research Council (Grant No. NRC 15-33).

Abstract No: OP04

Comparison between cDNA and DNA sequencing in detection of *TP53* variants in head and neck, breast and colorectal cancer

Manoharan V[1], Karunanayake EH[1], Tennekoon KH[1], De Silva S[1], Angunawela P[2], De Silva K[3], Lunec J[4]
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[2]Department of Pathology, Faculty of Medicine, University of Colombo.
[3]National Cancer Institute, Maharagama. [4]Northern Institute for Cancer Research, Newcastle University.

Knowledge on *TP53* mutation status is important in deciding treatment options for the cancer patients. In this study, we compared DNA and cDNA sequencing to find a better detection method of somatic *TP53* mutation. DNA and RNA were simultaneously extracted from fresh tissues of 42 patients which included 25 head and neck cancers, 9 breast cancers and 8 colorectal cancers. PCR amplification of DNA and synthesized cDNA were carried out using exonic primers and cDNA primer respectively. Amplification of DNA was successful with all 42 samples whereas, only 13 cDNA samples showed amplification with the cDNA primers. PCR products were purified and subjected to sequencing. Both the sequencing data were compared. There were 8 pathogenic/ likely pathogenic variants found in common which include 7 missense and 1nonsense variants. Five of the 7 missense variants which appeared as heterozygous in DNA were found to be homozygous variants in cDNA. The remaining 2 missense variants were heterozygous in both DNA and cDNA. However, the nonsense variant detected in heterozygosity in DNA was homozygous wild-type in the cDNA. Better sequencing results were obtained via cDNA compared to DNA which may possibly be due to (i) the silencing of wild-type P53 transcription, (ii) the degradation of wild-type P53 mRNA, or (iii) the selective overexpression of mutated p53 mRNA. Similarly, loss of detection of nonsense variant in cDNA may be due to the rapid degradation of mutant p53 mRNA due to non-sense mediated mRNA decay. However, the precise mechanisms still remain to be discovered. Though previous literatures state, cDNA sequencing improves the detection of p53 missense variants than DNA sequencing, samples with lower expression of p53 cannot be amplified using the cDNA primers. Thus, cDNA sequencing analysis alone appears to be of limited value in assessing the mutational status of *TP53.*

Keywords: Head and neck cancer, breast cancer, Colorectal cancer, TP53, Genetic analysis

This work was supported by National Research Council (Grant No. NRC 15-33).

Abstract No: OP05

Human nervous system cancer mutation analysis from protein sequences and structures

Senadheera SPBM [1], Weerasinghe AR [1], Wijesinghe CR [1], Porras P [2], Medal B[2]
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According to the central dogma, genomic mutations (SNP) will affect the protein function and structure. Human nervous system cancer data are rarely studied in protein sequence level and protein structure level. We focused on studying the protein sequence and structure changes in nervous system tumor mutation datasets available in cBioPortal. We have considered only SNP mutations which alter the protein sequences. Objective of this study is to map human neuron system SNP mutation into protein level data. Furthermore, we try to provide user friendly visualization for protein level mutations. Variant effect predictor, UNIPROT web tool, UNIPROT API and BISQUE tool were used for protein sequence level data annotation. BISQUE, PDB genomic mapper and Joseph Marsh Group PDB mapper tool were used to map the structure level data. In visualization panel, we used the PDB Topology Viewer, Sequence feature View, PDB Residue Interactions and LiteMol in the PDB component library. According to the results we identified unique 59148 genomic positions in 15574 genes. Moreover, 151402 unique protein positions were altered in 49132 proteins. We recognized a clinically significant position (C9J4N6_132_R>H) in protein sequence level. Furthermore, in the datasets, Alanine (A) > Threonine (T) (8%) mutation will change the protein position nonpolar (hydrophobic) to polar (hydrophilic) which will significantly change the protein structure. Secondly, we have developed script to connect BISQUE through R for large mutation list annotation. Finally, we design a protein structure visualization panel for nervous system caner mutations. As future work we are trying to implement the process as a single workflow.

Keywords: Cancer mutations, Human neurons, Data annotation, protein sequence,

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Abstract No: OP06

Expression of co-stimulatory molecules involved in T-cell dependent B-cell activation in rats orally treated with a polyherbal formulation

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Link Samahan® (LS) is a polyherbal formulation used for prophylaxis against cold related symptoms. This study was designed to investigate the effect of LS on the expression of co-stimulatory molecules (CD28, CD40L, CD80, CD86 and CD40) involved in T-cell dependent B-cell activation. Three groups of rats were immunized with bovine serum albumin (BSA) intraperitoneally on day 1 and 15. In each group both immunizations were followed by 5-consecutive day oral treatments with either LS, water or sugar. Water and sugar treated groups were used as two controls since LS contains water and sugar as excipient. Rat peritoneal cells (RPC) obtained on day 0, 2, 14 and 16 were used to perform real-time quantitative polymerase chain reaction (RT-qPCR) to quantify the expression of co-stimulatory molecules. RPC were used to extract RNA and synthesize cDNA and to amplify genes using primers specific for co-stimulatory molecules. The expression of CD28, CD40L, CD80, CD86 and CD40 on day 0 and 14 was comparable in all three groups. LS-treated rats showed a significant increase in expression of co-stimulatory molecules on day 2 and 16 compared to their levels on day 0 and 14 respectively ($p<0.05$). On day 2 and 16, the gene expression of co-stimulatory molecules in the LS group was significantly higher in T helper cells (CD28 - 5.1 fold and CD40L - 4.9 fold) as well as in B cells (CD80 – 2.4 fold, CD86 - 2.5 fold and CD40 - 2.3 fold) compared to the two controls. This shows that the effect of both controls was similar and that sugar had no significant effect on co-stimulatory molecule gene expression. These results suggest that LS may have induced a higher degree of enhancement of gene expression of co-stimulatory molecules on T-helper cells compared to the gene expression of co-stimulatory molecules on B cells. We have previously shown that LS has immunostimulatory activity and induce significantly high levels of IgG antibody against BSA. Collectively, RT-qPCR findings from the present study suggest that the enhancement of T-cell dependent B-cell activation may have induced a higher production of IgG antibodies against the protein antigen.

Keywords: co-stimulation, T-helper cells, B-cell activation, polyherbal formulation, RT-qPCR

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

Abstract No: OP07

In vitro study of IgE independent basophil degranulation due to selected food items

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Food allergy is a rising health problem worldwide. As opposed to the most common IgE antibody-dependent (indirect) basophil or mast cell degranulation in an allergy, pseudo-allergies occur in an IgE independent (direct) manner. Food is identified as a main cause for pseudo-allergies, which cause allergy-like reactions but with normal IgE levels, however, the causes and mechanisms of pseudo-allergies are not well understood. Since food allergy can be fatal, the appropriate diagnosis is essential. The current study is focused on IgE independent basophil degranulation caused by four common allergenic foods including skipjack tuna (*Katsuwonus pelamis*), pineapple (*Ananas comosus),* Asian tiger shrimp (*Penaeus monodon*) and peanut (*Arachis Hypogaea L.*). The concentration of crude food protein extracts was determined by Bradford assay. The non-toxic concentrations of food items to rat basophil cell line (RBL-2H3) were determined by using Sulforhodamine B (SRB) assay. Degranulation of basophils after treatment with 500 µg/ml of crude protein extracts was quantified microscopically using Toluidine blue dye. Significant induction of degranulation was shown by tuna (Mean percentage±SD, 73.51±6.75), peanut (57.92±22.12), pineapple (54.36±6.90) and shrimp (50.50±21.46) ($p<0.05$) compared to the untreated controls. In addition, green tea (*Camellia sinensis*) with 200 µg/ml concentration of crude proteins significantly inhibited compound 48/80 (positive control) induced rat basophil degranulation ($p<0.05$). Certain known allergenic proteins in tuna and peanut crude extracts were determined by SDS-PAGE. In conclusion, the tested food items including skipjack tuna, pineapple, Asian tiger shrimp and peanut can cause IgE independent basophil degranulation to cause allergies whilst green tea has the potential of stabilizing basophils and anti-allergic activity. Further research is emphasized to reduce the prevalence of food allergies by identifying the food allergens and their mechanisms, identifying native plants and modes of clinical treatments for pseudo-allergies.

Keywords: Allergy, IgE-independent basophil degranulation, Food allergy, Pseudo-allergy, Anti-allergic activity

The work was supported by Department of Zoology and Environment Sciences, University of Colombo and IBMBB.

Abstract No: OP08

Comparison of IgG and IgM responses in rats immunized with two types of antigen preparations of *Leptospira biflexa* serovar Patoc

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Leptospirosis is a zoonotic disease which is caused by the spirochaetes of genus *Leptospira*. At present, Sri Lanka has been identified as a leptospirosis endemic country. The clinical symptoms of Leptospirosis vary from mild fever to severe conditions. Hence for prompt treatment, rapid and appropriate laboratory diagnostic tests are needed however not available at present. Production of monoclonal antibodies is an essential step for the development of novel diagnostic tests for detecting *Leptospira* antigens in body fluids. The objective of this study was to compare the rat immune responses against two types of antigen preparations of *L. biflexa* serovar Patoc**.** The IgM and IgG responses in rats immunized, as follows, i) killed and sonicated antigen preparation (group 1), ii) heat-killed whole organisms (group 2) and iii) adjuvant control (group 3) were assessed. To immunize rats, five doses of antigen were injected intraperitoneally at two-week intervals using a combination of Freund's complete adjuvant (FCA) and Freund's incomplete adjuvant. Results indicated that there was significantly higher production of IgM in group 1 and 2 by day 7 compared to group 3 ($p<0.050$, ANOVA) and decreased by day 14. There was no significant difference in IgM levels between group 01 and group 02 ($p<0.050$)**.** IgM responses observed after 2nd and 3rd dose in groups 2 and 3 were similar to that after the first dose. In contrast, IgG level in group 1 and 2 increased after each immunization (from day 7 up to day 56) and was significantly higher compared to group 3 ($p<0.050$). Further, the IgG response in group 1 was significantly higher compared to group 2 until day 56 ($p<0.050$). In conclusion higher IgG response elicited against the sonicated antigen (group 1) compared to heat-killed whole organisms (group 2) suggests that the sonicated antigen preparation has induced a higher boosting humoral response. This indicate that the production of memory cells may have been more efficient following immunization with sonicated antigen which is more appropriate for monoclonal antibody production.

Keywords: Leptospirosis, *Leptospirosis biflexa*, Sonicated antigen, IgG response, Heat-killed antigen

This work was supported by International Centre for Genetic Engineering and Biotechnology (ICGEB Grant No. CRP/LKA 17-01).

Abstract No: OP09

In vitro study on the involvements of Angiopoietin-1 and Angiopoietin-2 in severe dengue patient sera leading to endothelial dysfunction and fluid leakage

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Lack of understanding of pathogenesis and its mechanisms leading to plasma leakage in severe dengue infections cause difficulties in early detection and management of patients developing dengue hemorrhagic fever (DHF) and dengue fever (DF). Angiopoietin-1 (Ang-1) and Angiopoietin-2 (Ang-2) are angiogenic factors that affect integrity of endothelium. The aim of this study was to assess the *in vitro* effects of serum Ang-1 and Ang-2 levels of DHF patients on endothelial integrity and functions. Our previous studies have shown increased serum Ang2 in DHF patient compared to that of DF. Monolayers of Ea.hy926 cell line (human macro-vascular endothelial cells; EC) were treated *in vitro* with sera of clinically confirmed DF patients, DHF patients at critical stage and healthy controls (HC) (n=6 in each category). Detachment of EC from monolayers and the distance between EC were measured following 1, 3 and 6 h incubations with sera. Serum Ang-1 and Ang-2 levels before and after incubation were measured using enzyme linked immunosorbant assay and the leaking rate through EC monolayer was measured using the "Transwell" plate. A significant Ang-1 reduction and a significant Ang-2 accumulation were observed after 3-hour incubation with DHF serum ($p<0.001$), whereas Ang-1 accumulation was observed after 3h incubation with HC serum ($P=0.001$). This indicates that remaining Ang-2 from DHF serum and Ang-1 from HC serum may be bound to EC via Tie-2 receptor and secrete more Ang-2 and Ang-1 by EC cells which were treated with DHF sera and HC sera respectively. The highest distances between cells in the EC monolayer was observed 3h after incubation with DHF serum compared to that of HC sera ($p<0.0001$) and conversely the highest leaking rate was observed with DHF serum ($p<0.0001$) indicating that the EC dissociation/disruption *in vitro* may have occurred due to Ang-2 rich DHF serum. Using this *in vitro* system of EC monolayer treated with DHF patient serum, this study was able to simulate *in vivo* the endothelial leaking phenomenon in DHF patients.

Keywords: Dengue, Angiopoietin, dengue hemorrhagic fever, endothelium, cellular dissociation

The study was supported by Department of Zoology and Environment Sciences, University of Colombo and IBMBB.

Abstract No: OP10

Diagnosis of *Vespa affinis* venom allergy: use of immunochemical methods

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Allergy to *Vespa affinis* venom is common in the Asia Pacific region. Venom preparations for diagnosis are not commercially available for this species. The aim of this study was to describe the allergens in *V. affinis* venom and the diagnosis of venom allergy using *in vitro* test. This study identified the allergens in *V. affinis* venom using immunochemical methods; sodium dodecyl sulphate gel electrophoresis (SDS-PAGE), immunoblots and high-performance liquid chromatography (HPLC). *In vitro* diagnostic tests (ImmunoCAP) of *Vespula vulgaris* crude venom and its components in the diagnosis of *V. affinis* venom anaphylaxis were further tested using sera from 30 patients. The double-positivity rate (bee and hornet) were determined using ImmunoCAP of *Apis mellifera* and *V. vulgaris* venom. High IgE reactivity was seen with five allergens in *V. affinis* venom; 96.7% (29/30) for 34 and 24 kDa, 93.3% (28/30) for 45 kDa and 90% (27/30) reactivity for the 100 and 80 kDa respectively. IgE cross-reactivity was low with ImmuunoCAP using *V. vulgaris* venom (43.3%; 13/30) and Ves v1 (3.3%; 1/30), but relatively high with Ves v5 (73.3%; 22/30). In ImmunoCAP, a high double-positivity rate (76.6%; 23/30) was seen with bee venom. In contrast, with immunoblots, 60% (18/30) double-positivity was seen and all of them were due to hyaluronidase (45 kDa). High IgE reactivity for five allergens of *V. affinis* points to the potential of using these allergens in component resolved diagnosis (CRD) of *V. affinis* venom allergy. Hyaluronidase with its high rate of double-positivity may hamper the specific diagnosis of *V. affinis* venom allergy. The low level of IgE cross-reactivity with *V. vulgaris* venom/components except Ves v5 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

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Abstract No: OP11

Effects of ion pump and ion channel modulators on activation and function of THP-1 derived macrophages

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Ion pump and ion channel modulators are used to treat a number of non-communicable diseases including diabetes and hypertension. They have receptors on different types of cells including macrophages, but their effects on macrophage function need further investigation. This study was conducted to assess the effect of ambroxol, amiloride HCl, diazoxide, digoxin, furosemide, glibenclamide, HCTZ, metformin, nifedipine, omeprazole, pantoprazole, phenytoin and verapamil on the activation and function of THP-1 derived macrophages. THP-1 derived macrophages were stimulated with inactivated *Mycobacterium bovis* BCG and then treated with the test drugs. Nitric oxide production, superoxide production and cytokine expression were then compared between treated macrophages and drug-free controls. Nine of the thirteen drugs had an inhibitory effect on nitric oxide production. Of the 9, phenytoin demonstrated the highest inhibitory effect on nitric oxide synthesis (36.7% reduction, $P<.001$). All 13 drugs caused a reduction in superoxide production and IL-12 expression. Verapamil exhibited the greatest inhibitory effect on superoxide production (38% reduction, $P<.001$). Omeprazole was the most potent inhibitor of IL-12 expression, reducing its RNA levels by 56%. Omeprazole also caused a reduction in the expression of IL-1 and TNF-α while the rest of the drugs enhanced their expression. Furosemide demonstrated the greatest effect on the production of TNF-α and IL-1β, causing a 9.9-fold and 5.8-fold increase in their expression respectively. In summary, ambroxol, amiloride HCl, diazoxide, digoxin, furosemide, glibenclamide, HCTZ, metformin, nifedipine, omeprazole, pantoprazole, phenytoin and verapamil displayed a wide range of immunomodulatory properties, some of which cannot be explained by their known mechanisms of action. There is need for further assessment of the effects of ion pump and ion channel modulators on co-morbid infections when used in people with non communicable diseases, in light of the high prevalence of non communicable diseases worldwide.

Keywords: Immunomodulation, ion channel blockers, verapamil, nifedipine, THP-1

This work was supported by a Scholarship and research funds received from Association of Commonwealth Universities, United Kingdom and constitutes a part of SCM-N M.Sc. studies.

Abstract No: OP12

In vitro effects of methanol-dichloromethane extract of *Vernonia zeylanica* and its solvent fractions on human, matured KU 812-derived basophils

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Vernonia zeylanica is an endemic Sri Lankan shrub used in Ayuverdic medicine for eczema, boils, bones fractures and asthma. Its anti-inflammatory, anti-bacterial and anti-nociceptive properties have been reported. The present study was aimed to determine whether methanol-dichloromethane extract (MDME) of *V. zeylanica* and its solvent fractions (hexane, ethyl acetate and methane) exert modulatory or inducing effect(s) in human basophils, i.e. KU 812 cells. KU 812 cells maturity was induced either with hydrocortisone treatment for 21 days or with cell- starvation treatment alone for 6 days in complete media. Results indicated that starvation treatment gave 8-12% higher yield of matured basophils compared to hydrocortisone treatment and was selected for further experiments. Mature KU 812 derived basophils were stimulated with *p*horbol-12-*m*yristate-13-*a*cetate and *c*alcium *i*onophore (A23187) (PMACI). The effect of *V. zeylanica* on non-IgE mediated degranulation, intracellular calcium concentration and IL-8 expression were measured using human basophil degranulation assay, Fura-2AM indicator and RT-qPCR respectively. MDME of *V. zeylanica* and its hexane and methanol fractions exerted significant dose-dependent inhibition on non-IgE mediated degranulation of KU 812 derived mature basophils ($r = 0.91$-0.96; $p < 0.05$). Ethyl acetate fraction did not show a significant inhibition. In contrast, treatment with MDME and its three solvent fractions resulted in an increase in both [Ca]i influx (35.74-47.01%; $p<0.05$) and IL-8 expression (0.38-1.80 fold change). In conclusion, these results indicate that *V. zeylanica* causes both inhibitory as well as inducing effects on human KU 812-derived mature basophils. Further, the extent of its inhibitory properties observed might not be a direct function of the two molecular parameters investigated in a non-IgE mediated pathway. Therefore, there is a need for further studies on the characterization of MDME and its solvent fractions of *V. zeylanica* and isolation of active components to provide specific therapeutic lead candidates for allergic-inflammatory indications.

Keywords: Basophils, KU 812 cells, *Vernonia zeylanica*, Interleukin-8, Intracellular calcium

This work was supported by a Scholarship and research funds received from Association of Commonwealth Universities, United Kingdom and constitutes a part of HCE M.Sc. studies.

Abstract No: OP13

Preliminary study on bacterial load of *Leptospira* among severe and mild leptospirosis patients

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Leptospirosis is a potentially fatal zoonotic disease caused by spirochetes belonging to the genus *Leptospira*. *LipL32* is the major outer membrane lipoprotein found in the pathogenic *Leptospira*. The aim of this study was to determine the *Lipl32* based q-PCR positivity among severe and mild leptospirosis patients confirmed by microscopic agglutination test, using 86 randomly selected blood samples. Clinical categorizations as of these patients indicated 35 and 51 having mild and severe leptospirosis. DNA was extracted using 300 µl of blood using Wizard(R) Genomic DNA Purification Kit, Promega, USA. Real-time q-PCR assay was carried out using genesig Real-time PCR detection kit for Leptospirosis (Primerdesign™ Ltd, UK) for targeting *Lipl32* gene. Standard curve was plotted using positive control provided with kit and *Leptospira* genome quantity (bacterial load) was calculated. qPCR results showed that, 63.9% (55/ 86) of blood samples were positive for DNA of pathogenic *Leptospira* spp. A significantly high percentage of severe patients (72.5%) were qPCR positive, compared to mild patients (51.4%) ($p=0.045$, Pearson chi-square). The qPCR positive patients had higher days of illness (8.53 days) compared to those who were q-PCR negative (7.22 days) ($p=0.016$, Mann-Whitney U test). There was a significant correlation between the days of illness and *Leptospira* genome quantity ($r=0.292$; $p=0.007$). However, the mean values of *Leptospira* genome quantity (Mild: 19075, Severe: 23461.4) and days of illness (Mild: 7.88, Severe: 8.18) between the mild and severe patient categories were not significantly different. This preliminary study indicates that *Leptospira* bacterial load was associated with the days of illness.

Keywords: *Leptospira*, Severe leptospirosis, *Lipl32,* qPCR, days of illness

This work was supported by National Research Council (Grant No. NRC – 17-098).

Abstract No: OP14

Development of a triplex Reverse Transcription-Real Time Polymerase Chain Reaction for simultaneous detection of dengue, Japanese encephalitis and chikungunya viruses

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A recent study has reported that Dengue virus (DENV) accounts for 41% of encephalitis cases reported in Sri Lanka while Japanese encephalitis virus (JEV) accounts for 26%. Apart from flaviviruses, Chikungunya virus (CHIKV) has also been reported as an encephalitis causing agent. Thus, a triplex PCR was established for DENV, JEV and CHIKV with different fluorescence labelled probes for simultaneous detection of these viruses. Extracts of the infected Vero E6 cell culture supernatant containing the respective viruses were used as positive controls. The RNA extracted from each cell line supernatant was diluted to prepare a dilution series and real time PCR was carried out for viral dilutions to find out the required dilution to obtain threshold cycle number (C_T value) of 30 for each virus. Optimum primer concentrations to maintain the detection of each virus at C_T value of 30, were determined. Performance of the assays for detecting the three viruses with the optimized primer concentrations, were tested with different target concentrations in separate assays. The RT-PCR with the optimal primer concentration combination was further optimized with respect to the concentration of the probe. In this experiment, 0.25 µM Forward primer (F), 0.50 µM Reverse primer (R) and 0.25 µM FAM-labelled probe combination was chosen as the optimal for CHIKV, 0.50 µM F, 0.25 µM R and 0.50 µM Cy5-labelled probe for DENV and 0.50 µM F, 0.50 µM R and 0.25 µM HEX-labelled probe was optimal for JEV. Three primer pairs showed no interaction when tested for possible primer-primer interactions. These optimized conditions will be used for validation of the assay using patient samples and will be later used for the diagnostic purposes in combination with an internal control.

Keywords: Triplex PCR, Reverse Transcription-Real Time Polymerase Chain Reaction, Dengue virus, Japanese encephalitis virus, Chikungunya virus

This study was supported by the Robert Koch Institute, Germany (Grant No. UOC/RKI/BV/17-01).

Abstract No: OP15

Isolation and molecular identification of ethanol producing microbes in fermented decoctions (Arishta)

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Arishta is a self-generated fermented form of polyherbal drugs used in Ayurvedic medicine and ethanol formation during fermentation has additional value in preservation and solubilization of active ingredients. As limited studies are available on investigation of microbiological aspects of ethanol formation, a study was conducted to isolate and identify ethanol producing microorganisms from four Arishta formulations (Arjunarishta, Abhayarishta, Dashamoolarishta and Ashokarishta). Arishta samples (2 samples from Arjunarishta and 5 samples each for other Arishta) were spread on Nutrient agar (NA) and Yeast Malt Extract agar (YM agar also known as YPD agar) comprising Bromothymol Blue (BTB). The microorganisms grown and displayed a color change from blue to yellow were isolated and categorized into 4 groups based on their colony morphology. The selected isolates were then grown for 5-days in YM broth at 25 °C and ethanol production was further analyzed by Pyridinium Chlorochromate (PCC) assay. All tested organisms were positive for ethanol in PCC assay. Ethanol production in fungal isolates positive for BTB and PCC assay (ARJ1SU1-3-S5, DAM1M1-1-S3. ARJ1SU1-2-S5. ASH1BO1-2-S3) were confirmed and quantified by Gas Chromatography Mass spectrometry (GC-MS). Baker's yeast (BY) was used as a positive control. GC-MS results revealed that except ARJ1SU1-2-S5, all the other isolates produced ethanol. Bidirectional sequencing of internal transcribe spacer (ITS) 1 and 2 of nuclear ribosomal RNA gene of fungal isolates was carried out by using ITS 1 and ITS 4 primers. The resultant sequences were searched over GenBank database at National Center for Biotechnology Information (NCBI) using Basic Local Alignment Search Tool (BLAST). Based on the sequence similarities in alignments, these isolates were identified as *Saccharomyces cerevisiae* (ARJ1SU1-3-S5 from Arjunarishta), *Aspergillus tubingensis* (DAM1M1-1-S3 from Dashamoolarishta), *Paecilomyces variotii* (ARJ1SU1-2-S5 from Arjunarishta) and *Monascus fumeus* (ASH1BO1-2-S3 from Ashokarishta). Highest ethanol production after 5-day fermentation period was shown by *Monascus fumeus* according to the GC-MS analysis.

Keywords: Arishta, *Monascus fumeus*, *Aspergillus tubingensis*, *Paecilomyces variotii*, PCC assay, Fermentation

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Abstract No: OP16

Identification of *Mimusops elengi* through DNA barcoding

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Mimusops elengi commonly known as 'Munamal'is a medicinal plant widely used in Ayurvedhic system of medicine. It belongs to the family *Sapotaceae* and the whole plant has medicinal value. The efficacy of Ayurvedhic drugs depend on the reliable identification of the correct source of plants. Usage of incorrect plant materials may result in inefficacy or toxicity. Species identification through morphological characteristics is a challenge as the plant parts are used as raw materials. Further, increased domestic and global demand has increased the probability of adulteration of raw materials. DNA barcodes are short DNA sequences of an organism's genome that can differentiate the species. The efficiency and accuracy of DNA barcodes provide a platform for reliable identification of authentic plant materials. The general objective of the study was to develop DNA barcodes to identify *Mimusops elengi*. The genomic DNA was extracted from fresh leaves of *Mimusops elengi*. Universal plant barcode regions ***i.e. matK, rbcL*** and *psbA-trnH* of chloroplast genome were amplified and bidirectionally sequenced. The barcode sequences obtained were searched over GenBank database. All the three barcoding sequences were aligned by sharing 99% identity with available sequences of *Mimusops elengi* in the database. The accession numbers for the deposited *rbcL*, *matK* and *psbA-trnH* sequences of *Mimusops elengi* were MK 947210, MK 947211 and MK 947212 respectively. Therefore the barcoding sequences deposited will be useful in the identification of *Mimusops elengi* in future.

Keywords: DNA Barcode, *Mimusops elengi*

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Abstract No: PP01

PNPLA3 I148M and *TM6SF2* E167K single nucleotide polymorphisms: Development of a genotype assay and characterization in a cohort of tamoxifen treated breast cancer patients

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Genomic Wide Association Studies (GWAS) have shown that there is a genetic background for the development of non-alcoholic fatty liver disease (NAFLD). Drug induced fatty liver may also occur in a similar genetic background. Patatin-like Phospholipase Domain-containing 3 Gene Variant I148M *(PNPLA3* I148M) and Transmembrane 6 Superfamily Member 2 Gene Variant E167K (*TM6SF2* E167K) are considered as major genomic variations associated with NAFLD. Primer extension-based method was developed to detect these two Single Nucleotide Polymorphisms (SNPs). Assay developed was used to study the prevalence of the two SNPs in a cohort of estrogen receptor positive (ER+) breast cancer patients who had been treated with tamoxifen. Prolong usage of tamoxifen is known to cause hepatic adverse effects. Polymorphic regions were amplified with SNP specific primers by polymerase chain reaction (PCR) and amplicons were analyzed via ABI PRISM® SNaPshot™ Multiplex Kit to identify the presence or the absence of two SNPs.Among 30 patients studied, 14 were heterozygous for the *PNPLA3* I148M SNP. None were homozygous for the variant. None of the patients carried the variant of *TM6SF2* E167K SNP either in heterozygosity or in homozygosity. In this cohort there were 4 patients who had developed fatty liver following tamoxifen treatment, where one carried the *PNPLA3* I148M SNP. Further studies are required to establish the role of these two SNPs in non-alcoholic fatty liver disease including drug induced fatty liver in Sri Lankans.

Keywords: NAFLD, *PNPLA3* I148M, *TM6SF2* E167K, Tamoxifen, ER+ breast cancer

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Abstract No: PP02

Development of a Y chromosomal SNP array with the use of minisequencing technique

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Studying of Y chromosomal single nucleotide polymorphisms (Y-SNPs) has become a widely accepted method in molecular anthropology to understand the origin and evolution of populations and to trace back the geographical distribution and paternal lineages of modern humans. Therefore present study was designed in order to develop a Y-SNP array based on multiplex PCR and minisequencing techniques, with the aim of investigating Y haplotypes diversity in contemporary Sri Lankan population. Sixteen Y-SNPs were selected according to the historical relationships and migration patterns of Sri Lankans and they were co-amplified using multiplex PCR technique. The sizes of the PCR products ranged from 66 to 532 bp. All PCR primers have theoretical melting temperatures (T_m) 53 ± 5 ^{0}C at a salt concentration 50 mM. Based on fragment length and T_m of PCR primers 3 multiplexes were designed and optimized accordingly to obtain a balanced yield with less number of nonspecific amplicons. The annealing temperatures of 3 multiplexes depended on the respective PCR primer combinations. Higher concentrations of $MgCl_2$ (3.0 mM, 4.0 mM and 6.0 mM for multiplex 1, 2 and 3 respectively) were required to obtain a higher yield of products while lower concentrations of template DNA ranging from 1 ng/µL to 4 ng/µL with the optimum levels of *Taq* DNA polymerase (1.5U), dNTP (400 µM) and PCR buffer (1X) yielded lower number of nonspecific amplicons. Genotyping of 16 Y-SNPs was performed by minisequencing using ABI Prism® *SNaPshot* Multiplex Kit. Fluorescently labeled 16 DNA fragments were then analyzed by capillary electrophoresis in the presence of LiZ-120™ internal size standard. Out of the selected Y-SNPs, 13 Y-SNPs gave successful results and three markers required further optimizations. Validity and reproducibility of the minisequencing assay were tested and confirmed in order to use the developed Y-SNP array for many samples.

Keywords: Y-SNP array, Multiplex PCR, Minisequencing

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Abstract No: PP03

Comparison of DNA extraction protocols for tooth samples

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Molecular archeological findings have shown teeth as the preferred source of a DNA due to its distinctive composition and location. Location within the jawbones helps to protect the teeth from physical and environmental conditions that influences the acceleration of decomposition and DNA decay. DNA extraction from tooth samples yields adequate amounts of high-quality DNA compared to bone samples which can be used for downstream applications such as PCR based diagnostic, sex determination, forensic applications etc. Samples used in this study were teeth removed due to medical reasons from elderly individuals over 60 years of age. After sample decontamination, DREMEL® 4000 was used to obtain the powder of mainly dentine and pulp of the tooth samples. Three protocols were attempted using 50 mg of tooth powder. Protocol A**:** Methods of preparing the tooth for DNA isolation by Preseeki et el (2000), protocol B: Ancient DNA extraction from bone and teeth by Rohland and Hofreiter (2007) and protocol C: QIAamp DNA investigator kit. All the DNA extraction protocols were carried out with a negative control. DNA was quantified using Quantus™ Fluorometer (Promega, USA) with QuantiFluor® dsDNA kit (Promega, USA). The highest DNA concentration (6.6 ng/µL) was recorded from protocol A followed by protocol B (2.56 ng/µL) and C (0.151 ng/µL). Use of sodium acetate in protocol A has a positive effect on DNA aggregation pelleting out procedure. As a result, extracted DNA has a higher quantity and a reasonable quality compared to protocol B and C. Hence, the method with highest yield can be further optimized to obtain DNA from ancient tooth samples obtained from archeological excavations.

Keywords: Molecular Archeology, DNA, PCR, Extraction, decomposition,

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Abstract No: PP04

Ion pump and ion channel modulators as antimycobacterial agents

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Mycobacterium tuberculosis owes most of its success as a pathogen to its ability to survive and replicate inside macrophages. There is growing interest in the development of therapeutic interventions that will enhance control of mycobacteria by host macrophages. This study was conducted to assess the efficacy of ambroxol, amiloride HCl, diazoxide, digoxin, furosemide, glibenclamide, HCTZ, metformin, nifedipine, omeprazole, pantoprazole, phenytoin and verapamil at controlling both free and phagocytosed mycobacteria. *Mycobacterium bovis* BCG was grown in liquid media and exposed to each of the test drugs. To assess the effects of the drugs on phagocytosed bacteria, THP-1 derived macrophages were exposed to *M. bovis* BCG for 1 hour, after which un-internalized bacteria were washed out and the test drugs were added. None of the drugs were bactericidal to free or phagocytosed *M. bovis* BCG. However, ambroxol, amiloride, diazoxide, digoxin, furosemide, HCTZ, metformin, nifedipine, phenytoin and verapamil inhibited the growth of both free and phagocytosed bacteria. Of these, nifedipine was the most efficacious inhibiting growth of free and phagocytosed bacteria by 69.5% (P=.008) and 49.8% (P<.001) respectively. Glibenclamide and omeprazole were only bacteriostatic to phagocytosed bacteria, indicating that their effect was largely due to their manipulation of macrophage function. Co-treatment of the bacteria with the anti-TB drug rifampicin and any of the test drugs resulted in higher bacteria clearance rates than when rifampicin was used alone. Verapamil and rifampicin had the highest synergistic effect against phagocytosed bacteria and yielded 8.3% (P=.001) higher bacteria clearance than rifampicin alone. In summary, all the test drugs displayed antimycobacterial properties that were synergistic to those of rifampicin. This makes them viable candidates as adjuncts for the current anti-TB regimens. There is need for further studies to assess the effects of these drugs on mycobacteria infections *in vivo*.

Keywords: Tuberculosis, ion channel blockers, verapamil, nifedipine, host-directed therapy

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Abstract No: PP05

A preliminary study on the roles of oxidative stress and anti-oxidant therapy in resistant hypertension patients in Sri Lanka

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Resistant hypertension is defined as uncontrolled blood pressure despite treatment (> 140 /90 mmHg) with concurrent use of three antihypertensive agents of different classes, one of which should be a diuretic. It has been hypothesized that oxidative stress is a key player in the kidney and development and persistence of hypertension. Reactive oxygen species (ROS) is known to increase afferent arteriolar tone and reactivity by both of its direct and indirect actions. This study was aimed to measure the burden of oxidative stress and anti-oxidant affect in patients with resistant hypertension. Forty resistant hypertensive patients, age between 18-75 years were recruited from outpatient medical clinics of the National Hospital of Sri Lanka. Patients with moderate to severe renal insufficiency and secondary hypertension were excluded. Subjects were randomly and equally assigned into the test and placebo groups by computer generated random numbers with a 1:1 allocation. The pro-oxidant state was assessed with estimation of serum nitrates and nitrites (NO_x), which are surrogate markers of serum NO levels using Griess and modified Griess assay respectively. The total anti-oxidant capacity (AOC) was measured using ABTS assay. A majority of patients in both propranolol group and the placebo group were females (72-73%) and males were 27-28%, both with a mean age of 56±9.5 and 56±10.5 years respectively. The mean NO_x levels in both propranolol (n=18) and placebo groups (n=15) were not significantly different at both baseline stage and after the follow up. Similarly, the baseline mean nitrite (NO_2) level in both propranolol and placebo groups were reduced after the 90-day follow-up but not significantly. In contrast, the mean AOC levels in both propranolol and placebo groups were significantly increased after the 90 day follow up ($p < 0.05$). These preliminary results indicate that Propranolol has a beneficial effect in hypertensive patients in increasing anti-oxidant capacity in the body.

Keywords: Hypertension, Oxidative stress, Propranol, Reactive Nitrogen Species, Antioxidant capacity

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