OB-1

MICROSPORIDIA INFECTION: A SERIES OF CASE REPORT

Zeehaida M, Siti Asma H and Kirnpal-Kaur BS

Department of Medical Microbiology & Parasitology, School of Medical Sciences, Universiti Sains Malaysia, Health Campus 16150 Kubang Kerian, Kelantan, Malaysia.

Introduction: Microsporidia are an obligate intracellular, spore-forming parasite that has been implicated in emerging infectious diseases. They are increasingly recognized as opportunistic parasites in AIDS patients as well as pathogens in immunocompetent individuals. Though more then 1000 species were named in Microsporidia phylum, only 11 to 14 species were known to infect human. A wide range of clinical presentation was associated with this infection. Chronic diarrhea is the commonest manifestation however myositis, keratoconjunctivitis and disseminated infection have been reported.

Objective: To review cases with microsporidia infection.

Patients and Method: All requests for suspected cases of microsporidia sent to the Medical Microbiology and Parasitology laboratory, School of Medical Sciences, USM from year 2003 to 2007 were reviewed. Patients were considered positive whenever the microsporidia oocysts were detected by Gram Chromotrope staining method. The detailed histories of patients with positive results were traced from the hospital record office.

Results: Five patients were detected as positive for microsporidia oocyst within 4 years study period. Four of them were children aged 3 months to 8 years old. One patient was an immunocompromised adult. All patients had gastrointestinal symptoms. No other clinical manifestations were reported.

Discussion and Conclusion: This study demonstrated that microsporidia are present in our local setting despite the fact that their detection is low. Education to the clinician is important to instill awareness on the diversity of its clinical presentations to support appropriate specimen collection and examination procedures.

OB-2

RAPID SCREENING OF ASYMPTOMATIC BACTERIURIA IN PREGNANCY : COST-EFFECTIVE ANALYSIS

[1]Md. Anayet U, [2]Md. Abdul MS, [3]Md. Iftikhar A, [3]Md. Abdus S

[1]Medical Education Department, School of Medical Sciences, Universiti Sains Malaysia, Health Campus 16150 Kubang Kerian, Kelantan, Malaysia. [2]Community Medicine Department, [3]Microbiology Department, Rajshahi Medical College, Rajshahi, 6200 Bangladesh

Objective: To assess the cost-effectiveness of rapid screening tests for detecting asymptomatic bacteriuria in pregnancy.

Patients and Method: This study was conducted among the apparently healthy pregnant mothers attending for their routine antenatal care at the Antenatal Clinic of Rajshahi Medical Collage Hospital Rajshahi, Bangladesh. A total of 600 apparently healthy pregnant mothers were selected by random sampling. Data were processed using SPSS for windows. Receiver Operative Characteristic (ROC) curve was applied to assess the effectiveness of the screening tests to identify the asymptomatic bacteriuria. Cost-effectiveness of each of the screening tests was determined by calculating the incremental patient cost of obtaining an additional asymptomatic bacteriuric mother.

Results: The prevalence of asymptomatic bacteriuria was 4%. Staphylococcus saphrophyticus was the commonest causative agent (41%). Leukocyte count / HPF by routine microscopic urine examination, which is the common practice to detect asymptomatic bacteriuria in pregnancy during antenatal care in Bangladesh, efficiently detect the healthy mothers as healthy but fail to detect asymptomatic bacteriuric mothers as bacteriuric in majority of cases. Bacteria count / OIF in Gram stained smear of uncentrifuged urine is the most cost-effective screening test for detecting asymptomatic bacteriuria in pregnancy.

Discussion and Conclusion: Bacteria count in gram stained smear of uncentrifuged urine may be integrated during the routine antenatal check up as the screening approach for detecting asmptomatic bacteriuric mothers in Bangladesh.

OB-3

DOES PARTICIPATION IN SPORTS IMPROVE SCHOLASTIC PERFORMANCE?

[1]Jagdeep S, [2]Adinegara L A, [2]Anthony B J, [2]Melissa M, [2]Sandra M, [2]Zawaniah B A, [2]Kavitha A

[1]No 24, Jalan BK 4/3E, Bandar Kinrara, 47100 Puchong, Selangor, Malaysia. [2]No 66K, Jalan Permai 6, Tmn Perkota, 75350 Batu Hampar, Melaka, Malaysia.

Introduction: There is little scientific data studying the effects of sports on academic achievement. The few studies that are available were conducted in western countries, primarily in high schools.

Objective: To determine if there is any correlation between sports and academic performance in medical school.

Subjects and Method: This is a cross sectional study of 174 randomly selected students of Melaka Manipal Medical College conducted over the month of January 2007. The participants were interviewed individually using a questionnaire designed to elicit information on their sports, sleep, and study patterns during the 6th and 7th semesters. These variables were then compared with their average score in all the examinations conducted over the same period.

Results: Variables such as gender, ethnicity and involvement in extracurricular activities other than sports (e.g. playing musical instruments, dancing & singing) did not have significant effects on academic performance (P>0.05). However, our data showed that students playing 3 or more different sports a week were 2.9 times more likely to fail as compared to those who participated in two or less sports (P=0.02). We also noted that students who were involved in high intensity sports such as football averaged 4 points (P=0.013) lower than their counterparts.

Discussion and Conclusion: Our study indicates that excessive indulgence in sports or participation in physically demanding sports can have a detrimental effect on academic performance. However this should not deter medical students from participating in sports because our data shows that moderate amounts of sports doesn't have a negative impact on academic performance.

OB-4

PRELIMINARY STUDY OF THE EFFECT OF MOMORDICA CHARANTIA (BITTER GOURD) ON THE KIDNEYS OF STREPTOZOTOCIN INDUCED DIABETIC RATS

Srijit D, Teoh SL, Azian AL

Department of Anatomy, Universiti Kebangsaan Malaysia, Jalan Raja Muda Abdul Aziz, 50300 Kuala Lumpur, Malaysia.

Introduction: *Momordica charantia* is a tropical and subtropical "Vine" vine of the family "Cucurbitaceae" Cucurbitaceae, known for its bitterness amongst all "Vegetable" vegetables. The extract of the plant is known to have antioxidant, anti-inflammatory, antiseptic, antihelminthic and antidiabetic properties.

Objective: To observe the protective role of *Momordica charantia* (bitter gourd) on streptozotocin-induced diabetic kidneys of experimental rats.

Subjects and Method: Eighteen Sprague Dawley rats weighing 200 ±50 gms were taken for the study. The rats were divided into three groups of six each (n=6). The animals were acclimatized to their surroundings for five days. Group I did not receive any streptozotocin nor any treatment with *Momordica charantia* extract. Group II received a single dose of 45 mg/kg body weight of streptozotocin administered intravenously through the lateral tail veins after fasting overnight. The level of the blood glucose was checked and the rats were labeled as diabetic if the fasting blood glucose level reached more than 8mmol/L. No treatment was provided to the animals of group II. Group III received single dose of 45 mg/kg body weight of streptozotocin and an extract of *Momordica charantia* via oral gavage (50 mg/kg body weight). The subjects were sacrificed on the tenth day following treatment. The kidneys were taken to observe for histological changes under the light microscope using H & E, VVG and PAS stains.

Results: Histological studies showed that in the diabetic rats, there were gross thickening of the mesangium due to the accumulation of PAS with deposits in the glomeruli stained pinkish yellow. The glomerular basement membrane increased in thickness. Cortical tubules showed dilatation, hyalinization and fibrosis. The treatment with *Momordica charantia* extract reduced these alterations.

Discussion and Conclusion: *Momordica charantia* shows a protective role in the progression of diabetes as evident from the histological study probably through its antioxidant properties. A diet laden with *Momordica charantia* extract may restrict the damages in the glomeruli, basement membrane and tubules in the kidney of diabetic rat.

OB-5

STUDY OF RENAL SYMPATHETIC NERVE ACTIVITY IN DIABETIC SPRAGUE DAWELY RATS

[1]Ibrahim MS, [2]Munavvar AS, [3]Nor AA, [2]Mohammed HA, [2]Omar ZA, [2]Hassaan AR, [2]Kolla RLAS, [2]Raisa NK, [2]Fathihah BB, [2]NurJannah MH, [4]Edward JJ

[1]3C-14-02 N-Park Condominium, Jalan Batu Uban, 11700 Gelugor, Penang, Malaysia. [2]School of Pharmaceutical Sciences, Universiti Sains Malaysia, Minden, 11800 Penang, Malaysia. [3]Department of Pharmacology, Faculty of Medicine, Universiti Malaya, 50603 Kuala Lumpur, Malaysia. [4]Department of Physiology Aras Windle, University College Cork, College Road, Cork, Ireland.

Objective: To study the early changes in the activity of renal sympathetic nerves in the presence and absence of acute renal sympathectomy (ARS) in diabetic and normal anesthetized Sprague Dawley (SD) rats.

Subjects and Method: Twenty four SD rats were used in this study. Diabetes mellitus (DM) was induced by an i.p injection of streptozotocin at a dose of 55 mg/kg. Diabetic rats were kept for seven days after which the overnight fasted rats were anesthetized with sodium pentobarbitone, 60mg/kg i.p and renal sympathectomy was produced by application of phenol to the left renal artery. Subsequently, the renal nerves were directly stimulated at a sequence of frequencies of 1, 2, 4, 6, 8, and 10 Hz at 0.2 ms duration and 15V for a period of 15s in ascending and descending manner. By means of Square-Wave Electromagnetic flowmeter, renal blood flow changes in response to nerves electrical stimulation were recorded.

Results: Both diabetic and normal rats showed a significant ($P<0.05$) attenuation in renal vasoconstrictor response in reaction to renal nerve stimulation. Although no considerable difference in renal vasoconstrictor response was seen between the non-sympathectomised diabetic and normal rats, a significant ($P<0.05$) difference was found between the sympathectomised diabetic and normal rats.

Discussion and Conclusion: The data showed that adding to the intact sympathetic nerves found in early DM, there is a remarkable increase in the sympathetic activity in the kidney, which thought to be due to increased adrenergic receptors sensitivity to noradrenaline released from nerve terminals in response to renal nerve stimulation following ARS.

OB-6

THE EFFECT OF CARVEDILOL ON ANGIOTENSIN II AND ADRENERGIC AGONISTS' VASOCONSTRICTOR RESPONSES IN THE RENAL RESISTANCE ARTERIES OF SPONTANEOUSLY HYPERTENSIVE RATS: INTERACTION BETWEEN RENIN-ANGIOTENSIN SYSTEM AND SYMPATHETIC NERVOUS SYSTEM.

[1]Mohammed HA, [2]Munavvar AS, [3]Nor AA, [2]Ibrahim MS , [2]Hassaan AR, [2]Kolla RLAS, [2]Raisa NK, [2]Fathihah BB, [2]NurJannah MH and [4]Edward JJ

[1]3C-14-02 N-Park Condominium, Jalan Batu Uban, 11700 Gelugor, Penang, Malaysia. [2]School of Pharmaceutical Sciences, Universiti Sains Malaysia, Minden, 11800 Penang, Malaysia. [3]Department of Pharmacology, Faculty of Medicine, Universiti Malaya, 50603 Kuala Lumpur, Malaysia. [4]Department of Physiology Aras Windle, University College Cork, College Road, Cork, Ireland.

Objective: To investigate the influence of adrenergic receptors blocking by carvedilol along with sympathetic effect inhibition by acute unilateral renal denervation to alter the renal vasoconstrictor response to exogenous angiotensin II and a set of vasoactive agents in spontaneously hypertensive rats (SHR).

Subjects and Method: Thirty two SHR rats underwent acute unilateral renal denervation and the denervation was confirmed by significant drop ($P<0.05$) in renal vasoconstrictor response to renal nerve stimulation along with marked diuresis and natriuresis following denervation. After 7 days treatment with carvedilol, the overnight fasted rats were anesthetized (sodium pentobarbitone, 60 mg/kg i.p.) and renal vasoconstrictor experiments were performed. The changes in the renal vasoconstrictor responses were determined in terms of reductions in renal blood flow caused by intrarenal administration of noradrenaline, phenylephrine, methoxamine and angiotensin II.

Results: The data showed a significant (all $P<0.05$) decrease in the vasoconstrictor response to Angiotensin II and all the adrenergic agonists given after treatment with carvedilol when compared to the untreated rats. Data for sympathectomisd rat treated with carvedilol showed a significant (all $P<0.05$) elevation of the vasoconstrictor response to Angiotensin II and all the adrenergic agonists given as compared to intact renal nerves rats treated with carvedilol.

Discusion and Conclusion: Carvedilol reduces the vasoconstictior response to Angiotensin II markedly while, the removal of the sympathetic impact on the kidney caused by surgical denervation enhanced the sensitivity of renal α1- adrenoceptors to the injected agonists along with AT1 receptors, indicating a crosstalk between α1- and AT1 receptors in SHR rats.

OB-7

OVER-EXPRESSION OF AN IN-HOUSE RECOMBINANT TAQ DNA POLYMERASE ENZYME

Ang KC, Hoe CH, Asma S, Basir M, Yahaya O, Tang TH

Advanced Medical & Dental Institute (AMDI), University Sains Malaysia, 13200 Kepala Batas, Pulau Pinang, Malaysia.

Introduction: *Thermus aquaticus* (Taq) DNA polymerase plays as an essential role in polymerase chain reaction (PCR). In addition, Taq DNA polymerase is also used for 3' A-tailing of blunt ends, primer extension as well as in DNA sequencing. Although the price for commercial recombinant Taq had reduced substantially, it can still be reasonably expensive for a small laboratory that work with Taq polymerase enzyme. By recombinant DNA technology, we had cloned the Taq DNA polymerase gene.

Objectives: To determine the over-expression of our pTaq by a simple purification step and the activity of our enzyme as compared to other commercial sources of recombinants Taq enzymes.

Materials and Method: In order to over-express Taq DNA polymerase enzyme, pTaq was transformed into *E.coli* strain DH5α in the ampicillin selective media. The culture was grown overnight; expression of pTAq protein was induced using IPTG (0.5mM). The cells were then harvested and lysed in Tris buffer containing a mixture of lysozyme, dextrose and EDTA. Lysate was then treated at 75^0C to denature heat labile proteins. Centrifugation was carried out to remove the unwanted debris and denatured proteins. The supernatant, containing Taq enzyme was collected and equal volume of storage buffer containing 50% glycerol buffer was added to preserve the Taq enzyme. In order to determine the activity of our recombinant Taq enzyme, we carried out PCR using genomic and plasmid DNA. We also compared with commercial Taq DNA polymerases from various companies.

Results: The recombinant pTaq was successfully expressed and tested with genomic and plasmid DNA. Moreover, the expressed Taq polymerase had significant enzyme activity when compared to commercial recombinant Taq polymerases.

Discussion and Conclusion: We have successfully expressed and purified recombinant Taq DNA polymerase. Our results show that our expressed Taq polymerase is highly efficient and cost effective as compared to others. The recombinant Taq Polymerase enzyme could be used for our in-house PCR as well as various researches which utilize this enzyme.

OB-8

SMALL NON-PROTEIN-CODING RNA AS NOVEL DIAGNOSTIC MARKER FOR INFECTIOUS DISEASES

[1]Tang TH, [1]Asma S, [1]Ang KC, [1]Hoe CH,[2] Raabe CA, [2]Chinni VS, [2]Brosius J, [2]Rozhdestvensky TS

[1]Advanced Medical & Dental Institute (AMDI), University Sains Malaysia, 13200 Kepala Batas, Penang, Malaysia. [2]Institut fur Experimentelle Pathologie/Molekulare Neurobiologie (ZMBE), Universitat Munster, D-48149 Munster, Germany.

Introduction: Traditionally, the molecular diagnostic of infectious diseases is largely based on proteins, protein-coding-genes or ribosomal RNAs. Non-protein coding RNAs (npcRNAs), are RNA transcripts that are not translated into proteins but they are involved in various aspect of cell regulations. Recently, npcRNAs have been suggested as markers for genetic diseases and cancer. However, none used npcRNA genes as markers for the detection of infectious diseases. By an Experimental RNomics approach, we analysed RNome of infectious agents such as *Plasmodium falciaparum* (*P. falciparum*), *Vibrio Chlolerae* (*V. Chlolerae*) and *Salmonella typhi* (*S. typhi*). Here we report the discovery of npcRNAs that have the potential to serve as diagnostic markers for the rapid detection of *S. typhi* infection.

Objectives: To elucidate the roles of npcRNAs in disease as well as to seek their potential as antimicrobial target or diagnostics markers.

Materials and Methods: To identify and characterize npcRNAs from *S. typhi*, we extracted total RNA and constructed a specialized cDNA library. The cDNAs clones were sequenced and aligned using MacDNAsis (Bioinformatic program). BLASTN databases were used to *in-silico* identify and map potential npcRNA genes and their location within the genome. Primers for PCR were designed from detected npcRNAs which shows specificity to *S. typhi*. PCR were carried out to experimentally verify their specificity.

Results: Among the novel npcRNA candidates, the expression of StyR161 was restricted to *S. typhi* only. Further bioinformatics searches also indicate the uniqueness of this gene. Thus, StyR161 could represent a potential diagnostic maker for *S. typhi*. We therefore designed primers in order to developed a PCR based diagnostic test for *S. typhi.* Our results show that this novel small npcRNA gene is specifically amplified in *S. typhi* only.

Conclusion: StyR161 npcRNA gene is specific for *S. typhi* and could be used as potential maker for the detection of *S. typhi*. This supports our notion that npcRNAs can be an excellent candidate for the specific detection of this pathogenic organism.

OB-9

MAXIMAL ACCUMULATED OXYGEN DEFICIT (MAOD) OF PHYSICALLY ACTIVE FEMALES DURING MID-FOLLICULAR (MF) AND MID-LUTEAL (ML) PHASES OF OVARIAN CYCLE

[1]Shazlin S, [1]Asok KG, [2]Ahmad AI

[1]Sports Science Unit, [2]Department of Obstetric and Ginecology, School of Medical Sciences, Universiti Sains Malaysia, Health Campus 16150 Kubang Kerian, Kelantan, Malaysia.

Objective: To evaluate the anaerobic capacity in repeated sprint cycling bouts during different phases of ovarian cycle.

Patients and Method: This intervention study involved twelve physically active females aged 22.41 ± 1.68 years, weight 52.06 ± 7.28 kg, height 158.17 ± 4.17 cm, and VO_2max of 34.92 ± 4.85 ml•kg-1•min-1. The method of measuring maximal accumulated oxygen deficit (MAOD) was implemented from Medb et al., (1988). Initially, the VO_2max of the participant were measured on cycle ergometer following a graded exercise protocol. Then, the participants did sub-maximal cycling exercise for 10 minutes at 50%, 60%, 70% and 80% of VO_2max on separate days. The linear regression determined from the VO_2–power relationship was used to approximate supra-maximal power output at 120% VO_2max. Next, the participants performed repeated sprint cycling at 120% of VO_2max intensity with 20 minutes rest between consecutive sprints during mid-follicular (MF) and mid-luteal (ML) phases. The menstrual phases were verified through daily basal body measurement and serum progesterone analysis.

Results: Results indicated there was no significant difference in maximal accumulated oxygen deficit (MAOD) and sprint performance between mid-follicular (MF) and mid-luteal (ML) phases in repeated sprint cycling. There was also no significant difference in plasma lactate and plasma ammonia concentration between mid-follicular (MF) and mid-luteal (ML) phases in repeated sprint cycling.

Discussion and Conclusion: This study showed that the ovarian phases of women with regular menstrual cycle have no significant effect on anaerobic capacity.

OB-10

DETERMINATION OF EXERCISE DURATION REQUIRED TO INDUCE BONE GAINS FOLLOWING A STANDARD JUMPING EXERCISE REGIMEN

[1]Ooi FK, [1]Rabindarjeet S, [2,3]Harbindar JS, [4]Yoshihisa U,

[1]Sports Science Unit, [2]Department of Physiology, School of Medical Sciences, Universiti Sains Malaysia, Health Campus 16150 Kubang Kerian, Kelantan, Malaysia. [3]Faculty of Medicine, Universiti Teknologi MARA, 40450 Shah Alam, Selangor, Malaysia. [4]School of Health and Sports Science, Chukyo University, Toyota, Japan.

Introduction: The duration required to induce bone gains vary depending upon the exercise regimen.

Objective: To determines the minimum duration required for a standard jumping exercise regimen to induce bone gains in female rats.

Subjects and Method: Ninety, 12-week old female rats were divided into 10 experimental groups, where the groups were given either no exercise or performed jumping exercises for 2, 4, 6, 8 or 10 weeks. The standard exercise regimen consisted of 40 jumps/day (40J/d) for 5 days/week(5d/w) at a jump height of 40 cm. At the end of the study, tibial fat free dry weight, ultimate bending load/strength, stiffness, length and mid shaft maximum diameter were measured. Serum osteocalcin and C-terminal telopeptide of type 1 collagen (1CTP) were determined. Statistical analysis was performed using one-way ANOVA.

Results: There were no significant differences in the tibial length, serum osteocalcin and serum 1CTP between exercised rats and their respective age-matched sedentary controls. The differences in tibial fat free dry weight, ultimate bending load and maximum diameter only became significantly evident after 6 weeks of jumping exercise. Tibial stiffness, however, only became significantly different from the sedentary control after ten weeks of jumping exercise.

Discussion and Conclusion: A minimum duration of 6 weeks of exercise at an intensity of 40 J/d for 5 d/w is required to increase tibial mass, strength, and maximum diameter. However, a longer duration is required to induce changes in bone stiffness.

OB-11

RB1 GENE MUTATIONS AT POCKET B OF E1A DOMAIN AND CLINICAL PRESENTATION OF RETINOBLASTOMA CHILDREN IN MALAYSIA

[1]Siti Raihan I, [2]Hanani H, [3]Joseph A, [3]Shuaibah AG, [1]Shatriah I, [1]Lai PS, [1]Liza Sharmini AT, [2]Zilfalil BA

[1]Department of Ophthalmology, [2]Human Genome Centre, School of Medical Sciences, Universiti Sains Malaysia, Health Campus 16150 Kubang Kerian, Kelantan, Malaysia. [3]Hospital Kuala Lumpur, 50586 Kuala Lumpur, Malaysia.

Objective: To determine the presence of exon 20 to 22 (pocket B E1A Domain) mutations of RB1 gene and their clinical presentation in Malaysian children with retinoblastoma.

Patients and Method: Children with retinoblastoma from two main tertiary centers in Malaysia, Hospital Universiti Sains Malaysia and Hospital Kuala Lumpur who fulfilled the inclusion criteria were enrolled in this study. Their clinical presentation was analyzed according to the age, sex, laterality, stage of presentation (ICRB classification), defaulted rate and outcome of disease. The peripheral blood was taken for the genetic analysis of RB1 gene mutation at exon 20 to 22 (pocket B E1A domain). A combination analysis consisting DNA extraction, PCR followed by Denaturing High Performance Chromatography (dHPLC) technique and DNA sequencing were used to detect RB1 gene mutations.

Results: Fifty retinoblastoma children (69 eyes) were recruited in this study. The most common presentation was leukocoria (65%) followed by other features, strabismus (9%), proptosis (7%), orbital inflammation (10%), vitreous haemorrhage (3%) and secondary glaucoma (3%). Majority presented at advanced stage (86.6% Stage E ICRB). Three mutations were found in our retinoblastoma children including two novel mutations at exon 22. T and insertion. The novel mutations were identified as transversion of 162108A of 162106_162107insC, which shifted the whole frame. Another mutation was G. identified at the flanking region of intron 19, substitution A.

Discussion and Conclusion: The novel mutations identified in pocket B of E1A domain of RB1 gene may play an important role in the pathogenesis of retinoblastoma in our population. Their correlation with clinical presentation is not established. Leukocoria remain the main clinical presentation, proptosis and orbital inflammation is not uncommon especially in the advanced stage of the disease.

OB-12

INSERTIONAL INACTIVATION OF HEMA GENE OF SHIGELLA FLEXNERI 2A: TOWARDS THE DEVELOPMENT OF LIVE ATTENUATED VACCINE

[1]Mehru N, [1]Kirnpal-Kaur B.S., [3]Mohd Zaki S, [2]Lau HY, [1]Manickam R

[1]Department of Medical Microbiology and Parasitology, [2]Reconstructive Science Unit, School of Medical Sciences, Universiti Sains Malaysia, Health Campus 16150 Kubang Kerian, Kelantan, Malaysia. [3]Faculty of Pharmacy, Universiti Teknologi Mara (UiTM), 40450 Shah Alam, Selangor, Malaysia

Introduction: *Shigella* is one of the most important aetiological agents causing severe form of bloody diarrhoea called bacillary dysentery or shigellosis. To date, no vaccine is available against shigellosis, despite many studies that have been conducted worldwide. *hem*A is a housekeeping gene which is involved in production of aminolevulinic acid (ALA). In Gram negative bacteria, ALA which is synthesis in C5 pathway is an important precursor for heme synthesis.

Objective: To determine that ALA auxotroph of *Shigella flexneri* could be used as an attenuated live oral vaccine against *shigellosis*.

Material & Method: In this study, *hem*A gene (1474bp) was PCR amplified from wild type *Shigella flexneri* 2a using *Hem*A-forward and *Hem*A-reverse primers. The amplified gene was cloned into pTZ57R PCR cloning vector. A unique *Nde*1 was identified in *hem*A gene and a kanamycin resistant gene cassette (*aphA*) was inserted at the *Nde*1 site of *hem*A gene. This cassette contained a promoter, ribosomal binding site and kanamycin resistance gene flanked by *Fse*I restriction enzyme site. The mutated gene (*hem*A*::aph*A) was then subcloned into pWM91 conjugative suicidal vector at the *Sma*1 and *Sac*1 site.

Result: The *hem*A gene has been successfully mutated using a kanamycin gene cassette and has been subcloned into a conjugative suicide vector, pWM91.

Discussion and Conclusion: The construct of *hem*A*::aph*A is an important approach towards the development of ALA auxotroph of *Shigella* that could be used as an attenuated live oral *Shigella* vaccine against shigellosis.

OB-13

A STUDY TO DETERMINE THE ASSOCIATION OF CD4⁺T-LYMPHOCYTE COUNTS WITH INTRACRANIAL TOXOPLASMOSIS INFECTION IN HIV/AIDS PATIENTS IN KELANTAN

[1]Abdul Kareem M, [1]Siti jusna, [2]Arif A, [2]Mahiran M

[1]Department of Radiology, School of Medical Sciences, Universiti Sains Malaysia, Health Campus 16150 Kubang Kerian, Kelantan, Malaysia. [2]Hospital Raja Perempuan Zainab II, 15586 Kota Bharu, Kelantan, Malaysia.

Objective: To identify the association between CD4 count with site and type of intracranial lesions of Toxoplasma infections in HIV/AIDS patients.

Patients and Method: This cross sectional study was carried out in Hospital USM and Hospital Raja Perempuan Zainab II (HRPZ II) after obtaining proper informed consent from the patients and ethical committee approval. The study sample comprised of 56 adult HIV patients, with focal or non-focal neurological deficit. $CD4^+$ T lymphocyte count was done in all the cases. Plain and post-contrast cranial axial CT scan were performed on all these patients from foramen magnum to the vertex as per the standard department protocol. A consultant experienced Radiologist did the reporting as regards to the presence of edema, atrophy, mass, nodule, ring lesion, meningeal enhancement, hydrocephalus and assess the site and number of lesions.

Results: Late stage of HIV infection with a. CD4 count <200 was found in 49. Toxoplasma serology was positive in 29 cases of late stage HIV. Simultaneous multiple opportunistic infections were found in 17 late stage patients with CD4 count <50. CT scans were normal in 5 and abnormal in 12 patients. The cerebrum was affected in 65.5% of cases. In the order of frequency, white matter hypodensity, edema, meningeal, ring enhancing lesions and nodular lesions were seen. Only one case presented with hydrocephalus. Focal lesions were found in 31% at frontal lobe, 58.6% at parietal lobe, 34.5% at occipital lobe, 34.5% at basal ganglia, 20.7% at temporal lobe, 20.7% at midbrain and 3.4% in cerebellum. Majority lesions were multiple.

Discussion and Conclusion: In our study, Toxoplasmosis focal mass lesion and atrophy are commonly associated with late stages of HIV infection with CD4 count less than 200cells/μl. White matter hypodensity, meningeal and parietal lobe involvement are commonly seen in our with HIV patients with toxoplasmosis. No significant correlation was found between toxoplasmosis with CD4 cell count.

OB-14

GENETIC RELATIONSHIP & DISTRIBUTION OF ANCESTRAL GENETIC COMPONENT AMONG PENINSULAR MALAYSIA MALAY SUB-ETHNIC GROUPS

[1]Wan Nur Hatin WI, [1]Nur Shafawati AR, [1]Zahri MK, [2]Shuhua XU, [3]Mohammed Rizman I, [1]Zilfalil BA

[1]Human Genome Center, School of Medical Sciences, Universiti Sains Malaysia, Health Campus 16150 Kubang Kerian, Kelantan, Malaysia. [2]CAS-MPG Partner Institute for Computational Biology, Shanghai Institutes for Biological Sciences, Shanghai, China.[3]Institute of Biological Sciences, Faculty of Science, Universiti Malaya, 50603 Kuala Lumpur, Malaysia.

Objectives: To determine a probabilistic genetic structure of 4 Malay sub-ethnic groups in Peninsular Malaysia which are *Melayu Kelantan, Minang, Jawa* and *Bugis* as well as to infer the population ancestry based on the single nucleotide polymorphism (SNP) microarray multilocus genotype data.

Patients and Method: The analysis of genotype data of these 4 Malay sub-ethnic groups were compiled together with 11 other populations' genotype data from Indonesia, China, India, Africa and *Orang Asli* sub-groups in Peninsular Malaysia obtained from the Pan Asian SNP Initiative (PASNPI) Database. Two major approaches which are distance-based clustering method and model-based clustering method were implemented by several specific bioinformatics tools such as PEAS v1.0, MEGA 4 and STRUCTURE v2.2. Phylogenetic tree for 4 genetic distances algorithm were constructed by Neighbor Joining method using all 54,794 autosomal SNPs encompassing the entire genome which are shared by 434 individuals. Bootstrapping was done for 1000 replications and clades with bootstrap values less than 80% were condensed. STRUCTURE analysis for 5 dataset running with 20,000 burn-in period and 20,000 MCMC iterations from K=2 to K=9 were done using admixture model and assuming that allele frequencies were correlated.

Results: All resulted phylogenetic tree performed more than 95% of bootstrap value at each node with very similar topologies. The phylogenies showed that *Jawa*, *Bugis* and *Minang* have a very close relationship and tend to cluster together with Indonesians, meanwhile the position of *Melayu Kelantan* is far apart on the tree indicated that they are not sharing the most recent common ancestral. According to the distribution of Ln Probability and estimated membership coefficient (Q) from STRUCTURE, the most probable and appropriate number of clusters in the 15 populations should be 6 (K=6) and all the studied Malay and Indonesian population sub-groups are in the same cluster but the *Melayu Kelantan* are still slightly different from the other modern Malays.

Discussion and Conclusion: The individual and population ancestry of all studied population also been inferred from the Q plot and knowledge of individual ancestry will be important for biomedical studies.

OB-15

GROWTH CHARACTERISTIC PATTERN OF NORMAL DERMAL FIBROBLASTS AND HYPERTROPHIC SCAR FIBROBLASTS IN PRIMARY HUMAN SKIN CULTURE

[1]Arffah SK, [1]Zahri MK, [2]Mahirah SY, [3]Aida Hanum G.R, [2]Halim AS, [1]Zilfalil B.A

[1]Human Genome Center, [2]Reconstructive Sciences Unit, [3]Department of Pharmacology, School of Medical Sciences, Universiti Sains Malaysia, Health Campus 16150 Kubang Kerian, Kelantan, Malaysia.

Objective: To compare the growth characteristic of human skin fibroblasts derived from normal and hypertrophic scar culture.

Patients and Method: Discarded tissues from surgical procedures in 5 patients with hypertrophic scars were sampled. In addition, a small excision biopsy of normal skin near the excised scar was obtained as a control from the same patient. Fibroblasts were maintained in DMEM supplemented with 10% Fetal Bovine Serum (FBS) and 1% Penicillin in humidified condition at 37ºC with 5% CO2. Normal human dermal fibroblasts (nHDF) and hypertrophic scar fibroblasts (hSCF) were seeded at 1 X 10^4 cells in 5ml medium in T75 culture flasks. Flasks were incubated for 1, 2, 3, 4 and 5 days and the number of viable cells of nHDF and hSCF were counted with haemacytometer according to the Trypan Blue Exclusion Test method.

Results: Mean cell count for nHDF at day 1, 2, 3, 4 and 5 were 5.9 X 10^4 cells/ml, 8.1 X 10^4 cells/ml, 12.0 X 10^4 cells/ml, 14.7 X 10^4 cells/ml and 20.4 X 10^4 cells/ml respectively. In contrast to nHDF, mean cell count for hSCF at day 1, 2, 3, 4 and 5 were 7.3 X 10^4 cells/ml,12.9 X 10^4 cells/ml, 18.1 X 10^4 cells/ml, 21.8 X 10^4 cells/ml and 26.4 X 10^4 cells/ml respectively.

Discussion and Conclusion: The calculation of cells growth showed a significant difference in growth characteristic between nHDF and hSCF culture. Samples of nHDF and hSCF were used to display a contact inhibition pattern and fibroblasts from hypertrophic scar exhibited linear growth and sustained a higher cellular viability compared to normal skin fibroblasts.

OB-16

MUTATION ANALYSIS OF DYSTROPHIN GENE IN MALAYSIAN DUCHENNE MUSCULAR DYSTROPHY (DMD) PATIENTS

Mahyoob R, Zahri MK, Marini M, Salmi AA, Siti Mardzia MD, Zabidi-Husin AMH, Zilfalil BA

Human Genome Centre, School of Medical Sciences, Universiti Sains Malaysia, Health Campus 16150 Kubang Kerian, Kelantan, Malaysia.

Objective: To develop a multiplex PCR to detect the distal hotspot of dystrophin gene in Malaysian DMD patients.

Patients and Method: The blood of 25 clinically diagnosis DMD patients from varies hospitals in Peninsula Malaysia were taken and sent to Human Genome Center. Two hundred μl of DNA were extracted using QIAGEN kit and analyzed using multiplex PCR to detect deletion in seven exons. Two sets of multiplex PCR were developed to screen these exons, which were exon 43, 44, 45, 46 and 50 for set 1, and exon 49 and 51 for set 2. These exons represent for the distal hotspots of the dystrophin gene.

Results: We detected deletions of the distal hotspot of the DMD gene in 13 patients (52%). Out of these, 4 had no family history of DMD. The most frequently deleted exons were exons 49, 50 and 51 with 20% of deletion for each exon. The remaining 12 patients did not show any deletion for these exons. The samples that had no mutations detected using our developed method are planned for further analysis of the mutation in the proximal hotspot, or the mutation might be duplication or point mutation.

Discussion and Conclusion: Multiplex PCR assay allows a rapid molecular diagnosis for DMD patients in the country. The deletion frequency of the distal hotspot in our Malaysian DMD patients is similar to the frequency of other population.

OB-17

FRAGILE X SYNDROME IS UNDERDIAGNOSED THROUGH CYTOGENETIC ANALYSIS ALONE: 6 YEARS OF HUSM EXPERIENCE

Marjanu HE, S Mariam I, Suhaida MA, N Hashimah M, M Zaki H, Noratifah MA, Zilfalil BA, Ravindran A.

Human Genome Center, School of Medical Sciences, Universiti Sains Malaysia, Health Campus 16150 Kubang Kerian, Kelantan, Malaysia.

Objectives: To detect chromosomal abnormalities of Fragile Xq27.3 in inviduals suspected to have Fragile X Syndrome using cytogenetic technique and to investigate the phenotype (clinical characters) correlates with the Fragile X Syndrome.

Patients and Method: Cytogenetic analysis were done by employing the standard method of blood culture for fragile site using folic acid deprived culture media, followed by harvesting, slide preparation, staining and karyotyping. Minimum of 20 metaphases were screened for the slides stained with G banding technique and 100 metaphases were screened for the slides stained with Unbanded-Giemsa Stain technique.

Results: This report presents the data on cytogenetic analysis carried out in Human Genome Center, USM, during 2002-2007 periods, on 39 male patients and 1 female patient suspected of Fragile X Syndrome. The phenotypic features were variable. Out of these 40 patients, Fragile Xq27.3 was detected cytogenetically in 5 patients (12.5%) only.

Discussion and Conclusion: The low percentage of Fragile Xq27.3 detection by cytogenetic analysis could be attributed to the high degree of variability in fragile Xq27.3 expression between individuals, variability among the cytogeneticist and the laboratories. Hence, Fragile X Syndrome may go under diagnosed by cytogenetic analysis alone. This report warrants the importance of the molecular studies to be performed for the accurate diagnosis of Fragile X Syndrome.

OB-18

MOLECULAR ANALYSIS TEST OF SURVIVAL MOTOR NEURON GENE IN 118 MALAYSIAN SPINAL MUSCULAR ATROPHY (SMA) PATIENTS

[1]Fatemeh H, [1]Watihayati MS, [1]Marini M, [1]Atif AB, [2]Che Badariah AA, [1]Zilfalil BA

[1]Human Genome Centre, [2]Department of Physiology, School of Medical Sciences, Universiti Sains Malaysia, Health Campus 16150 Kubang Kerian, Kelantan, Malaysia.

Objectives: To determine the frequency of SMN1 exon7 deletion in Malaysian SMA Patients and to establish the molecular analysis method for diagnosing SMA in Malaysia

Patients & Method: A total of 118 blood samples were received from August 2003 until April 2008. The samples were taken from clinically suspected SMA patients (86 malay, 17 chinese, 6 indian and 9 other,) from various hospitals in Malaysia. DNA were extracted from blood samples using DNA extraction kit and deletion analysis of exon 7&8 of SMN1 gene were done using the method described by van der Steege et al (1995). PCR product was digested with *Dra I* and *Dde I* restriction enzymes respectively and digested PCR product were analyzed by electrophoresis in 3% agarose gel.

Results: Fifty eight percent (68) of the patients fulfilled the criteria for SMA described by the International SMA consortium (1998). Out of these 68, 46% were type I SMA, 40% were type II and 14% type III SMA. Seventy eight percent (53) of these patients (22 type I, 23 type II, 8 type III) were found to have homozygous deletion of exon 7 SMN1 gene and 22% of patients (9 type I, 4 type II, 1 type III) showed the presence of exon of the SMN1 gene.

Discussion and Conclusion: SMN1 deletion has been found to be a major cause for SMA in Malaysia. SMN1 deletion analysis has been proven to be useful for establishing the diagnosis of SMA and can be used as alternative method for diagnosing SMA compared to the more invasive clinical investigations of muscle biopsy and EMG.

OB-19

DEVELOPMENT OF A CHEMICAL INVENTORY MANAGEMENT SYSTEM (CIMS) FOR THE INSTITUTE FOR RESEARCH IN MOLECULAR MEDICINE USM

Phua KK, Badrul SMZ

Institute for Research in Molecular Medicine (INFORMM), Universiti Sains Malaysia, Health Campus 16150 Kubang Kerian, Kelantan, Malaysia.

Introduction: For any chemical-using organisation that is under the scrutiny of regulatory standards, such as ISO 9001, there is a need to closely monitor the usage of chemicals, including expiry dates, inventory stock control, and safety issues.

Objective: To develop a web-based CIMS to address these issues.

Materials and Method: Filemaker Pro™ 8.5 was used to develop the relational database on a desktop computer, and following prototype testing the database was deployed on an Apple Xserve™ , that enabled an unlimited number of users access to the database on the USM network. The effectiveness of the system was evaluated based on cost-benefit analysis, as well as laboratory-user satisfaction surveys before-and-after CIMS implementation using Likert attitude rating.

Results: A total of 320 chemical records with 140 fields of data, were successfully stored in the CIMS. The server provided 24-hour access to the database which also permitted data searches, formatting and printing of summary reports with unprecedented accuracy and speed. Audit report of before-and-after CIMS indicated an 82% reduction in chemical wastage between 2006 and 2007, and client survey Likert analyses showed a significant increase ($p<0.0001$) in laboratory-user satisfaction rating from a mean of 2.0 ± 0.2 to 4.3 ± 0.2 ($n=22$).

Discussion and Conclusions: The task of ensuring ISO compliance and managing an optimal chemical inventory stock control has been made possible by a team of dedicated staff with the assistance of CIMS. CIMS represents a new medium, as a convergence of the web and PC technologies together to solve the problems in chemical inventory management.

OB-20

THE POSSIBLE ROLE OF PTGFR GENE POLYMORPHISM IN PREDICTING AN ACUTE ATTACK (AAC) OF PRIMARY ANGLE CLOSURE (PAC).

[1]Kodisvary RM, [2]Mohd Nizam Z, [2]Zilfalil A, [1]Liza Sharmini AT

[1]Department of Ophthalmology, [2]Human Genom Center, School of Medical Sciences, Universiti Sains Malaysia, Health Campus 16150 Kubang Kerian, Kelantan, Malaysia.

Objectives: To identify the presence of prostanoid receptor gene (PTGFR) polymorphism in primary or acute angle closure (PAC/AAC) patients and its role in predisposing these susceptible eyes to PAC/AAC.

Patients and Method: A comparative cross sectional study was conducted on 27 newly diagnosed PAC/AAC patients with age and sex matched 30 non-glaucoma controls. Ocular examination and biometry were done for all participants. A total of 3 cc of venous blood was obtained for genetic study. Genomic DNA was extracted using the QIAGEN DNA Mini Kit and the amplification of DNA regions were done using PCR technique. Screening for PTGFR polymorphism in 4 exons were performed using denaturing High Performance Chromatography (dHPLC). Segments with heteroduplex peak were further analyzed by DNA sequencing to confirm any nucleotide base sequence changes.

Results: We found 2 regions of polymorphism at IVS3-97A>T and EX41209A>G, with allele frequency of the IVS3-97A>T among PAC/AAC was Adenosine (A) (0.74), Thiamine (T) (0.26), in controls (A) (0.73) and (T) (0.27) (p=0.5). The allele frequency of the EX41209A>G polymorphism in PAC/AAC was (A) (0.80) and Guanine (G) (0.20), in controls it was (A) (0.77) and (G) (0.23) (p=0.3). The genotype frequency of the IVS3-97A>T polymorphism was statistically significant among AAC patients compared to PAC patients within the study group (p=0.03), this mainly consisted of the A/T genotype of the IVS3-97A>T polymorphism.

Discussion and Conclusion: PTGFR gene polymorphism at IVS3-97A>T may have a role in increasing the susceptibility of PAC patients to develop an acute attack. The mechanism through which this occurs is not known. Future research on this gene is warranted to determine its role in PAC/AAC.

OB-21

MUTATION AND ABERRANT METHYLATION OF THE DOWNSTREAM EFFECTORS AND REGULATORS OF RTK/RAS SIGNALLING IN MYELOID MALIGNANCIES

[1]Muhammad Farid bin Johan [2]Anne Goodeve, [3]Professor John Reilly

[1]Department of Hematology, School of Medical Sciences, Universiti Sains Malaysia, Health Campus 16150 Kubang Kerian, Kelantan, Malaysia. [2]Academic Unit of Haematology,School of Medicine & Biomedical Sciences, The University of Sheffield, Sheffield S10 2JFUnited Kingdom. [3]Royal Hallamshire HospitalSheffield S10 2JF, United Kingdom

Purpose : The role of downstream effectors of RTK/RAS signaling and effectors of JAK/STAT pathway were investigated to determine if point mutation is involved in pathogenesis of myeloid leukaemia. The role of negative regulators of RTK/RAS signaling were also investigated to determine if aberrant promoter methylation is also involved in myeloid malignancies.

Method : The study population consisted of a total of 326 patients diagnosed with AML, CMML, MDS and JMML. Genomic DNA was obtained at presentation from 214 cases of AML entered into the Medical Research Council (MRC) AML X and XII trials (United Kingdom). Patients were screened for mutations in NRAS, KRAS and PTPN11 genes using conformational sensitive gel electrophoresis (CSGE). Methylation specific polymerase chain reaction (MS-PCR) was employed to screen DNA promoter methylation in RASSF1A, SHP1 and SOCS1 genes. The relative expression of SOCS1 was also analysed using RQ-PCR to confirm the role of SOCS1 methylation in haematopoietic cell lines.

Result : NRAS mutation was found in 20%(8/40) of inv(16) AML and 19%(4/21) of t(8;21). KRAS mutation was detected in 3%(1/40) of inv(16) AML. PTPN11 mutation was detected in 1/67 AML and 1/5 JMML. Of 50, only one CMML patient possessed a Val617Phe mutation in JAK2 gene. Aberrant RASSF1A methylation was found in 9%(5/55) MDS and in 1/5 JMML. Aberrant SHP1 methylation was present in 11%(13/121) AML. Aberrant promoter methylation in SOCS1 was present in 11%(8/74) MDS patients. Aberrant methylation in exon 2 of SOCS1 was found in 40%(19/47) AML and was associated with the transcriptional silencing of SOCS1 gene in haematopoietic cell lines.

Conclusions : Ninety-two percent of activating mutations in AML and inv(16) were not associated with one another, thus suggesting the mutual exclusivity of RTK/RAS signalling pathway mutations. The findings lead to support the two-hit model of leukaemogenesis and moreover for the possibility of targeted therapy for patients with CBF AML especially inv(16).

OB-22

THE PROGNOSTIC SIGNIFICANCE OF ANTIGEN EXPRESSION BASED ON CYTOGENETICS IN 246 ADULT PATIENTS WITH ACUTE MYELOID LEUKAEMIA (AML)

[1]Hussein AR, [2]Richards SJ

[1]Department of Haematology, School of Medical Sciences, Universiti Sains Malaysia, Health Campus 16150 Kubang Kerian, Kelantan, Malaysia. [2]Haematological Malignancy Diagnostic Service (HMDS), 3rd Floor, Bexley Wing, St James's University Hospital, Leeds LS9 7TF, UK.

Introduction: Chromosomal abnormalities are known to be one of the most important independent prognostic factors in AML. Other parameter which may influence the prognosis is the expression of certain surface markers on the blast cell populations.

Objectives: To determine the various antigen expressions in AML and to determine the relationship between cytogenetics and antigens that being expressed.

Patients and Method: Antigen expression profiles of 13 cellular antigens of the blast cell populations from samples of peripheral or bone marrow in 246 patients were determined by a 4-colour flow cytometric immunophenotyping. The determination of the antigen positivity of the blast cell populations was based on the criteria of the cut-off point of 20%. Conventional cytogenetic analysis from bone marrow aspirate or peripheral blood was carried out in all the patients.

Results: The expression of the 13 cellular antigens in 246 patients with descending trend of CD33+, CD13+, CD117+, HLA-DR+, MPO+, CD64+, CD34+, CD15+, CD56+, CD7+, CD19+, CD14+ and CD2+ occurred in 94%, 92%, 86%, 73%, 71%, 69%, 57%, 55%, 19%, 18%, 16%, 15% and 7%, respectively. There were significant associations between CD34 expression and karyotype ($p < 0.05$), between expression of CD34 and CD7 ($p < 0.05$) and between expression of CD34 and CD56 ($p < 0.05$). However, the associations between expression of CD34 and CD19 ($p = 0.684$) and with CD2 ($p = 0.608$) were not significant. CD19+, MPO+ and CD2+ were predominantly occurred in 43%, 100% and 22%, respectively in patients with favourable outcome group with CD7+, 0.1% the least expressed. On the other hand, CD34+ was predominantly occurred in 93% of patients with adverse prognosis. Other antigens expressions were relatively variable with regard to the prognosis. Sixty eight percent of patients with t(15;17) had the composite of CD34-/CD33+/CD13+/HLA-DR-, whereas 46% and 92% of patients with t(8;21) had the phenotypes of CD19+/CD34+ and CD19+/CD15+, respectively.

Discussion and Conclusion: The three most common antigens expressed in the patients were CD33, CD13 and CD117. The expression of CD34 and CD7 were found mostly in the adverse prognostic group of patients. Individual expression of MPO, CD19 and CD2 were the three most common expressed antigens in the favourable group. The majority of AML patients with t(15;17) expressed the composite of CD34-/CD33+/CD13+/HLA-DR-, whereas the composite of CD19+/CD15+ was found in the majority of AML patients with t(8;21).

OB-23

EFFECTS OF MELATONIN SUPPLEMENTATION ON THE DEVELOPMENT OF HYPERTENSION IN SPONTANEOUSLY HYPERTENSIVE RATS (SHR)

[1]Lee SK, [1]Arun K, [1]Rahimah Z, [2]Sirajudeen KNS, [3]Singh HJ

[1]Department of Physiology, [2]Department of Chemical Pathology, School of Medical Sciences, Universiti Sains Malaysia, Health Campus 16150 Kubang Kerian, Kelantan, Malaysia.[3]Faculty of Medicine, Universiti Teknologi MARA, 40450 Shah Alam, Selangor, Malaysia.

Introduction: Hypertension has been increasingly linked with oxidative stress, and melatonin has been found to have significant antioxidant properties. The effect of melatonin on the development of hypertension however remains unclear.

Objective: To examine the effects of melatonin supplementation on the development of hypertension in SHR.

Materials and Method: Ten pregnant SHR were given melatonin in drinking water at a dose of 10mg/kg body weight from day 1 of gestation. After delivery, melatonin treatment was either terminated after 3 weeks postnatal, i.e. at weaning, or the pups continued to receive melatonin for a further 13 weeks postnatal. Gestation period, litter size and birth weight was recorded. At birth each litter was culled to 8 per dam, and only male pups were included in this study. Systolic arterial blood pressure of the pups was measured at 4, 6, 8, 12 and 16 weeks of age using tail-cuff plethysmography. Data were analyzed using Analysis of Variance (ANOVA).

Results: No significant differences were evident in gestation period, birth weight and litter size between the controls and melatonin treated rats. Systolic blood pressure in rats receiving melatonin during gestation and for 3 weeks postnatal was significantly lower till the age of 8 weeks when compared to untreated SHR. Systolic blood pressure in rats that continued to receive melatonin till the age of 16 weeks was also significantly lower till the age of 16 weeks when compared to the untreated SHR. In both groups however there was a tendency for a gradual increase in blood pressure despite melatonin treatment.

Discussion and Conclusion: It appears that antenatal and postnatal melatonin administration significantly delays the development of hypertension in SHR, but does not ameliorate hypertension completely. The precise mechanism responsible for this effect is unclear and further studies are required to confirm if the effect is via anti-oxidant properties of melatonin.

OB-24

DEVELOPMENT OF ALLELE SPECIFIC PCR FOR THE DETECTION OF HOMOZYGOUS DELETION OF THE SMN1 GENE IN SPINAL MUSCULAR ATROPHY (SMA) PATIENTS IN MALAYSIA.

[1]Marini M, [1]Watihayati MS, [1]Atif AB, [1]Fatemeh H, [2]Ravichandranm, [1]Zilfalil BA

[1]Human Genome Centre, [2]Department of Microbiology, School of Medical Sciences, Universiti Sains Malaysia, Health Campus 16150 Kubang Kerian, Kelantan, Malaysia.

Objective: To develop an alternative method using allele-specific PCR for the rapid detection of homologous deletion of the survival of motor neuron 1 (SMN1) gene and for the confirmation of the clinical diagnosis in SMA.

Patients and Method: A total of 125 blood samples were obtained from patients clinically suspected to have SMA in various hospitals in Malaysia. Genomic DNA was extracted and analyzed by PCR-RE digestion method using *Dra I* (exon 7) and *Dde I* (exon 8). Both deleted and non-deleted samples resulted from PCR-RE methods were then analyzed using Allele-Specific PCR (AS-PCR). An internal control has been derived and seeded in the AS-PCR mixture for the validation of false negative result. The PCR products were analyzed using 2% agarose gel. The cost and time of running the tests for both methods were compared.

Results: Results from both methods were compared. All samples (100%) showed the same result. The cost for running PCR-RE was RM286 while the cost for AS-PCR was RM118. The AS-PCR method was able to complete within 3 hours while PCR-RE took longer than 5 hours.

Discussion and Conclusion: The AS-PCR method is more rapid and cost effective which the cost was reduced by about 58%, compared to PCR-RE method. This method only required a single PCR step and it has been developed upon a single nucleotide polymorphism in the exon 7 of the SMN1 and SMN2 gene.

OB-25

MUTATIONAL ANALYSIS OF CREB BINDING SITES IN SMN2 GENE

Atif AB, Watihayati MS, Fatemeh H, Marini M and Zilfalil BA

Human Genome Centre, School of Medical Sciences, Universiti Sains Malaysia, Health Campus 16150 Kubang Kerian, Kelantan, Malaysia.

Objectives: To analyze the bioinformatics characteristics of the SMN2 (Survival of Motor Neuron) promoter region and to determine the mutational analysis of the CREB binding sites within the promoter region of the healthy individual and clinical types of SMA (Spinal Muscular Atrophy).

Patients and Method: Three SMA patients (Type I, II and III) with homozygous deletion of the SMN1 genes were subjected to the mutational analysis. The cloned amplified PCR products with in the pTOPO 2.1e cloning vector were subjected to direct sequencing. The sequences from the samples of healthy and the clinical types were aligned through CLUSTALX and were presented as Gene Doc file.

Results: Total of 39 ORFs (Open Reading Frames) contained 15 TATA box sequences reflecting the diverse function integrity of SMN promoter region. Out of these 15 TATA boxes, 11 were TATA, 2 tata and 2 Goldberg-Hogness sequences. The positive strands', essential Cis element binding sites were LSF, ERE, Tef, Sp1 and CRE and on the negative strand were Mef2, ERE, Ets, AP1 and SRF. The mutational analysis of the CREB binding sites in healthy control and the clinical types of SMA revealed that there were no mutations detected in any of the clinical types.

Discussion and Conclusion: We characterized SMN2 promoter region using bioinformatics soft wares. There was no mutation detected in the CREB binding sites within any of the clinical types of SMA in our HUSM SMA patients' promoter regions.

OB-26

SAFETY EVALUATION OF ETHANOLIC EXTRACT OF ANDROGRAPHIS PANICULATA ON TESTICULAR HISTOLOGY IN MALE RATS

[1]Dasuki MS, [2]Siti Amrah S, [3]Hasnan J, [4]D' Souza UJ, [1]Mohsin SSJ

[1]School of Health Sciences, [2]Department of Pharmacology, [3]Department of Pathology, School of Medical Sciences, Health Campus, 16150 Kubang Kerian, Kelantan, Malaysia. [4]School of Medical Sciences, Universiti Malaysia Sabah, Kota Kinabalu, Sabah, Malaysia.

Introduction: The usage of *Andrographis paniculata* (AP) as a traditional medication is still wrought with controversy. There are multiple claims that AP has anti-viral, anti-cancer, anti-thrombotic as well as anti-diabetic properties. However, various researchers had reported that AP also has anti fertility effects and show some toxicity. Therefore, investigation into its possible toxicity on testicular histology become extremely important as this may affect the normal functioning of male reproductive system.

Objective: To evaluate the effects of 50% ethanolic extract of AP on testicular histology using qualitative and quantitative techniques in male rats.

Materials and Method: Fifty male rats were given a 50% ethanol extract of AP (APE) by gavaging for more than 77 days, while another 10 male rats were given distilled water. Post male reproductive performance (MRP) and male sexual behavior (MSB) procedures, the male rats were sacrificed and testes were collected. Histological evaluations of testes were then done using light microscope and Image analyzer.

Results: The qualitative observation of the testes showed minor changes in terms of interstitial oedema and Leydig cell hyperplasia. However, no significant changes in tubular differentiation index (TDI), seminiferous tubular diameter (STD) and seminiferous epithelium height (SEH) were observed among APE treated groups when compared quantitatively to the control group.

Discussion and Conclusion: APE did not adversely affect the testicular histology of the male rats.

OB-27

AN INNOVATIVE DIAGNOSTICS DATABASE FOR TYPHOID FEVER

Phua KK, Foong SY, Amy AA, Norhafiza MN, Siti Norazura M, Asma I

Institute for Research in Molecular Medicine (INFORMM), Universiti Sains Malaysia, Health Campus 16150 Kubang Kerian, Kelantan, Malaysia.

Introduction: An important strategy for irradication of typhoid fever include a centralized database for storage, retrieval and processing of laboratory results from patients during an acute attack, and follow-up investigations to identify the carriers of the disease.

Objective: To develop a relational database for this purpose.

Material and Method: Filemaker Pro™ 8.5 was used to develop the database according to user requirements. An aesthetically appealing graphical user interface was designed to enable easy navigation and usage of the database.

Results: A total of 531 patients admitted to various hospitals and clinics in Kelantan during the April 2005 typhoid outbreak, and 554 suspected carriers from the Kelantan Public Health Department in June 2007, were included in the study. Subject biodata and serological, microbiological, biochemical and PCR test results were entered in fields on layouts that use pull-down menus, check-boxes and value-lists to ensure error-free data entry. Algorithms developed to cater for specific user requirements, such as laboratory test sensitivity and specificity calculations, results reporting on standard proforma, statistical analysis, and integrated charting for project monitoring; all worked flawlessly.

Discussion and Conclusion: Manual paper-ledger systems are incapable of meeting the demands of a modern diagnostic research laboratory. An aesthetically appealing relational database that is user-friendly, with automation of reports, statistical analyses and customizable functions, all together provided a positive user experience and improve efficiency.

OB-28

PRELIMINARY REPORT OF STUDY OF LEPTIN AND ASSOCIATED RISK FACTORS IN BREAST CANCER

Karami A, Arunkumar S, Sirjudeen, Zainal Mohmood KNS, Hasnan J, Shariza AR, Wan Manan, Than Win, Ravichandran M, Kavitha M and Bhavaraju VMK

Universiti Sains Malaysia, Health Campus 16150 Kubang Kerian, Kelantan, Malaysia.

Introduction: In 2002, about 4332 new cases of breast cancer have been reported by the National Cancer Registry Malaysia and carcinoma is the commonest breast cancer among women in Malaysia. Leptin has been proposed as a risk factor for breast cancer via stimulating regulation of neoplastic cells primarily by activation of estrogen receptors. However the association between circulating leptin levels and breast cancer risk still unclear.

Objectives: To assess the levels of serum leptin, lipid profiles and total antioxidant status, and to measure the estrogen and progesterone receptors and Cerb-2 genes in breast cancer patients.

Patients and Method: In this pilot study, a total of 20 patients comprising of 10 breast cancer patients and 10 controls were recruited. All the subjects were taken up for demographic information, anthropometric measurements, dietary intake, biochemical parameters involving serum total leptin levels, lipid profile, total antioxidant levels. Molecular parameters like estrogen and progesterone receptors and C-erb2 genes were estimated. In the study group, the clinical features and stage of breast cancer were documented.

Results: The mean age of study group was 43.9 and control was 34.5 years. The dietary analysis showed more body fat, fat intake and waist hip ratio in breast cancer patients compared to the controls. The leptin levels were lower in breast cancer patients compared to control subjects. The biochemical results were same in both study group and also control group. Ninety percent of patients were diagnosed in advanced stage of disease.

Discussion and Conclusion: Our preliminary results indicated that both control group and study group had same anthropometric measurements, lipid profile and biochemical parameters. However, the study group had higher fat intake and body fat compared to the control group.

OB-29

ST14 VNTR POLYMORPHISM IN THE CARRIER DETECTION OF HEMOPHILIA A IN PAKISTANI POPULATION

[1]Beenish Arif, [2]Atif AB, [1]Saqib M, [1]Naeem M

[1]Department of Microbiology and Molecular Genetics, University of the Punjab, Pakistan.
[2]Human Genome Centre, School of Medical Sciences, Universiti Sains Malaysia, Health Campus 16150 Kubang Kerian, Kelantan, Malaysia.

Objective: To determine the St14 VNTR in a cohort of Pakistani Punjabi hemophilic patients and normal controls.

Patients and Method: Venous blood was taken with consent of families. DNA was extracted using inorganic method. The PCR-based analysis of St14 VNTR (DXS52) was carried out in order to determine allele frequency and the carrier status. 78 blood samples (Hemophiliac=23, Normal=55) from 15 families were analyzed to determine the St14 VNTR. The allele frequencies were calculated and Hardy-Weinberg equilibrium was estimated.

Results: A total of nine alleles (2400, 2100, 1750, 1690, 1630, 1570, 1390, 1300, 1220 bp) were detected in the pool of subjects. Nineteen (19) out of 40 females were found to be carriers with respect to the St14 VNTR polymorphic marker. The marker was informative in 73.33% of families. The expected heterozygosity rate of the St14 VNTR was 0.86 while the observed heterozygosity was 0.7.

Discussion and Conclusion: St14 VNTR is 70% informative in the Pakistani (Punjabi) population, allowing it to be a useful marker in carrier detection, as informativeness is the direct reflection of heterozygosity of a polymorphic marker.