University of Colombo

IBMBB

Institute of Biochemistry, Molecular Biology and Biotechnology

Fifth Annual Scientific Sessions

27th April 2012

Programme and Abstracts

Institute of Biochemistry, Molecular Biology and Biotechnology

University of Colombo

PROGRAMME

9.30 h	Inauguration
9.30 h	Lighting of the traditional oil lamp
9.40 h	Welcome address by Director IBMBB
9.50 h	Address by Vice Chairman-University Grants Commission
10.00 h	Address by Vice Chancellor- University of Colombo
10.10 - 11.00 h	Institute lecture by Prof Ira Thabrew – "Discovering Natural Leads for Cancer Therapy"
11.00-11.05 h	Vote of Thanks
11.05 - 11.30 h	Tea
11.30 -11.55 h	Update I – "Cancer stem cells: implication for the treatment of cancer"
11.55 -12.20 h	Update II – "Breast cancer genetics: BRCA status and effect on breast cancer"
12.20 - 13.20 h	Lunch
13.20 -14.35 h	Free papers – Oral presentations Session I – Plant Molecular Biology & Medicinal Plants
14.35 -15.00 h	Update III - "Seeking answers to lingering questions on endometriosis using *in vitro* models: A review"
15.00 -15.25 h	Update IV – "DNA Markers in Forensic Analysis"
15.25 -16.25 h	Free papers – Oral presentations Session II – Molecular Biology & Immunology
16.15 h – 17.15 h	Viewing Poster presentations & Tea

Message from the Director/ IBMBB

I take great pleasure in writing a few words to mark the occasion of the 5th 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 8th Anniversary. IBMBB, the only state Institute in Sri Lanka dedicated to postgraduate training and research in Molecular Life Sciences was established with a soft loan from the Swedish Government in 2004. This was the culmination of a SAREC supported research programme led by its Founder Director, Professor Eric H Karunanayake. 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 several other MPhil and PhD students have registered at the IBMBB, all of them supported either by the SAREC Grant or by other competitive research funds secured by academics of the IBMBB. There have been several firsts in Sri Lanka among its MPhils and PhDs. These include 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 foetal growth, first study on the tea genome, first study to genetically characterize traditional and weedy rice. Amongst the MPhil/PhD students who have completed are three academic staff members from the University of Jaffna and one academic staff member from the Eastern University. In addition to the students registered at the IBMBB, several other MPhil/PhD students registered at other Higher Educational Institutes also carry out all or part of their research work at the IBMBB. Two probationary lecturers, one from the Faculty of Medicine, University of Kelaniya and the other from the Faculty of Medical Sciences, University of Sri Jayawardenapura are among such students. Thus the IBMBB is providing the higher educational sector with much needed academics with extensive research training.

IBMBB commenced two MSc programmes in 2005, one in Molecular Life Sciences and the other in Cellular and Molecular Immunology. Both MSc courses boast a more than 80% completion rate and to date 57 students have graduated.

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. In 2009 and 2010 IBMBB did not hold Annual Scientific Sessions as it organized an International Conference in September 2009 to mark IBMBB's 5th Anniversary. IBMBB students have won several International Awards and its Founder Director, incumbent Director, other permanent

academic staff and four other currently serving Board Members are Presidential Research Award winners.

High point of the 5th Annual Scientific Sessions will be the Institute Lecture on "***Discovering Natural leads for Cancer Therapy"*** to be delivered by Professor Ira Thabrew, Emeritus of Biochemistry**,** University of Kelaniya and Visiting Professor/IBMBB. Prof. Thabrew commenced her research at the IBMBB on a National Science Foundation Fellowship and has continued to supervise research students thereafter. There will also be four update lectures by senior PhD students on current topics related to their work and 25 research presentations including a few from students/researchers from other Higher Educational Institutes. From the authors and Institutes given in the abstracts, wide collaboration of IBMBB with other Academic Institutes, Health Sector and Agricultural Sector is clearly evident.

I take this opportunity to thank Prof. Ranjith Senaratne, Vice-Chairman, University Grants Commission and Prof. Kshanika Hirimburegama, Vice Chancellor, University of Colombo for their support and gracing the inauguration, Prof Ira Thabrew for accepting the Institute 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 thank you to Prof. Eric Karunanayake, Prof. Ira Thabrew, Dr. Shiroma Handunetti and Dr. Jagath Weerasena for reviewing abstracts, Dr. Shiroma Handunetti for editing the abstract book, Kanchana and Sashika for designing the abstract book and coordinating printing, Anoma and Abeysinghe for coordinating sponsorships and Champika and Nadeesha for secretarial assistance.

Professor Kamani H Tennekoon
Director / IBMBB

Updates I - IV

11.30 – 11.55 h (Abstract 01):

Update I – Cancer stem cells: implication for the treatment of cancer

Samarakoon SR, Thabrew I, Tennekoon KH

11.55 – 12.20 h (Abstract 02):

Update II – Breast cancer genetics: BRCA status and effect on breast cancer

De Silva WS, Tennekoon KH and Karunanayake EH

14.35 h - 15.00 h (Abstract 03):

Update III - Seeking answers to lingering questions on endometriosis using *in vitro* models: A review

Silva N, Tennekoon K, Senanayake H

15.00 - 15.25 h (Abstract 04):

Update IV – DNA Markers in Forensic Analysis

Ranasinghe RACR, Tennekoon KH, Karunanayake EH

Oral Communications

Session I - Plant Molecular Biology & Medicinal Plants

13.20 – 13.35 h (Abstract 05): Morphological and Molecular comparisons of *O. rhizomatis* accessions collected from different locations in Sri Lanka.
Rajkumar G, Weerasena OVDSJ, Fernando KKS, Ratnasiri PW

13.35 – 13.50 h (Abstract 06): Identification of *Macrophoma theicola* Petch in Tea using ITS Region Sequencing
Pradeepa NHL, Weerasena OVDSJ, Liyanarachchi CJ, Wijesundera RLC, Abeysinghe ISB

13.50 – 14.05 h (Abstract 07): Anti-proliferative effects of *Mangifera zeylanica* bark in human hepatoma (HepG2) cells
Samarakoon SR, Thabrew I, Galhena P, Tennekoon KH

13.05 – 14.20 h (Abstract 08): Use of microsatellite markers to identify genetically diverse individuals from old seedling tea (*Camellia sinensis* L.) populations in Sri Lanka
Meran EPK, Mewan KM, Abeysinghe ISB

14.20 – 14.35 h (Abstract 09): Scavenging of free radicals and inhibition of superoxide anion production by leaf extracts of *Ixora coccinea* and *Vitex negundo*
de Silva DSD, de Silva ED, Handunnetti SM

Session II - Molecular Biology & Immunology

15.25 -15.40 h (Abstract 10): Identification of functionally important DNA element in the mouse B7-1 promoter
Vivehananthan K, Sharma M, Sahoo NC, Rao KVS

15.40 -15.55 h (Abstract 11): Quorum sensing regulation of virulence gene expression in *Vibrio harveyi*
Ruwandeepika HAD, Jayaweera TSP, Karunasagar I, Bossier P

15.55 -16.10 h (Abstract 12): Production of antiserum for uncharacterized protein of *Setaria digitata* using New Zealand White rabbits
Rodrigo WWP, Dassanayake RS, Karunanayake EH, Weerasena OVDSJ, Thammitiyagoda M

16.10 -16.25 h (Abstract 13): Localization of the neural profiles of human lymphoid tissues – an immunohistochemical study
Thayabaran M, Yasawardene SG

Poster presentations

Parasite and Molecular Biology

PP 01 (Abstract 14): Physicochemical, functional and structural characterization of Yip1 domain containing Golgi membrane protein from filarial parasite *Setararia digitata* using *in silico* methods
Senathilake KS, Samarakoon SR, Karunanayake EH

Immunology, Inflammation and Infectious Diseases

PP 02 (Abstract 15): Comparison of serological diagnostic methods for leptospirosis
Eugene EJ, Wickramasinghe SA, Kalugalage TL, Rodrigo C, Wickremesinghe H, Dikmadugoda N, Somaratne P, De Silva HJ, Rajapakse S, Handunnetti SM

PP 03 (Abstract 16): Seroconversion following vaccination with tetanus toxoid, to diagnose specific antibody deficiency in patients with recurrent infections
Niloofa MJR, Munasinghe TMJ, Senanayake MP, De Silva NR

PP 04 (Abstract 17): Assessment of oxidative stress in severe leptopsirosis patients
Wickramasinghe SA, Eugene EJ, De Silva HJ, Rajapakse S, Handunnetti SM

PP 05 (Abstract 18): Immunomodulatory activity of combined hot water extract of *Coriandrum sativum* L. and *Coscinium fenestratum* L. in rats
Kothalawala SD, Premakumara GAS, Handunnetti SM, Ratnasooriya WD

PP 06 (Abstract 19): Anti-inflammatory activity of aqueous leaf extract of *Wallidda antidysenterica* L. (*Wrightia antidysenterica*) in rat model
Wahalathanthri YS, Ratnasooriya WD, Handunnetti SM, Premakumara GAS

Growth

PP 07 (Abstract 20): 1737 A/G Polymorphism in *H19* gene in a Sri Lankan cohort of mother-baby pairs: effect of maternal and newborn genotype on birth size

Hewage AS, Jayanthiny P, Tennekoon KH, Karunanayake EH, Kumarasiri JM, Wijesundere APDeS

PP 08 (Abstract 21): Screening for selected mutations and sequence variants in the Growth Hormone 1 gene in children with growth hormone deficiency – A preliminary study

Kariyawasam UGIU, Hewage S, Marikar F, De Silva KSH, Tennekoon KH

PP 09 (Abstract 22): Screening for selected mutations and sequence variations in growth hormone releasing hormone receptor (GHRH-R) in children with growth hormone deficiency: A preliminary study

Fernando MDM, Hewage S, De Silva KSH, Marikar F, Tennekoon KH

Cancer

PP 10 (Abstract 23): A study of *BRCA1* genomic rearrangement in familial breast cancer in Sri Lanka

De Silva S, Tennekoon KH, Karunanayake EH, Amarasinghe I, Angunawala P

PP 11 (Abstract 24): Effect of a decoction of *Fluggea leucopyrus* on the expression of Caspase 3 and Caspase 9 in endometrial carcinoma cells

Kotigala SB, Gammana-Liyanage I, Samarakoon SR, Thabrew I, Tennekoon KH

PP 12 (Abstract 25): Effect of a herbal extract on *Bcl-2* and *Bax* expression in endometrial carcinoma cells

Gammana-Liyanage IMP, Samarakoon SR, Thabrew I

Plant Molecular Biology/Molecular Biology

PP 13 (Abstract 26): Differentiation of selected *Piper nigrum* L. (black pepper) cultivars in Sri Lanka using morphological and AFLP markers
Silva SIC , Weerasena OVDSJ , Senavirathne JM

PP 14 (Abstract 27): Genetic differentiation and identification of varietal specific AFLP markers for some *Artocarpus heterophyllus* Lam. (Jackfruit) varieties in Sri Lanka
Thanthrige MRL, Weerasena OVDSJ, Pushpakumara DKNG, Senavirathne KGS

PP 15 (Abstract 28): Variation in Sri Lankan aromatic rice: progress towards identification of a new haplotype
Peiris WAM, Kottearachchi NS, Weerasena OVDSJ

PP 16 (Abstract 29): A new cost effective method for extracting genomic DNA from fungi
Kariyawasam GK, Mithrasena YJPK, Fernando THPS, Wijesundera RLC, Wijesundera WSS

Institute Lecture

Discovering Natural Leads for Cancer Therapy

Ira Thabrew

Emeritus Professor, University of Kelaniya / Visiting Professor,

IBMBB, University of Colombo

Cancer is one of the major causes of mortality in the world. While modern surgery has significantly reduced cancer mortality, the use of additional treatments such as chemotherapy, hormonotherapy and radiotherapy have not resulted in the expected reduction in the number of deaths; they also produce some very unpleasant side effects in patients. Therefore, during the past fifty years, there has been a continuing search for alternative therapies that would not only reduce cancer mortality more effectively, but would also produce less unpleasant side effects. Since natural products have throughout history, been found to be a rich source of compounds that have found many applications in the field of medicine, the main focus of investigations for the discovery of effective alternative anticancer therapies have been natural compounds isolated from plants and other living organisms. Investigations during the past fifty years have led to the identification of a multitude of lead compounds that can be developed for cancer therapy, from a variety of plants, microorganisms, marine organisms and terrestrial arthropods. Many drugs developed from such natural leads are in routine clinical use today for treatment of cancer patients. In fact, a review of the cancer drug development process during the past half century clearly shows that drugs derived from natural resources represent a significant segment of the pharmaceutical market (>60%) as compared to randomly synthesized compounds. With unraveling of the human genome in the early part of this decade, and a better understanding of genes involved in the process of carcinogenesis, a number of new assays and techniques have been developed in different laboratories not only for the purpose of rapid identification of novel compounds with anticancer activity, but also for developing molecularly targeted anticancer drugs. Investigations carried out by the author using some of the above methods to discover the anti-cancer potential of natural agents will also be presented.

Update I - (Abstract 01)

Cancer stem cells: implication for the treatment of cancer

Samarakoon SR, Thabrew I, Tennekoon KH
Institute of Biochemistry, Molecular Biology and Biotechnology, University of Colombo

Cancer stem cells (CSC) are rare cell population in all cancers, which behave like normal stem cells. This cell population can differentiate into a variety of cell types corresponding to the tissue of origin of the cancer, supporting tumor growth and cell heterogeneity typical of many solid tumors, while maintaining the ability to self-renew. Although the concept of CSC is not new and was proposed many years ago, there is currently much controversy and debate about the origin of CSC. According to one hypothesis certain types of CSC originate from normal stem cells or progenitor cells, or even bone marrow-derived cells. Cancer stem cells are intrinsically resistant to chemotherapeutic agents and radiation therapy, there is at present significant interest in CSC biology and development of new drugs targeting CSC. In this review, the concept and origin of CSC, similarity between somatic stem cells and CSC, markers of CSC used for their identification and isolation, and chemotherapy and radiotherapy resistance of CSC are discussed. Compound library screen using CSC model and identification of CSC active agents that inhibit molecular pathways such as signaling pathways, epigenetic pathways and miRNAs preferentially expressed or active in CSC are also discussed. In addition, immune-based strategies such as monoclonal antibodies targeting CSC specific markers, or vaccination of host with cancer CSC or their components are also reviewed. Finally, based on the current knowledge on CSC, we discuss potential approaches for developing new drugs that may selectively target CSC and propose new methods for evaluating potential drugs that can acts against CSC.

SRS is a PhD student partially supported by National Science Foundation.

Update II - (Abstract 02)

Breast cancer genetics: BRCA status and effect on breast cancer

De Silva WS, Tennekoon KH, Karunanayake EH
Institute of Biochemistry Molecular Biology and Biotechnology, University of Colombo.

Breast Cancer is a complex, multifactorial disease which is the commonest malignancy among women and the primary cause of death in women worldwide. Germline mutations in two human tumor suppressor genes *BRCA1* or *BRCA2* confer the susceptibility to breast and ovarian cancers. These may cause hereditary forms of breast cancer which accounts for about 5-10% of all breast cancer cases. It has been suggested that breast cancer prognosis may differ with the degree of germline mutations in *BRCA1/2* genes. For example women with breast cancer and *BRCA2* mutations have worse prognosis than women diagnosed with breast cancer at the same age, who do not carry a *BRCA2* mutation. Likewise the BRCA status of the breast cancer can have an impact on the degree of prognosis, age of onset, receptor status of the breast cancer thereby leading to differences in administration of therapy, survival rate of the patient etc. A strong association between *BRCA1* mutation carriers status and estrogen receptor negative cancer is reported. Most of the *BRCA1* mutations carriers develop triple-negative breast cancer, especially before the age of 50 years, considered as early onset of the disease. These triple negative subtype of breast cancer have a poor survival rate and a high recurrence rate. Knowledge of the carrier status at diagnosis might influence treatment decisions, leading to different outcomes. Thus BRCA testing for selected patients such as patients with early onset of the disease and/or with strong family history will facilitate management protocols.

WSDeS is a PhD student Supported by Sida/Secretariat for Research Cooperation (formerly SAREC) Grant for Molecular Biology and Biotechnology/

Update III - (Abstract 03)

Seeking answers to lingering questions on endometriosis using *in vitro* models: A review

Silva N[1,2], Tennekoon K[2], Senanayake H[3]
[1]Faculty of Medical Sciences University of Sri Jayewardenepura, [2]Institute of Biochemistry, Molecular Biology and Biotechnology, University of Colombo, [3]Faculty of Medicine, University of Colombo

The exact aetiology of endometriosis still eludes the clinicians and scientists despite extensive research. In 1927, Sampson proposed that endometriosis results from implantation of endometrial tissue which gains access to the peritoneal cavity by retrograde flow during menstruation. Although Sampson's theory is widely accepted as a plausible explanation, lingering questions still remain unanswered. The objective of this review is to describe the use of *in vitro* models to gain insight into the distinct molecular, biochemical and cellular mechanisms of endometriosis in order to address some pertinent issues related to Sampson's theory. Monolayer cell culture models, attachment models such as chicken chorioallantotic membrane and invasion chambers are among the *in vitro* tools used by scientists. Experiments using these *in vitro* models have provided valuable clues on the mechanisms used by endometrial stromal and epithelial cells to survive in the peritoneal cavity to get attached to pelvic and intra-abdominal surfaces, subsequently invade the extracellular matrix of the mesothelium to proliferate and become vascularized, ultimately forming the ectopic deposits. Furthermore, monolayer cell culture models have been instrumental in defining the molecular pathways of common medication used in the management of endometriosis as well as devising new treatment options. In addition, cell monolayers have also been used to test the effects of endocrine disruptor chemicals such as dioxins and cadmium. Reliable *in vitro* models have significantly contributed towards the current concepts on the pathogenesis of endometriosis and may prove useful to evaluate current as well as novel therapies for the disease which may lead to therapeutic advances.

NS is a PhD student partially supported by University Grants Commission and National Coordinating Committee on Reproductive Health Research.

Update IV - (Abstract 04)

DNA Markers in Forensic Analysis

Ranasinghe RACR, Tennekoon KH , Karunanayake EH
Institute of Biochemistry, Molecular Biology and Biotechnology, University of Colombo

Genetic profiling or DNA fingerprinting is commonly performed by forensic scientists using multiple DNA markers to support individual identification by their respective DNA profiles. Unlike other physical information used in species identifications, DNA sequencing profile is unique to an individual. Thirteen specific DNA regions of the genetic makeup that vary from person to person can be analyzed for DNA- based individual identification. This encrypted set of data reflects persons DNA make up and it is referred to as genetic profiling. After the initial development of DNA fingerprinting based on the highly polymorphic Variable Number of Tandem Repeats (VNTRs) by Alec Jeffreys, DNA typing methodology based on highly polymorphic DNA markers revolutionized the human identity testing in the field of forensic science remarkably. Progress and development of DNA identification applied to criminal forensics provide impressive demonstrations of the amazing potential of the method, not only to offender the guilty but also to defend the innocent. Also these applications are widely used in paternity and other relationship testing, evolutionary studies, identification of individuals in mass disasters, historical investigation and studying fossil skeletal remains. With the development of Polymerase Chain Reaction (PCR) - based assays for DNA typing procedures, using VNTRs was replaced. Assays for other types of repetitive markers such as Short Tandem Repeats (STRs), Single Nucleotides Polymorphic based markers (SNPs), X and Y chromosomal polymorphisms, Mitochondrial DNA analysis and Low Copy Number (LCN) testing contributed greatly for enhance the accuracy of DNA markers in Forensic applications.

RACRR is a PhD student supported by National Research Council Grant (09-20) and Sida/Secretariat for research Cooperation (formerly SAREC) Grant for Molecular Biology and Biotechnology.

Abstract No: 05

Morphological and Molecular comparisons of *O. rhizomatis* accessions collected from different locations in Sri Lanka

Rajkumar G[1], Weerasena OVDSJ[1], Fernando KKS[2], Ratnasiri PW[3]

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Oryza rhizomatis Vaughan (family: Poaceae) is an endemic wild rice species in Sri Lanka. Several morphological differences have been observed in *O. rhizomatis* plants growing under different agro-ecological conditions of the island. Therefore, they may have genetic differences as a result of continued adaptation to climatic conditions to consider them as 'ecotypes'. Morphological and molecular comparisons of *O. rhizomatis* accessions collected from different locations were carried out by using forty five Morphological Descriptors (qualitative and quantitative) and Amplified Fragment Length Polymorphism (AFLP) analysis of DNA followed by cluster analysis. Mean values for different morphological traits and standard errors were calculated from mean values of each accession. Scatter diagram of PCoA of *O. rhizomatis* accessions based on morphological data, and the standard errors showed that there were no significant differences among the accessions. According to molecular analysis by AFLP all the accessions were genetically similar. Jaccard genetic similarity coefficients varied with a very narrow range (0.915 to 1.000) between the accessions. According to the results, the *O. rhizomatis* accessions tested did not show significant morphological and genetic differences. In this study representative samples from different locations were grown in the green house**,** exposing them to the same environmental conditions. This may have contributed to results obtained with no observable morphological differences. Further studies have to be carried out with *in situ* collections from different locations when extreme weather conditions are prevailing to see whether the morphological characters are changed as an adaptation.

This work was supported by National Research Council of Sri Lanka (Grant No: 05-61) and constitutes part of the PhD studies of GR.

Abstract No: 06

Identification of *Macrophoma theicola* Petch in Tea using ITS Region Sequencing

Pradeepa NHL[1,2], Weerasena OVDSJ[1], Liyanarachchi CJ[1], Wijesundera RLC[3], Abeysinghe ISB[2]

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Stem canker in tea causes heavy crop losses in low country tea gardens of Sri Lanka. In the past this disease has been attributed to *Macrophoma theicola* Petch (family: Botryosphaeriaceae) and tea is the only known host for this fungus. *Macrophoma* is an anamorphic genus and its teleomorph belongs to the genus *Botryosphaeria*. The present study was carried out to delimit the causal agent (*M. theicola*) and to distinguish it from other morphologically similar species. *M. theicola* was isolated from symptomatic material collected from five low country tea growing districts. Isolates were grouped into four morpho types based on morphology and culture characteristics. Two *M. theicola* isolates were obtained from Tea Research Institute of Vietnam for the morphological comparisons. Twelve *M. theicola* isolates were selected from each morpho type to confirm their pathogenecity. Genomic DNA was extracted from the isolates and amplification of Internal Transcribed Spacer region (ITS) of rDNA was carried out using fungal specific ITS1F and ITS4R primers. Amplified products were sequenced and sequences were searched over the GenBank database using BLASTn search tool. ITS sequences of 07 isolates (including the Vietnam isolates) showed highest similarity to that of *Botryosphaeria mamane.* Three isolates were highly similar to *Botryosphaeria dothidia.* Remaining 02 isolates showed highest similarity to *Lassiodiplodia theobroma*. *Lassiodiplodia theobroma* has been reported in tea previously as a canker causing fungus and it is considered as the anamorphic stage of *Botryosphaeria dothidia*. Therefore, it may be concluded that stem canker in tea is caused by anamorphs of *Botryosphaeria* spp.

This work was supported by Tea Research Institute of Sri Lanka and constitutes part of the PhD studies of NHLP.

Abstract No: 07

Anti-proliferative effects of *Mangifera zeylanica* bark in human hepatoma (HepG2) cells

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Hepatocellular carcinoma is the most frequent primary liver cancer and the fifth commonest neoplasm in the world. A number of compounds derived from medicinal plants have been developed into anti cancer drugs. *Mangifera zeylanica* (family: Anacardiaceae), commonly known as "Etamba", is a slow growing, large medicinal plant endemic to Sri Lanka, distributed in intermediate and wet zones. Although many medicinal properties of other *Mangifera* species have been reported, such effects of *M. zeylanica* have not been studied. The present study has investigated whether an extract of *M. zeylanica* can exert anti-proliferative effects in human hepatoma (HepG2) cells, which if proven can lead to development of anti cancer therapy. Hot water (decoction) and methanolic extracts of *M. zeylanica* bark were prepared, and different concentrations of these extracts incubated with HepG2 cells (2.5×10^4 cells/ml) and cultured in DMEM medium. Anti-proliferative effects of the extracts were evaluated at the end of 24, 48 and 72 h incubations, by (a) observations of cell morphology and (b) overall cell activity by 3-(4, 5-dimethyl-2-thiazolyl) -2, 5-biphenyl tetrazolium bromide (MTT) assay. Results demonstrated that the methanolic extract, but not the decoction, could exert a significant ($p< 0.001$) dose and time dependent anti-proliferative effect on HepG2 cells. IC_{50} valves were 128.4 µg/ml, 78.5 µg/ml, 53.7 µg/ml respectively for 24, 48, 72h incubations. Further investigation of the methanolic extract may result in identification of novel anti carcinogenic components from *M. zeylanica* bark.

This work was supported by the IBMBB, University of Colombo.

Abstract No: 08

Use of microsatellite markers to identify genetically diverse individuals from old seedling tea (*Camellia sinensis* L.) populations in Sri Lanka

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This study was primarily aimed to identify genetically diverse individuals from old seedling tea population (OSD) in Sri Lanka. Twenty-four OSD accessions from four tea estates (Concordia (CON), Court-Lodge (CL), Kirkoswald (KL) and Oliphant (OLI) and were assessed with 10 genomic and EST- SSR primers. To compare the results, three TRI recommended tea cultivars i.e. TRI777 ("Cambod" "China" mixed origin), TRI2024 (ASM4/10 OP) and TRI4052 (ASM4/10 x CY9) were also included. SSR loci were separated on 6% polyacrylamide gels, and were visualized by silver staining. Bands were scored and Nei and Li's genetic distance matrix was generated. Highest diversity within a population (0.14) was observed in CL while highest diversity between populations (0.15) shown between CL and CON. OSDs, KL50, KL1, OLI25 and CON35 displayed the highest diversity from improved cultivars (0.38, 0.29, 0.29 & 0.29 respectively) whereas accession KL50 showed the highest average diversity (0.24) against all accessions. The dendrogram constructed using diversity values, has two major groups (A and B). Cultivars TRI2024 and TRI4052 were grouped in the main cluster A, proving their origins (ASM4/10- 'Cambod' hybrid). Clustering pattern of cultivar TRI777 in group A clearly proves its ancestry, which shares both 'China' and 'Cambod' characters. Group B mainly consisted of OSDs selected from four estates, assuming their close genetic relationships, which is apart from TRI cultivars, but a closer affinity to TRI777. This finding showed OSDs share a close affinity to 'China' and 'Cambod' types.

This work was supported by IBMBB, University of Colombo and Tea Research Institute, Talawakelle and constitutes part of the MSc studies of EPKM.

Abstract No: 09

Scavenging of free radicals and inhibition of superoxide anion production by leaf extracts of *Ixora coccinea* and *Vitex negundo*

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Neutrophils migrate to sites of injury and release reactive oxygen species (ROS) which kill pathogens during inflammation. Excessive neutrophil activation leads to inflammatory disorders. *Ixora coccinea* L. (family: Rubiaceae) and *Vitex negundo* L. (family: Lamiaceae) have been scientifically proven to have anti-inflammatory effects *in vivo.* The objective of the study was to quantify the antioxidant effects of various leaf extracts (aqueous – ALE; methanol – MLE; ethyl acetate – ELE; chloroform – CLE; hexane – HLE) of *I. coccinea* and *V. negundo* by determining their capacity to (a) scavenge free radicals (2, 2-Diphenyl-1-picrylhydrazyl assay) and to (b) inhibit ROS (i.e. superoxide anion) production by human neutrophils (quantitative nitro blue tetrazolium assay). In DPPH scavenging investigations, the potency of *I. coccinea* extracts (EC_{50} µg/ml) were: ALE (7) > MLE (65) > CLE (127) > ELE (252) > HLE (329). Potencies of *V. negundo* extracts were: MLE (66) > ALE (127) > ELE (247) while that of the positive control vitamin C was 6. No significant activity was demonstrated for CLE and HLE of *V. negundo.* *I. coccinea* at 500 µg/ml inhibited ROS production with the following potencies: MLE (56%) > ALE (51%) > CLE (48%) > ELE (46%) > HLE (32%). Potencies of *V. negundo* extracts were: ALE (55%) > MLE (52%) > CLE (44%) > ELE (41%) > HLE (22%). The positive control Diphenyleneiodonium chloride demonstrated a potency of 70%. All activities for both plants were dose-dependent ($r=0.737 – 0.970$; $p <0.001$). In conclusion, free radical scavenging activity and inhibition of ROS production may contribute significantly to the underlying anti-inflammatory mechanisms of *I. coccinea* and *V. negundo*. The polar extracts (ALE and MLE) are more active than the non polar extracts (ELE, CLE and HLE).

This work was supported by National Research Council - Grant No 05-52 and constitutes part of the MSc studies of DSDdeS.

Abstract No: 10

Identification of functionally important DNA element in the mouse B7-1 promoter

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The B7-1 (CD80) is the most potent co-stimulatory molecule expressed on the surface of B cells upon antigen stimulation. Co-stimulation is required for the productive T cell activation. The expression of B7-1 is tightly regulated. Regulation of gene expression occurs primarily at the transcriptional level. Transcriptional activation involves the interplay of many transcriptional factors and a combinatorial array of cis-regulatory DNA elements in eukaryotes. Understanding the molecular mechanisms controlling the CD80 gene expression upon immune response is quite interesting and high-throughput strategies are absolutely required to identify the important control regions of the gene and characterizing the relevant protein-DNA interactions within control region. *The in vivo* DNase I-mediated foot printing approach was employed to detect the protein–DNA interactions that activate the CD80 gene expression. DNase I foot printing analysis of J558L cells resulted only single protected element which was centered around 70 nt in the upstream proximal CD80 region providing information about the DNA sequences that are inaccessible to digestion by the nuclease (DNase 1) confirming the protein bound to this DNA sequences. EMSA analysis was performed to determine binding properties of the protein for the core promoter element which resulted super shift due to the formation of a protein-DNA complex suggesting that a nuclear factor binds to this defined region. In conclusions, the location of functionally important DNA element in CD80 promoter was detected. Further, nuclear factor binding to this DNA element was identified as NF-1. The regulatory role of NF-1 was determined by analyzing the data together with the NF-kB recruitment and suggested as NF-1 functions as a repressor in gene activation program and its dissociation is necessary for the transcription activation.

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Abstract No: 11

Quorum sensing regulation of virulence gene expression in *Vibrio harveyi*

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Vibrio harveyi causes luminescent vibriosis, leading to severe losses in the aquaculture industry. Several virulence factors have been associated with pathogenicity. Quorum sensing, the bacterial cell-to cell communication regulate the expression of certain genes. Three quorum-sensing signal molecules have been identified in gene regulation (Harveyi Autoinducer 1(HAI1), Autoinducer 2 (AI2) and Cholerae Autoinducer 1 (CAI1)). This study investigated quorum sensing regulation of virulence factors and virulence regulators by determining the expression levels of these genes in wild type *V. harveyi* and different quorum sensing mutants *in vitro*. The expression of haemolysin (*vhh*), metalloprotease (*vhp*), serine protease (*srp*), the quorum sensing master regulator LuxR and the virulence regulator ToxR was measured with reverse transcriptase real-time PCR with specific primers. *V. harveyi luxO* mutants JAF483 (QS+) and JAF548 (QS-), which mimic the high and low cell density conformation respectively were used, to check the quorum sensing regulation. HAI-1, AI-2 mutants and wild type isolate also were used. The quorum sensing master regulator *luxR* and the *vhp* showed around 3-fold and 5-fold higher expression levels in a *luxO* mutant with maximum quorum sensing activity than in a *luxO* mutant with minimum quorum sensing activity. There was no difference in expression of *vhh* between the two mutants. There was however more than 2.5-fold lower expression in an AI-2-negative mutant, suggesting that this gene is specifically regulated by AI-2 quorum sensing through a yet unknown signal transduction cascade. There was no difference between the strains in expression levels of the virulence regulator gene *toxR* and the *srp*, providing that these genes are independent of quorum sensing.

This study was supported by the Flemish Interuniversity Council–University Development Cooperation (VLIR), Belgium.

Abstract No: 12

Production of antiserum for uncharacterized protein of *Setaria digitata* using New Zealand White rabbits

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Setaria digitata is a filarial parasite living in the peritoneal cavity of cattle. Preliminary bioinformatic analyses of cDNA clones from *S. digitata* revealed a parasitic nematode-specific growth factor like protein, named as uncharacterized protein of *S. digitata* (*SDUP*). The objective of this study was to produce anti-*SDUP* antibodies to determine tissue localization of SUDP by histochemical staining. Open Reading Frame of *SDUP* was cloned to a bacterial expression vector pET45b(+) to achieve inframe fusion of ORF with vector derived N-terminus 6XHis and the construct was transformed into *Escherichia coli* BL21(DE3). The recombinant *SDUP* was expressed and purified using Ni-NTA column under denaturing conditions. Purity and the molecular weight of *SDUP* were determined by SDS-PAGE and western blot analysis. Purified *SDUP* was concentrated using Amicon® Ultra-15 centrifugal filter devices and concentration was determined by Bradford method. Before immunization, 2.0 ml of blood was taken from test and control rabbits to detect the baseline antibody levels. Purified protein (100 μg) was suspended in 1.0 ml of PBS and inoculated intravenously to ear vein whilst control rabbit was given 1.0 ml of saline. One week after, 1:1 of mixture of Fruends incomplete adjuvant (FIA) and recombinant protein in PBS was injected to intramuscularly. Control rabbit was injected with the mixture of saline and FIA. After second week, booster injection was given. Protein concentration was increased in subsequent boosters (up to 500 μg) while keeping constant volume (500 μl). After third booster, antibody titers were detected by dot-ELISA assay. Significant antibody titers were observed after 4th booster and 25ml of blood was drawn from each animal. Serum was separated and stored in -80^{o}C until used for immunohistochemical staining.

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Abstract No: 13

Localization of the neural profiles of human lymphoid tissues – an immunohistochemical study

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Immune system is documented to be regulated by autonomic innervation. The neuronal cell bodies, axons, and peripheral nerves are exclusively stained by immunohisto-chemistry using neuron specific enolase (NSE), which is an isozyme of 2-phopho-D-glycerate hydro-lyase. The distribution of peripheral nerves in immune tissues was studied using NSE as a neuronal marker. Fresh human (n=5) postmortem samples of spleen, liver, lymph nodes and terminal ileum were processed and indirect immunohistochemistry was performed on 4µm tissue sections. Heat-induced antigen retrieval was done (Citrate buffer, pH=6) and slides were incubated in 3% H_2O_2 and were blocked with 5% BSA. Rabbit anti-NSE antibodies and biotinylated anti-rabbit IgG were used as primary and secondary antibodies (1:750) respectively (Sigma-Aldrich) to label tissue sections (5-10 sections/each sample). The labelled streptavidin biotin technique was used; with diaminobenzedene to detect the immunoreactivity (IR) by developing brown colour and counterstained with Mayers' hematoxylin. Cerebral cortex was processed as positive control. Qualitative computerized image analysis was performed on slides labeled from each paraffin block and the photomicrographs were taken at different magnifications. The IR was determined under x400 magnification, based upon a score of 0, 1+ (focal staining), 2+ (focal to diffuse staining) and 3+ (diffuse staining). In spleen the IR of NSE was mainly in capsule and perivascular supporting sheaths, with less IR in red pulp and absent IR in white pulp. The liver showed 2+IR in the portal triad, while in lymph nodes the capsule, subcapsular sinuses and the medullary cords showed 2+IR. The marginal zones of Peyers' patches expressed 1+diffuse IR. This study confirms the innervation of human immune tissues by autonomic nerves and their distribution through the supporting framework of reticulin fibres to the parenchyma.

Abstract No: 14

Physicochemical, functional and structural characterization of Yip1 domain containing Golgi membrane protein from filarial parasite *Setararia digitata* using *in silico* methods

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Human lymphatic filariasis (LF) that affects over 120 million people worldwide is a debilitating infectious disease mainly caused by filarial parasite *Wuchereria bancrofti*. Present study aimed to characterize yet uncharacterized proteins of filarial parasites using cattle parasite *Setaria digitata* as a model organism. A cDNA library of *S.digitata* was constructed in lambda-Zap vector. *In vivo* excised cDNA clones were subjected to DNA sequencing. I*n- silico* characterization of the cDNA sequences was carried out. Bioinformatics analysis of sequences revealed a complete coding sequence (CDS) of the nematode homolog of essential yeast Golgi membrane protein (Yip1p), that involved in membrane fusion during endoplasmic reticulum to Golgi membrane traffic and COP11 vesicle biogenesis. Deduced protein (Predicted Mw = 28264 Da, theoretical pI- 5.44) had a highly conserved trans-membrane domain of five helixes in C-terminal region. Cytosolic N-terminal region was predicted to have strong protein-protein interactions. Three highly conserved motifs were identified. Structurally and functionally important residues of each motif were predicted. Tertiary structures of potentially important regions were attempted to model by homology modeling. Three dimensional structures of any of the Yip1domain protein are not yet resolved and no reliable template structure is available for comparative modeling of full length Yip1p. This *in-silico* characterization will be useful in further wet lab characterization of the protein and for the bigger goal of predicting accurate 3D structure, as a step-by-step approach to first predict intermediate structural attributes. Since the protein is highly conserved results of this study would be fairly adapted to homologs from other organisms.

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Abstract No: 15

Comparison of serological diagnostic methods for leptospirosis

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In Sri Lanka, leptospirosis patients are often treated based on clinical diagnosis. Serological confirmation is not usually obtainable during the acute stage of the disease. There is a need for rapid immunodiagnostics for confirmation of leptospirosis. Two immunodiagnostic assays, ie enzyme linked immnnosorbent assay (ELISA) and an immunochromatographic technique- Lepto*c*heck-WB test (LCT) are used to detect leptospira specific IgM antibodies which are prevalent in early stages of acute infections. In the present study, the efficacy of these two rapid immunodiagnostics assays was compared with microscopic agglutination assay (MAT) to determine their usefulness. A set of sera (n=83) collected in 2010 for which MAT titers were available was used to perform IgM ELISA and LCT. Percentage positivity for LCT and IgM ELISA were 55.4% and 48.2% respectively and both assays detected acute infection by day 3 of illness. MAT ≥400 was used as the reference standard. For LCT, the overall sensitivity, specificity, accuracy, PPV and NPV (86.5%, 75.0%, 79.6%, 69.6% and 89.4% respectively) were higher compared to the respective values for IgM ELISA (50.0%, 62.3%, 57.1%, 50.0%, 62.3%). The highest of these values were observed during the first week for LCT and during the second week for IgM ELISA. The highest agreement was observed between LCT and MAT≥400 (κ=0.568) and there was a good agreement between LCT and IgM ELISA (κ=0.520). The high sensitivity and specificity, ease of use, and no requirement of specialized skills and equipment, makes LCT a good choice for screening, while IgM ELISA is an appropriate test for confirming acute Leptopsirosis.

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

Abstract No: 16

Seroconversion following vaccination with tetanus toxoid, to diagnose specific antibody deficiency in patients with recurrent infections

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Specific antibody deficiency (SAD) is a type of primary immune deficiency where B cells do not produce antibodies against certain antigens. The causes of SAD are still not clear. SAD clinically presents as recurrent infections. The objective of this study was to determine whether assessment of antibody production against a protein antigen (tetanus toxoid) could be used to diagnose SAD against protein antigens. Pediatric patients (age 0.5-15 years) with recurrent infections (n=35) with a majority (94.3%; 33/35) having normal or near normal immunoglobulin levels and two patients identified as immunoglobulin deficient were recruited for this study; their total immunoglobulin levels were measured using radial immune diffusion method. Seven positive (immunized) and two negative (non-immunized) controls were also recruited. Anti-tetanus antibodies were measured using an enzyme linked immunosorbent assay. Eleven out of 35 patients and the negative control sera did not have detectable levels of anti-tetanus antibodies indicating that 31.4% of patients had not seroconverted. Sera from 24 patients had 6.34 ± 2.67 IU/ml anti-tetanus antibodies which were comparable to that of the positive control sera 7.76 ± 1.97 IU/ml (mean ± SD) ($P>0.05$). There was no correlation found between the number of Diptheria-pertusis and tetanus vaccine doses and anti-tetanus antibody levels in both positive control and seroconverted group ($r=0.302$ and 0.111 respectively; $P>0.5$). Excluding the two immunoglobulin deficient patients, 27.3% (9/35) of patients with recurrent infections had SAD against protein antigen. Based on these results, this study also suggests seroconversion against protein antigens could be used to diagnose SAD.

This work was supported by IBMBB and Medical research Institute, Colombo 08 and constitutes part of the MSc studies of MJRN.

Abstract No: 17

Assessment of oxidative stress in severe leptopsirosis patients

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Release of reactive oxygen and reactive nitrogen species (ROS and RNS) contribute to increased oxidative stress and to tissue damage which is thought to lead to multi-organ failure in Leptospirosis. The antioxidant capacity of serum provides a measure of overall protection against oxidative damage. Level of oxidative stress caused by ROS and RNS (ie., nitric oxide ($NO^{\bullet}$) was assessed in leptospirosis patients in the present study. Of 81 clinically suspected leptospirosis patients recruited, 40 were confirmed by Leptocheck WB test (LCT). They were clinically categorized as severe (n=24) and mild (n=16) cases. LCT negative patients were considered as non-leptospirosis fever (NLF) controls (n=41) and a healthy control group (n=20) was also included. $NO^{\bullet}$ was measured by determining serum NO_2^- and NO_x ($NO_2^-+NO_3^-$) levels using direct Griess and modified Griess assays respectively. To assess the damage caused by ROS, serum anti oxidant capacity (AOC) was measured using ABTS decolorization assay and results were expressed as Trolox equivalent (TE) μM/mg proteins. Severe leptospirosis patients had significantly high serum NO_2^-levels compared to NLF and healthy controls (1.8±0.11μM, 1.2±0.08μM and 1.1±0.08μM respectively; $P<0.001$). Serum NO_x levels (18.5±1.50μM) of severe patients were significantly higher compared to mild leptospirosis patients, NLF and healthy control groups (14.3±0.94μM, 12.3±0.89μM and 5.5±0.27μM; $P<0.05$ respectively. Both severe and mild leptospriosis patients had comparable AOC levels (1.05±0.04 and 1.03±0.09 μM/mg protein respectively) but the levels were significantly higher compared to NLF and healthy control groups (0.97±0.04 and 0.92±0.03 μM/mg protein; $P<0.05$ respectively). Thus, serum NO_x could be used as a prognostic indicator of severity of leptospriosis.

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

Abstract No: 18

Immunomodulatory activity of combined hot water extract of *Coriandrum sativum* L. and *Coscinium fenestratum* L. in rats

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The combination of *Coriandrum sativum* Linn. (family: Umbelleferae) and *Coscinium fenestratum* Gaertn. (family: Menispermaceae) has been used for centuries by Sri Lankan traditional medicinal practitioners to treat inflammatory conditions. The aim of this study was to investigate the immunomodulatory effect of combined hot water extract (HWE) of *C. sativum* and *C. fenestratum* in rats by testing *in vivo* for i)anti-inflammatory activity in a carrageenan-induced rat paw edema model, ii)rat peritoneal cell infiltration and production of nitric oxide (NO) and iii)production of specific antibodies, and *in vitro* for anti-oxidant activity. The three doses of freeze-dried HWE used (human equivalent dose (HED-183 mg/kg), 2xHED-366 mg/kg and 1/2 HED-92 mg/kg; n=6 rats/group) showed an inverse dose-dependency at 4^{th} and 5^{th} ($r=-0.99$ and -1.00 respectively; $P<0.05$) h and HED which exhibited a marked anti-inflammatory action (72% inhibition) was selected as optimum dose to assess its effect on cell migration in peritoneal cell infiltration assay. HED mediated an enhanced cell infiltration (32% increase; $P<0.05$) and inhibited NO produc-tion by 94.5%. HED resulted in a significant increase in production of specific antibodies against sheep red cell antigens compared to control as detected by hemagg-lutination assay ($P<0.05$). HWE demonstrated a low anti-oxidant capacity (IC_{50}=290 µg/ml), but the DPPH scavenging activity was dose-dependent ($r=0.908$; $P=0.01$). In conclusion, the HWE demonstrated immunomodulatory activities which were immuno-suppressive (anti-inflammatory and reducing NO production) and immunostimulatory (enhanced cell migration and production of antibodies). This study scientifically validates the therapeutic use of combined *C. sativum* and *C. fenestratum* extract in Sri Lankan traditional medicinal system for treatment of inflammatory conditions.

This work was supported by the IBMBB and *Department of Zoology, Faculty of Science, University of Colombo and Industrial Technology Institute and constitutes part of the MSc studies of SDK*

Abstract No: 19

Anti-inflammatory activity of aqueous leaf extract of *Wallidda antidysenterica* L. (*Wrightia antidysenterica*) in rat model

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Wallidda antidysenterica (family: *Apocynaceae*), an endemic plant of Sri Lanka is used as a treatment for inflammatory conditions in traditional medicine. In order to scientifically validate the anti-inflammatory activity of *W. antidysenterica* following were carried out using an aqueous leaf extract (ALE). (a) the anti-inflammatory activity using a carageenan-induced rat paw edema model, (b) inhibition of infiltration of rat peritoneal cells and their production of nitric oxide, (c) *in vitro* anti-oxidant activity and (d) toxicity of ALE in rats. To investigate the anti-inflammatory activity, three different doses of ALE: low dose (LD) (182.5mg/Kg), human equivalent dose (HED) (365mg/Kg) and a high dose (HD) (730mg/Kg) were used. A 4^{th} group was treated with the reference drug indomethacin (5mg/Kg) and a 5^{th} group (controls) was treated with water (n=6/group). In the carageenan-induced rat paw-edema model, HD of ALE produced the highest activity (85.03% ±0.02) at the 5^{th} hour. This activity was similar to that of indomethacin. HD of ALE was thus selected to elucidate the anti-inflammatory mechanisms. HD of ALE did not significantly decrease the rat peritoneal cell infiltration compared to control and prednisolone (reference drug) groups. How-ever, a significant decrease in production of NO by HD of ALE which was comparable to that of the prednisolone group was observed ($P<0.05$). *In vitro* anti-oxidant activity carried out using the DPPH method showed an IC_{50} value of 180µg/ml and 3.2µg/ml for ALE and vitamin C respectively. Fourteen day oral treatment of rats with HD of ALE did not produce any overt signs of toxicity, haemato-, reno-, hepato- or neurotoxicity. In conclusion, this study demonstrates for the first time that the HD of ALE of *W. antidysenterica* is a relatively safe, orally active agent with a moderately potent anti-inflammatory activity and scientifically validates its use in traditional medicine.

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Abstract No: 20

1737 A/G Polymorphism in *H19* gene in a Sri Lankan cohort of mother-baby pairs: effect of maternal and newborn genotype on birth size

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Common genetic variations of imprinted genes that are maternally expressed could elucidate the maternal-specific inheritance of low birth weight. Human *H19* is an imprinted growth regulatory gene located at chromosome 11p15.5 exclusively expressed from the maternal allele coding for a regulatory RNA implicated in the regulation of insulin-like growth factor II. We have previously described *H19* 1737A/G genotype frequencies in 24 healthy mother-baby pairs. PCR based RFLP method was used for genotyping. Here we report data for 167 mother-baby pairs including the previous subjects. Distribution of genotypes AA, AG, GG was 5%, 42%, 52% for mothers and 8%, 42%, 50% for newborns. The allele frequencies of A and G were 0.275and 0.725 for mothers, 0.125 and 0.875 for newborns. The commonest allele and genotype for the *H19* 1737 A/G polymorphism were G and GG respectively for Sri Lankan population. Birth weight, crown heel length and head circumferences were not affected by maternal or newborn genotype (One way ANOVA $p > 0.05$). Thus *H19* 1737 A/G polymorphism is unlikely to affect birth weight in Sri Lankans.

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Abstract No: 21

Screening for selected mutations and sequence variants in the Growth Hormone 1 gene in children with growth hormone deficiency – A preliminary study

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Growth hormone 1 gene (*GH1*) is one of the hot spots for many types of mutations causing growth hormone deficiency. A group of children (N=10) with clinical and biochemical growth hormone deficiency were screened for two mutations reported from Asian populations, namely *GH1* gene deletion and mutation in the intron 3 region. DNA was extracted from peripheral blood samples and target regions were amplified by PCR. Restriction digestion with the *Sma1* enzyme was carried out to detect GH deletion. G>A transition in the 1st nucleotide of the invariant GT dinucleotide of the 5' donor site of intron 3 region was analysed by RFLP using *Nla III* enzyme. Two samples showed heterozygous condition for the deletion indicating that these patients have the possibility to acquire another abnormality in the residual allele causing growth retardation. G>A transition was not detected in any of the samples. These results revealed that the common mutations in the other Asian populations were not present in this study group. A larger group need to be studied to confirm that the occurrence of GH deficiency in Sri Lankan population may be due to other mutations present in the *GH1*gene.

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

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Abstract No: 22

Screening for selected mutations and sequence variations in growth hormone releasing hormone receptor (GHRH-R) in children with growth hormone deficiency: A preliminary study

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Growth hormone deficiency (GHD) is a recognized cause of severe postnatal growth failure and proportionate dwarfism. Although, the cause of isolated GHD (IGHD) in majority of cases is idiopathic, 3-30 % of GHD cases have a familial occurrence. In view of that, exploration of genetic factors underlying GHD has a significant importance that will eventually lead to early and accurate diagnosis, prevention of familial occurrence and exploration of effective clinical practices. Among the mutations identified to cause familial IGHD, a G>T transversion at codon 72 in exon 3 in GHRH-R gene is emerging as a relatively common cause of IGHD in Indian subcontinent. Current study was mainly concerned in screening for this mutation and C>T, G>A transversions in exon 2 and intron 3 respectively. DNA was extracted and 437 bp fragment containing exons 2 and 3 and the intervening intronic region of GHRH-R gene was amplified by PCR. Amplicons were subjected to RFLP with Bfa 1 enzyme. The G>T transversion on codon 72 was absent in all the samples (n=10) analyzed by RFLP. Sequencing of a selected sample confirmed that the targeted mutations were not present. As all cases studied were sporadic, GHD in these patients might be caused by structural defects or de-novo mutation in other region of GHRH-R gene or other relevant genes.

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

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Abstract No: 23

A study of *BRCA1*genomic rearrangement in familial breast cancer in Sri Lanka

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Majority of mutations reported in the *BRCA1*/*BRCA2* genes in breast and/or ovarian cancer families are point mutations, small insertions/deletions scattered over the coding sequence and splice junctions. We previously reported such mutations and sequence variants of *BRCA1* and *BRCA2* genes from Sri Lankan breast cancer patients. Large genomic rearrangements in *BRCA1* gene have been reported in breast cancer patients in several populations. In Netherlands, more than 30% of *BRCA1* related breast cancers occur due to copy number changes of one or more exons. Here in this study we carried out for the first time, detection of deletions and duplications of *BRCA1* in a group of 55 familial breast cancer patients, 21 at risk individuals and 23 healthy controls. Multiplex ligation-dependent probe amplification (MLPA) was used to detect deletions and duplications (SALSA MLPA KIT P002-C1 BRCA1®, MRC-Holland). The resulting amplification products were separated by using MegaBACE1000 DNA sequencer and Genetic Profiler software. Data obtained were analyzed using coafflyser® software and manual spreadsheet methods. One familial breast cancer patient showed a ratio of 0.62 as the ratio of intra normalisation of a sample for the exon 6 probe amplification product indicating an ambiguous deletion. To confirm the deletion, sequencing was carried out with appropriate primers designed for the 5'and 3' flanking regions of exon 6 and for the exon 6 MLPA probe annealing site. Sequencing results showed that the exon 6 was intact. Thus *BRCA1* rearrangements do not appear to significantly contribute to breast cancer in Sri Lanka.

Supported by Sida /Secretariat for Research Cooperation (formerly SAREC) Grant for Molecular Biology and Biotechnology and constitutes part of PhD studies of SDeS

Abstract No: 24

Effect of a decoction of *Fluggea leucopyrus* on the expression of Caspase 3 and Caspase 9 in endometrial carcinoma cells

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Endometrial carcinoma is a neoplasia of the epithelial lining of the uterus and has become one of the common gynecological malignancies in the world. Primary treatment for endometrial cancers is surgical dissection. In view of the recent interest in finding alternative therapies for cancer, we evaluated the effect of a decoction of *Fluggea leucopyrus* (Willd.) in an *in vitro* model of chemoresistant and hormone resistant endometrial carcinoma cells (AN3CA). AN3CA cells maintained in DMEM medium (4 x 10^5 cells/ml) were incubated for 24 h with 0 – 400 µg/ml of decoction and apoptotic changes were evaluated by (a) light microscopic observation of cell morphology and DNA fragmentation analysis, (b) activity of caspase-3 and caspase-9 enzymes by colorimetric protein assay and (c) mRNA expression of caspase-3 and caspase-9 genes by RT-PCR. Incubation of AN3CA cells with the *F. leucopyrus* decoction resulted in (a) DNA fragmentation and characteristic morphological changes associated with apoptosis and (b) enhancement of caspase-3 ($p<0.001$) and caspase-9 ($p<0.001$) protein activities, in a dose (r values, 0.9159 and 0.8227 respectively) dependent manner. Further, the decoction significantly induced the caspase-3 ($P<0.05$) and caspase-9 ($P<0.05$) gene expression in a dose (r values, 0.9504 and 0.9082 respectively) dependent manner. The decoction may therefore mediate its anti-carcinogenic effect, at least in part, through apoptosis via up regulating caspase 3 and caspase 9. Elucidation of active principles responsible for up regulation of caspases might yield novel drug candidates for cancer treatment.

Supported by IBMBB and constitutes part of the MSc studies of SBK.

Abstract No: 24

Effect of a herbal extract on *Bcl-2* and *Bax* expression in endometrial carcinoma cells

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Endometrial carcinoma is a common gynecological malignancy. Use of a decoction of aerial parts of *Flueggea leucopyrus* (Willd.) for cancer treatment has recently become popular among Sri Lankan traditional medical practitioners, despite the lack of scientific evidence to validate its anti-carcinogenic potential. The main aim of this study was to evaluate the effect of a decoction of *F. leucopyrus* on expression of two of the key genes (*Bcl-2* and *Bax*) involved in apoptosis, in an *in vitro* model of chemoresistant and hormone resistant endometrial carcinoma cells (AN3CA). Primarily, the optimization of PCR conditions for the detection of *Bcl-2* and *Bax* gene expression in AN3CA cells was carried out, followed by the evaluation of the effect of a decoction of *F. leucopyrus* on the expression of these genes in AN3CA cells (4×10^5 cells/ ml) by RT-PCR analysis. Subsequently, to confirm that the decoction could modulate apoptosis in AN3CA cells, morphological changes in the cells exposed to different concentrations of the decoction for 24 h were observed using light and fluorescent microscopy, followed by DNA fragmentation analysis. Decoction of *F. leucopyrus* exerted a significant ($P<0.05$) up regulation of *Bax* gene at higher dose, while the regulation of *Bcl-2* gene was inconclusive as primers failed to amplify target DNA. Nevertheless, microscopic observations of stereotypical morphological changes associated with apoptosis and DNA fragmentation patterns in treated cells confirmed that the decoction has the ability to induce apoptosis. Further investigation of the active components of the decoction, may help in the future to develop therapeutic agents for treatment of cancer.

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Abstract No: 26

Differentiation of selected *Piper nigrum* L (black pepper) cultivars in Sri Lanka using morphological and AFLP markers

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Black pepper (*Piper nigrum* L. (family: Piperarceae) or the "King of spices", native to damp jungles of the Kerala coast. Today, it is widely used in the international spice trade. There are two types of black pepper cultivars grown in Sri Lanka, the local selections and the introduced cultivars. Significant differences can be seen in morphological and yield characteristics of those cultivars. Therefore the objective of this study was to genetically and morphologically differentiate those cultivars. A preliminary study was conducted to assess the morphological and molecular variation in 10 cultivars of *P. nigrum* using morphological descriptors, yield characteristics and Amplified Fragment Length Polymorphisms (AFLP). Morphological and yield characters were analyzed and dendrogram was constructed by using Minitab package (Version 15). DNA extraction was carried out and AFLP analysis was performed using standard methods with minor modifications. Amplified products were subjected to fragment analysis by capillary electrophoresis. Polymorphic fragments were scored and cluster analysis was carried out using PHYLIP 3.69 software. According to the dendrograms cultivar KU is separated, from all other cultivars in both morphological and AFLP dendrograms. But other cultivars did not show similar clustering pattern in AFLP analysis. The cultivars of PAN and IW5 with closely related morphological and yield characters as spike length and weight of the spike were clustered together only in morphological analysis. Therefore reliability of AFLP based clustering pattern cannot be explained in this preliminary study by their morphological information. This may be due to, the use of only five primer combinations in AFLP. Therefore construction of AFLP dendogram for more primer combinations may be suggested.

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Abstract No: 27

Genetic differentiation and identification of varietal specific AFLP markers for *Artocarpus heterophyllus* Lam. (Jackfruit) varieties in Sri Lanka

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Artocarpus heterophyllus Lam. (Jackfruit; family Moraceae) is native to the rain forests of the Western Ghats of India and the Malaysian Archipelago. It is a naturalized species in Sri Lanka and found in association with permanent human settlements. Despite importance of *A. heterophyllus*, coordinated efforts to improve the species have been initiated only recently. Except for morphological, no genetic information is available for their identification. The objective of this study was to identify varietal specific AFLP markers for genetic differentiation of *A. heterophyllus* varieties in Sri Lanka. The selected 14 varieties were taken from the Horticultural Crop Research Institute of Department of Agriculture at Gannoruwa namely Father Long (FL), Kothmale (KM), Maharagama (MG), Hirosa/Rosa Kos (RS), Kuru Kos (KK), Horana (HR), AV Dias (AV), Kalpitiya (KP), Kuruwita (KW), Ganegoda (GG), 18 Months variety (M18), Thellippalai (TP), Gannoruwa (GN) and Mandoor (MD). Genomic DNA was extracted from fresh leaves and AFLP amplification was carried out. A dendrogram was constructed using Dice distance method. According to the dendrogram, 14 varieties are clustered in to three groups at 75% phenon level. Variety RS, HR and GN clusterd together and varieties FL and KM clustered into one group. Other varieties were separated from them and further divided into two groups as KW, MD, TP, AV, KK in one cluster and GG, MG, M18 and KP varieties in another cluster. Furthermore, 36 varietal specific AFLP markers were identified for 11 types/varieties whereas no specific markers were detected for RS, HR and GN types/varieties. The high polymorphism observed with AFLP markers for varieties/types used in this study demonstrate the suitability of AFLP as a tool for developing molecular markers to identify *A. heterophyllus* varieties and also suggest the need to include more primer combinations to increase the efficiency of identification of jack fruit types/varieties.

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Abstract No: 28

Variation in Sri Lankan aromatic rice: progress towards identification of a new haplotype

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Aroma is one of the important quality traits attracting consumers. Early investigations have revealed that the mutation of the Betaine Aldehyde Dehydrogenase 2 gene (*badh2.1*allele), occur at the 7th exon and is responsible for fragrance in most of the popular aromatic rice. Phenotypic assessment method for fragrance, KOH sensory test, revealed that Sri Lankan traditional aromatic rice contains pleasant aroma although their fragrance is not resulted by the presences *badh2.1* allele. Recently it was found that exons 1, 2, 10, 13 and 14 of the *badh2* gene could be hot spots for functional mutations for fragrance in rice. Therefore, this study was carried out to sequence the exon 14 of the *badh2* gene in Sri Lankan aromatice rice. A 476 bp DNA fragment covering the exon 14 region was amplified using specific primers and the amplified fragments were sequenced. The sequences were aligned with the wild type of the *badh2* gene and known sequence of Basmati. A new haplotype was identified as the ‘‘G’’ insertion in *Suwandal*, *Kuruluwee* and *Suwanda Al* suggesting that it could be a possible non functional mutation leading to the fragrance in Sri Lankan aromatic rice. This insertion changes the *Bsl* 1 restriction enzyme recognition sequence and would generate 188bp band that would discriminate from wild type varieties. Hence a cleaved amplified polymorphic sequence (CAPS) marker could be developed based on the fragments to screen Sri Lankan rice for aromatic properties. This investigation would be useful for the utilization of Sri Lankan aromatic rice germplasms in quality enhancing breeding programs.

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Abstract No: 29

A new cost effective method for extracting genomic DNA from fungi

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Analysis of DNA has now become an important aspect of mycological studies. Extraction of fungal genomic DNA is one of the most important steps in such studies. However, because of the thickness of the cell wall, the high polysaccharide content of mycelia and the presence of high quantities of fungal spores have made DNA isolation from fungi difficult. Several DNA extraction protocols have been developed to extract DNA from fungi and DNA extraction kits that are commercially available give promising results. However, these methods are time consuming and/or expensive and require skills to acquire large quantities of DNA. This investigation reports a new, cost effective protocol developed to extract DNA from plant pathogenic fungi. The fungi used were leaf pathogens *Colletotrichum acutatum* and *C. gloeosporioides* and the rice blast fungus *Magneporthe grisea*. The protocol includes a 72 hour incubation of the fungal mycelia in 600 µl of an extraction buffer at room temperature prior to maceration in the extraction buffer. After centrifugation small quantities of iso-propanol and sodium acetate was added to precipitate the DNA. Even though there was no significant difference between the yields of DNA extracted from the new protocol and the commonly used Treco DNA extraction protocol, the new protocol requires only smaller quantities of fungal biomass and chemicals. Further the new protocol does not require freeze drying and liquid nitrogen to macerate the mycelia. The new protocol which is simple and inexpensive is therefore ideally suited for extraction of DNA of fungi in laboratories in Sri Lanka.