POSTER BASIC SCIENCES SECTION

PB-1

THE RELATIONSHIP BETWEEN ANTI APOPTOTIC MARKER (BCL-2) AND BIOCHEMICAL MARKERS IN TYPE 2 DIABETES PATIENTS

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Purpose :
To investigate the expression of anti apoptotic marker (bcl-2) and the level of biochemical markers in type 2 diabetes patients.

METHODS :
A cross-sectional study was conducted from August 2003 to November 2005. Forty one type 2 diabetes patients and 36 non diabetes (control) subjects aged between 20 to 70 years were included in this study. Blood samples were collected for fasting plasma glucose (FPG), triglycerides (TG), Total cholesterol (TC), High density lipoprotein cholesterol (HDLC), Low density lipoprotein cholesterol (LDLC) and analyzed in the Chemical Pathology laboratory, while glycosylated hemoglobin A_{1c} (A1C) was analyzed in the Endocrine laboratory. The skin biopsy tissue samples were stained with immunohistochemistry (IHC) stain for expression of bcl-2 in the Pathology laboratory.

RESULTS :
There was a significant difference ($p<0.001$) between both groups for mean FPG (diabetics=11.02±4.25, control=4.41±1.12 mmol/L), HDLC (diabetics=1.00±0.38, control=1.47±0.72 mmol/L) and A1C (diabetics=9.50±2.24%, control=5.00±0.67%). However, there was no significant difference for TG, TC, and LDLC between both groups. Interestingly, the difference of mean bcl-2 expression were very highly significant ($p<0.001$) when compared between both groups. Mean bcl-2 expression was dibetics= 1.88±0.33 and control= 1.47±0.51. Positive bcl-2 expression was found in only 5 (12.2%) diabetics while 36 (87.8%) diabetics showed negative expression. Positive bcl-2 expression was observed in 19 (52.8%) controls while 17 (47.2%) showed negative expression.

CONCLUSION :
The expression of anti apoptotic marker bcl-2 was increased in non diabetic subjects in order to prevent cell death. However, the reduced expression of bcl-2 in diabetic patients may be associated with programmed cell death. The detailed mechanism for the gene expression of bcl-2 may help us to understand how bcl-2 is involved in apoptosis in diabetic microvasculature complications.

PB-2

A STUDY OF RHESUS POSITIVE PHENOTYPES / GENOTYPES AMONG REGULAR BLOOD DONORS OF DIFFERENT ETHNIC GROUPS IN HOSPITAL UNIVERSITI SAINS MALAYSIA, KELANTAN

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PURPOSE :
The rhesus blood group system was discovered in 1940 by Levine and Stetson; they characterized 85% of the population as rhesus positive and 15% as rhesus negative. Approximately more than 99.2% of the blood donors in Hospital Universiti Sains Malaysia, Kelantan are Rhesus (D) positive. The frequencies of rhesus phenotype/ genotype vary from one population to another. Because of high immunogenic, it is very importance to transfuse RBC with the same rhesus genotype particularly those who require regular blood transfusion such as thalassaemia patient. The objective of this study was to determine the rhesus phenotype/ genotype among Malay and Chinese blood donors in Hospital Universiti Sains Malaysia.

METHODS :
Two mls of donor's blood were collected into EDTA container. Rhesus phenotyping were performed using tube method. Anti-D, -C, -e and -Cw were obtained from Dominion Biological.

RESULTS :
A total of 100 Malay and 100 Chinese regular blood donors were studied. The rhesus genotypes distributions among the Malay were R1R1 (62%), R1R2 (19%), R1r (8%), R1Rz(5%), R2r(3%), R2Rz (2%), and Ror (1%). R2R2 and RzRz were not detected among the Malay. Whereas the rhesus genotypes distributions among the Chinese were R1R1 (55%), R1R2 (24%), R1r (4%), R1Rz(5%), R2r(2%), R2R2 (9%), and RzRz (1%). R2Rz and Ror were not detected among the Chinese.

CONCLUSION :
Our results showed that the commonest rhesus genotype among the Malay and Chinese are R1R1 and followed by R1R2. Others rhesus genotypes were less than 10%. This data is important for the Transfusion medicine Unit to make a good planning for blood transfusion management.

PB-3

INFLUENCE OF STRESS ON TESTOSTERONE LEVELS AND ITS EFFECT ON TESTICULAR MORPHOLOGY

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INTRODUCTION :

The noise stress, after it passes through the hearing apparatus, not only affects the auditory areas of brain but also other body functions through its numerous neural connections. Noise stress inhibits the production of serotonin, which has effect on sperm count and causes piddling ejaculation in human. This implies that the reproductive system is also indirectly vulnerable to noise stress. Our aim is to determine the influence of noise stress on male hormone and to find out changes in testicular morphology

METHODS :

Male albino rats weighing 200 to 250 gms were used for this study and were grouped as acute and chronic. In acute group, the rats were exposed to 120 dB of noise for 1hr, 2hrs and 3hrs. In chronic group, the rats were exposed to 120 dB of noise for 30 days, 45 days and 60 days.There were 6 animals in each group The control groups were also treated like experimental group in all aspects except exposure to noise. The blood samples were collected after the experiment by retro-orbital puncture for hormonal study and the rats were dissected out and testis were collected, weighed and washed in ice-cold saline and fixed in 10% formal saline. The tissues were processed and embedded in paraffin wax, sections were taken and examined under light microscope.

RESULTS :

There was significant reduction in serum testosterone levels in acute and in chronic groups. The architecture of arrangement of semenifereous tubules showed shrinkage, and this is well pronounced in the center core rather than periphery, maturation arrest in some germ cells , thinning of basement membrane and tubular degeneration.

CONCLUSION :

120dB of noise stress of varying duration had its effect on rat testicular morphology and the serum testosterone levels were decreased. These results suggest that the feed back action of testosterone on the hypothalamus and pituitary gland may be impaired. This present findings reveal the fact that noise stress is a potential threat to the propagation of human species. Adequate measures are urgently required to save humanity from this disaster.

PB-4

NERVE CELL GROUPS IN THE VENTRAL GREY HORN OF CERVICAL SPINAL CORD OF RAT

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Purpose: There is considerable disagreement regarding the arrangement of neuron somata in the ventral grey horn of cervical spinal cord in many mammalian species including rat and this prompted the present study. The aim of this study is to study the groups of neuron somata and their longitudinal extent in ventral grey horn of cervical spinal cord.

Methods: Ten Sprague-Dawley rats were used in the study. They were killed, circulation flushed with normal saline and perfused with 10% formal-saline. After perfusion, the cervical segments of spinal cord were embedded in paraffin wax, serial transverse sections cut at 40 micrometers and stained with thionine. From serial transverse sections, a reconstruction of cell groups of ventral grey horn of each cervical segment was made.

Results: The neuron somata of ventral grey horn were arranged in three groups, lateral, central and medial. The lateral group included three longitudinal cell columns: ventrolateral (VL), dorsolateral (DL), and retrodorsolateral (RDL). The VL extended from the cranial end of C-2 (second cervical segment) to caudal end of C-8. The DL extended from the cranial end of C-4 to caudal end of C-8 whereas the RDL occupied the whole length of C-8. The central group was represented by a single longitudinal column which had two parts, cranial and caudal. The cranial part was present in the caudal three-fourths of C-1 and whole length of C-2, whereas, its caudal part was present in the whole lengths of C-4 and C-5. The medial group included two longitudinal columns, the ventromedial (VM) and dorsomedial (DM). Both these columns extended from cranial end of C-1 to caudal end of C-8.

Conclusion: In the ventral grey horn of spinal cord of rat, the neuron somata are arranged in three groups of longitudinal columns, lateral, central and medial. No laminar arrangement of these neuron somata was found.

PB-5

LOCALIZATION OF MOTOR NEURON SOMATA OF THE SPINAL PART OF ACCESSORY NERVE IN RAT

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PURPOSE :
Motor neuron somata of spinal part of accessory nerve supply sternocleidomastoid and trapezius muscles and form group(s) of neuron somata called spinal nucleus of accessory nerve (SNA). There are many conflicting views regarding the longitudinal extent and topography of SNA. Some investigators have localized it in upper cervical segments of spinal cord whereas others have located it in lower part of medulla oblongata also. Our aim in this study is to locate the site and longitudinal extent of SNA.

METHODS :
Twelve Sprague-Dawley rats were used in the study. Under general anaesthesia (Nembutal sodium, intraperitoneally), the trunk of right accessory nerve was exposed in the neck before its entry into sternocleidomastoid and a part removed to prevent re-union. After 21 to 28 days, the animals were killed, circulation flushed with normal saline and perfused with 10% formal-saline. The medulla oblongata and first (C-1) to sixth (C-6) cervical segments of spinal cord were removed, embedded in paraffin, serial transverse sections cut at 40 micrometers and stained with thionine. Sections were examined microscopically to identify chromatolysed neuron somata and to compare the experimental right side with control left side.

RESULTS :
Chromatolysed neuron somata were located in caudal part (caudal 0.9-1.2 mm) of medulla oblongata, the whole lengths of C-1, C-2, and C-3, C-4 and C-5 and rostral-fourth of C-6. In medulla oblongata, they were located at a site immediately ventrolateral to pyramidal fibres that pass dorsolaterally after decussation. In C-1, they were located in dorsomedial and central columns of ventral grey horn. In C-2, they were located in dorsomedial, central and ventrolateral columns. In C-3, C-4, C-5 and rostral-fourth of C-6, they were located in ventrolateral column.

CONCLUSION :
The motor neuron somata of spinal part of accessory nerve of rat are located in caudal part of medulla oblongata and upper six cervical segments of spinal cord.

PB-6

C-KIT EXPRESSION IN SYNOVIAL SARCOMAS

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PURPOSE :
c-Kit is a 145 kDa transmembrane tyrosine kinase encoded by the c-Kit proto oncogene. c-Kit acts to regulate a variety of biological responses including cell proliferation, apoptosis, chemotaxis and adhesion. Mutations in c-Kit have been found to be important for tumor growth and progression in a variety of cancers. Synovial sarcoma is so named because of its resemblance to developing synovial tissue under light microscopy. It arises from pluripotential mesenchymal cells near joint surfaces, tendons, tendon sheaths, juxta-articular membranes, and fascial aponeuroses. This study was undertaken to find out the presence of c-Kit in synovial sarcomas in our setup.

METHOD :
A total of 28 cases of synovial sarcomas including referred have been reported in our hospital from 1996 till 2005. 22 blocks, which were available, retrieved from the archives and immunohistochemical staining for c-Kit was performed on one block in each case. The c-Kit antibodies used were from DAKO. Recommended dilutions and recommended procedures were followed. The stain was considered positive if there was visible cytoplasmic or membranous staining. When only a few cells were unequivocally positive it was called focally positive.

RESULTS :
It was found that only 7 of the 22 cases were positive for c-Kit and also the positivity was only focal and seen in spindle cells. Of these 7 cases 5 were monophasic type of synovial sarcoma and the other two were of the biphasic type.

CONCLUSION :
The results of our study are in keeping with the earlier published reports but the difference is that there is more number of monophasic types of synovial sarcomas being positive when compared to the earlier published reports. As there is a highly effective and specific drug available to treat malignancies where c-kit gene is present. More studies have to be done to find out which of these malignancies are c-Kit positive so that appropriate treatment can be administered.

PB-7

CONSTRUCTION OF VACIV: TOWARDS THE DEVELOPMENT OF DNA VACCINE AGAINST TUBERCULOSIS

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PURPOSE :
The failure of Bacille Calmette Guerin (BCG) and variable efficacy of protection in certain population against multi-drug resistance *Mycobacterium tuberculosis* strain has led to the development of more effective vaccine candidate. Therefore, we had constructed a multi-epitopes DNA vaccine encoding Mtb8.4, 30kDa (Ag85B) and 32kDa (Ag85A) genes of *Mycobacterium tuberculosis* as a vaccine strategy against Tuberculosis (TB).

METHOD :
In this study, we had performed an assembly polymerase chain reaction (PCR) technique to assemble an 34 overlapping oligonucleotides to produce a synthetic gene, designated as VacIV gene, sized 0.7kb. The VacIV gene was then cloned into a cloning vector, pTOPO for dioxyribonucleotides acid (DNA) sequencing and restriction enzyme analysis. The VacIV gene was digested with *Sac*I and *Xba*I restriction enzymes and subcloned into pROEX Htb which contained 6XHistidine gene to develop a recombinant plasmid designated as pROVacIV.

RESULT :
The DNA sequence of the cloned VacIV gene was confirmed by DNA sequencing analysis. For the expression study, the pROVacIV was transformed into *Escherichia coli* DH5_ strain. Immunoblotting analysis showed that, a 30.4kDa VacIV fused with 6XHistidine gene was successfully expressed in *Escherichia coli*.

CONCLUSION :
This DNA vaccine candidate is being tested for its immunogenecity in mice for further development as a potential vaccine candidate against TB.

PB-8

A REVIEW OF THE USEFULNESS OF ACETYLCHOLINESTERASE ENZYME (ACHE) HISTOCHEMISTRY IN THE DIAGNOSIS OF HIRSCHSPRUNG'S DISEASE

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INTRODUCTION :
Hirschsprung's disease is a congenital condition affecting newborns resulting from the absence of ganglion cells in the distal portion of the recto-sigmoid area. Rectal suction biopsy is an investigation used in the diagnosis of such condition in infants 1 year and below, while open rectal biopsy is employed for children above the stated age. Acetylcholinesterase enzyme (AchE) histochemistry is used to demonstrate the presence of abnormal nerve fibres in such biopsies. It is currently the main method used in our institution. However, AchE results are difficult to interpret and can be confounded by many external factors such as the freshness of the tissue and adequacy of the biopsy. Our aim was to review the AchE histochemistry results in order to evaluate the usefulness of this stain in the diagnosis of Hirschsprung's disease.

METHODS :
This is a cross-sectional study. Reports of all AchE histochemistry on rectal biopsy cases in the year 2005 were retrieved. The subsequent histopathological examination of the cases was then reviewed and compared with the AchE enzyme histochemistry result. Histopathology diagnosis was taken as the gold standard.

RESULTS :
There were 65 AchE examination performed during the period on 65 separate rectal biopsies taken from 57 patients. The gender distribution is approximately equal (28 girls and 29 boys). Age range is between 3 days of life to 5 years, with a mean age of 6.5 months. AchE histochemistry was reported as positive in 24 cases, negative in 22, equivocal in 11 and non-contributory in 8 cases. Seventeen cases had subsequent histopathological examination, where 13 were confirmed as Hirschsprung's disease. Of these, 8 had positive AchE study and 3 were negative. Two other cases had equivocal and non-contributory results. Sensitivity of the test was 81.82%; specificity 66.67% and false positive rate is 11.1%. The reasons behind these problems are discussed.

CONCLUSION :
Acetyl cholinesterase enzyme histochemistry is difficult to interpret. Nevertheless, it has a fairly high sensitivity for the diagnosis of Hirschsprung's disease, although the specificity is rather low. Based on this evaluation a few recommendations are made to improve the diagnosis of Hirschsprung's disease in this institution.

PB-9

POST-OCCLUSIVE REACTIVE HYPEREMIA MODEL TO ASSESS MICROVASCULAR FUNCTION – A DESCRIPTIVE STUDY

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PURPOSE :
The process of post occlusive reactive hyperaemia [PORH] using laser doppler fluximetry [LDF] is a non invasive method to assess microvascular function. This study describes the process and results obtained in 178 healthy subjects.

METHOD :
This prospective study involved 72 male and 106 female subjects between the ages of 18 – 40years. Females were studied between days 1 - 5 of their menstrual cycle. Subjects were studied in the supine position while fasted. Forearm blood flow was occluded at a supra-systolic pressure of 200mmHg for 3 minutes. Skin blood flow [SBF] was monitored before, during and for 2 minutes after occlusion release using the LDF. This process was performed twice [separated by 15 minutes], average of 2 readings was recorded. Results are presented as mean±sem.

RESULTS :
Baseline SBF was 10.17±0.28AU, maximum SBF post occlusion was 59.37±1.54AU. There was a 5.8 fold increase in SBF upon occlusion release; mean change in SBF was 49.20±1.42AU. Significant differences were seen between males and females in their baseline [10.90±0.49 vs 9.64±0.32AU, $p=0.026$] and change in SBF [57.306±2.33 vs 42.785±1.33AU, $p<0.001$] after PORH.

CONCLUSION :
The described process of PORH produced a 5.8 fold increase in SBF. Significant differences were seen between males and females in their baseline, and change in blood flow due to occlusion. These differences may be due to the higher systolic blood pressure and body mass index in the males. Follow up study will assess the PORH response to polymorphism at the nitric oxide synthase gene.

PB-10

BIOCHEMICAL AND HISTOLOGICAL STUDIES ON THE TOXICITY OF AQUEOUS EXTRACT OF LEAVES OF PHYLLANTHUS AMARUS, *IN RAT KIDNEY*

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PURPOSE :
Phyllanthus amarus (P.amarus), herbal species of *Euphorbiaceae* family is widely used in traditionally in the treatment of diabetes, hepatitis, dysentery and uro-genital disorders. However, toxicity study on this plant is rare and not well documented. Earlier we have reported the non-toxic nature of this plant in rat liver. Kidney is also an organ suspected to be vulnerable for damage on herbal administration, so the aim of this study was to assess the toxicity effects of aqueous extract of leaves of *P.amarus* (grown locally) on rat kidney by biochemical and histological evaluation.

METHOD :
Male and female Sprague-Dawley rats were used as experimental animal and the *P.amarus* extract was administered orally by gavage at the doses of 0 (control), 100 and 800 mg/Kg body weight/day for six weeks. Animals sacrificed at the end of experimental period and serum was analyzed for renal function tests (urea, creatinine, sodium, potassium, calcium and phosphate). Kidney tissues were fixed in 10% formal saline for histological examination (Hematoxylin and Eosin stain).

RESULT :
No significant changes ($p>0.05$) were observed between control and *P.amarus* extract administered rats in the biochemical parameters studied and histologically also no observable changes were found between them.

CONCLUSION :
This study suggests the non-toxic nature of *P.amarus* aqueous extract in rat kidney.

PB-11

PREMINILARY STUDY ON APOLIPOPROTEIN E EPSILON 4 IN HEMORRHAGIC STROKE

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PURPOSE :
Stroke is a major neurological disease in Malaysia that causes death and disability. In the average Malaysian population, the annual incidence of new stroke is 2 per 1,000 people. The objective of this study is to review DNA analysis of apolipoprotein E epsilon 4 used to assess hemorrhagic stroke patients.

METHOD :
The diagnosis of hemorrhagic stroke was based on clinical data and in the computed tomographic (CT) scan study. Molecular investigation of apolipoprotein E epsilon 4 included extraction from peripheral blood lymphocytes, followed by polymerase chain reaction (PCR) and direct sequencing. The PCR condition included denaturation for 2 minutes at 95^0C, annealing for 2 minutes at 65^0C and extension for 2 minutes at 72^0C. The cycles were repeated 30 times.

RESULTS :
The apolipoprotein E epsilon 4 were evaluated in patients with hemorrhagic stroke and controls. There were no mutations detected by using PCR- direct sequencing. The DNA sequencing results in all hemorrhagic stroke samples showed that all base sequences were normal.

CONCLUSIONS :
In this study, the presence of the epsilon 4 allele was not associated with hemorrhagic stroke. Stroke is determined by multiple factors, both genetic and environmental.

PB-12

DETECTION OF CYCLIN D1 *GENE ALTERATIONS USING DENATURING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (DHPLC)*

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PURPOSE :
To establish denaturing High Performance Liquid Chromatography (denaturing HPLC) method as a screening tool for detecting exon 4 and exon 5 alterations in *Cyclin D1* gene.

METHODS :
Fifteen gliomas and fifteen meningiomas samples were extracted using QIAamp tissue extraction kit prior to Polymerase Chain Reaction (PCR). The PCR products were prepared prior to screening analysis by performing slow re-annealing method to allow the formation of heteroduplex peaks. Intended for the purpose of detection of exon 4 and exon 5 (*Cyclin D1* gene) gene alterations, samples were analyzed using denaturing HPLC (VarianInc, USA) under 62^{o}C and 66^{o}C respectively. Samples which showed heteroduplex peaks during the screening were sent for DNA sequencing to confirm the allelic involved in the alterations.

RESULTS :
Based on the portion elution profiles, 2 (13.3%) out of 15 gliomas and 3 (20.0%) out of 15 meningiomas samples of exon 4 showed heteroduplex peaks. No heteroduplex was observed in exon 5 of the gene. From the DNA sequencing results, all samples showed C to T allelic variation at codon 223 (Lys223Lys), three samples showed T to C transition at codon 215 (Aspartic acid215Gly), 2 showed T to C transition at codon 217 (Aspartic acid215Gly) and 1 sample showed G base deletion at codon 214 (Pro214Arg).

CONCLUSION :
From the results, we suggest that denaturing HPLC is a sensitive method in detecting *Cyclin D1* gene alterations. Changes detected in the gene sequence propose that normal functions of Cyclin D1 protein might be affected due to the gene abberations.

PB-13

DETECTION OF MITRAGYNINE FROM MITRAGYNA SPECIOSA *KORTH CRUDE EXTRACT BY USING GAS CHROMATOGRAPHY MASS SPECTROMETRY (GC-MS) FOR LEARNING AND MEMORY PROCESS: PRELIMINARY RESULT*

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PURPOSE :
To confirm the presence and percentage of mitragynine from *M. speciosa* Korth crude methanol extract and to test the effect of *M. speciosa* Korth crude methanol extract on the animal learning and memory process.

METHODOLOGY :
The leaves were dried in an oven at 50°C for 12 hours. The dried leaves were then grinded into powder by a mill machine and the powder was then weighed. The powdered leaves underwent soxlet extraction using methanol for 4 hours. The brownish extract was concentrated under reduced pressure at 40°C using rotary evaporator and the resultant methanol leaf extract was screened for the presence of the alkaloid mitragynine using GC-MS.

RESULTS :
More than 23 peaks were detected after running the crude extract sample for 40 minutes in GC-MS. The suspected mitragynine peak was noted at a retention time of 31.99 minutes. The suspected mitragynine peak was identified using the peaks MS spectrum by matching it with the NIST 02 Library and confirmed by matching the retention time with the mitragynine standard provided by the Institute of Medical Research. At the same retention time, Corynan-16-carboxylic acid,16,17-didehydro-9,17-dimethoxy-,methylester, (3.beta.,16E)- and Corynan-16-carboxylic acid,16,17-didehydro-9,17-dimethoxy-,methylester, (16E)- were simultaneously detected. Using the above method, the crude extract of *M. Speciosa* Korth was found to contain approximately 30.802% mitragynine as compared to the other alkaloids.

CONCLUSIONS :
Previously, the crude extract crystal was not properly formed due to the incomplete drying process. In this experiment, the incomplete dry crude extract was further dried at 80°C for 3 hours. The GC-MS chromatogram showed that the percentage of mitragynine in the crude extract contained enough mitragynine to run animal behavior test.

PB-14

SOLVENT PARTITIONING FROM C. ASIATICA METHANOL EXTRACTS USING TLC TECHNIQUE FOR PATCH CLAMP STUDY

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PURPOSE :
C. asiatica from family Umbelliferae is considered a vital in Ayurvedic medicine where it is used to stimulate learning and memory capabilities. To date, the trends move towards extracting *C. asiatica* for learning and memory experiments. In this study, we had successfully determined the best solvent using solvent partitioning to isolate Asiatic acid from *C. asiatica* methanol extract with Thin Layer Chromatography (TLC) technique. This Asiatic acid compound will be used in patch clamp technique and animal behavioral test to determine the effect of learning and memory on rat hippocampus. Our aim is to determine the best solvent partitioning to detect Asiatic acid from *C. asiatica* using methanol extraction for patch clamp and animal behavioral test in animal model in future.

METHODS :
C. asiatica was purchased from a market in Kuala Terengganu and the sample was authenticated for their correct botanical identity by herbal expertise from KUSTEM. Then, the extract of *C. asiatica* was prepared according to the methanol extraction method which previously described by Jayashree *et. al* 2003 with certain modifications. Solvent partitioning from 4 solvent; Hexane, Chloroform, Ethyl acetate and n-Butanol was perform using methanol extraction. Then, Thin Layer Chromatography was executed to detect Asiatic acid from 4 solvent with reference of Asiatic acid, purchased from Sigma.

RESULTS :
Asiatic acid successfully detected from *C. asiatica* extracts with solvent partitioning from n-Butanol and another 3 solvent absent.

CONCLUSIONS :
Previously, the best solvent to isolate Asiatic acid was a big matter in getting the best solvent from *C. asiatica* methanol extract. Finally, we decided to make 4 solvent, from high polarity to non polarity solvent to determine the best solvent. Eventually, we had successfully determined the best solvent was n-Butanol after sprayer with Adenisaldehyde spray reagent. With this finding, solvent n-Butanol showed promising results in obtaining good Asiatic acid for patch clamp and animal behavioral test in animal models in the future.

PB-15

APOLIPOPROTEIN E3 AND NEUROPSYCHOLOGICAL OUTCOME POST TRAUMATIC BRAIN INJURY: AN INITIAL REPORT

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PURPOSE :
To determine whether apolipoprotein E3 (APOE-3) genotype influences neuropsychological outcome in patients with mild and moderate traumatic brain injury (TBI).

METHODS :
The preliminary data comprised 9 mild and moderate patients (one 18-year old female and 8 males, 30.50±6.33 years) diagnosed with TBI based on the Glasgow Coma Scale at the time of admission and resuscitation. Blood samples (2cc) were analyzed for the APOE gene utilizing the polymerase chain reaction (PCR) method. Executive function, verbal learning and memory, verbal fluency, and abstract reasoning were assessed twice at approximately 6 weeks and 6 months post injury. Data were analyzed using a 1-way ANOVA with repeated measures. Distributional characteristics were assessed by the Kolmogorov-Smirnov test and the coefficients for skewness and kurtosis. For normally distributed data, a Bonferroni-corrected alpha was used to determine the differences between the psychological measures.

RESULTS :
Executive function improved from pre- to posttest (36.67±13.30 vs. 42.67±13.77, $p=0.030$, $eta^2=0.464$) but this was no longer significant after the Bonferroni correction. There were no differences over time in verbal learning and memory (38.56±11.43 vs. 39.78±11.09, $p=0.363$, $eta^2=0.104$), verbal fluency (19.00±9.86 vs. 20.44±7.56, $p=0.457$, $eta^2=0.071$), and abstract reasoning (27.67±7.04 vs. 25.67±6.80, $p=0.279$, $eta^2=0.144$).

CONCLUSIONS :
It is encouraging that the executive functioning score improved over time, which would have been statistically significant with a larger sample size although the effect size is small. The initial results seem to agree with the current findings, i.e., there might be no clear APOE genotype influence on the recovery curves of patients with mild and moderate TBI.

PB-16

IMMUNOGENICITY OF SYNTHETIC ENTEROVIRUS 71(EV71) DNA VACCINE CANDIDATES

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PURPOSE :
Enterovirus 71 (EV71) is known as one of the major causative agents of hand, foot and mouth disease (HFMD). Furthermore, EV71 infection sometimes is associated with serious central nervous system diseases such as aseptic meningitis, encephalitis and polio-like paralysis. In Malaysia, the first epidemic occurred in 1997 in Sarawak and caused thirty-four deaths from the severe neurological syndrome. Our group is exploring the development of a candidate vaccine involving the construction of a synthetic VP1 gene of the EV71 fused to a a ubiquitin gene, for expression in a strong eukaryotic promoter, pVax (invitrogen) in the format of DNA vaccine.

METHODS :
In this project we evaluated the immunogenicity of constructed DNA vaccine in mice. Two delivery methods of DNA vaccines were used, DNA vaccine (designated as PVaxUbGRVP1) was delivered intramuscularly. While for DNA vaccine carrier format, live attenuated bacteria Salmonella typhi Ty21a was used as a carrier for oral delivery of DNA vaccine (designated as- STUbGRVPI-c). We also evaluated a prime-boosting strategy in our study using these two vaccine formats.

RESULTS :
The results indicated that the production of Th1-type cytokine (IFN-_) was significant in homologous strategy using DNA vaccine formats. While Th2-type cytokine (IL-4) relatively low in all group of immunization. Relative ratio of IgG_{2a} to IgG_1 showed a significantly higher in the same strategy than the other three groups.

CONCLUSION :
This study demonstrated that homologous strategy using DNA vaccine formats induced both cellular (Th1 type) and humoral immune responses effectively compared to other group of immunization.

PB-17

CYTOLYTIC EFFECT OF NEWCASTLE DISEASES VIRUS (NDV) STRAIN V4UPM AND AF2240 ON HT29 COLON CARCINOMA CELL LINES

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PURPOSE :
Newcastle disease virus (NDV) is a virus of *Paramyxovirus* family and in the genus of *Rubulavirus*. To date, the trends move towards study an effect of virus as virotherapy for the cancer as alternative approach for the treatment of cancer in human. In this study, we successfully determined the inhibition concentration fifty percent (IC50) of V4UPM and AF2240 against HT29 colon carcinoma cell line. We also proved the ability of NDV to suppressed cell proliferation as compared to untreated cells. This IC50 will be used for ex vivo study to determine the effect NDV on colon carcinoma growth in athymic mice. The objectives of this study were mainly to evaluate the cytolytic effect and subsequently determine the mode of cell death induced by this strain.

METHODS :
The NDV strain V4UPM and AF2240 were propagated in the allantoic fluid of 9 days-old embryonated chicken eggs. The allantoic fluid was harvested, purified and stored at _20ºC. The haemagglutination (HA) test was conducted on the purified viruses to determine the HA titre of the virus. V4UPM, the lentogenic strain and AF2240, the velogenic strain of NDV was screened for the cytolytic activity towards HT29 (colon carcinoma) using microtitration 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.

RESULTS :
The inhibition concentration fifty percent (IC50) value using monolayer method measured at 72 hours was 60HAU/ml and 93HAU/ml, respectively. Further study were done to observe cell cycle arrest and apoptosis of the infected cells by flowcytometry and revealed the apoptosis feature of the treated cells.

CONCLUSION :
From this study, we can conclude that the NDV, strain V4UPM and AF2240 able to inhibit the proliferation of HT29 colon carcinoma cell line with the IC50 values at 60HAU/ml and 93HAU/ml.

PB-18

EFFECT OF A SELF-EMULSIFYING FORMULATION OF PALM VITAMIN E ON ARTERIAL COMPLIANCE AND VITAMIN E BLOOD LEVEL

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PURPOSE :
Tocotrienol [T3] is the predominant vitamin E in palm oil. This study assessed the effect of a self-emulsifying preparation of tocotrienol rich vitamin E [SF-TRE] on arterial compliance [index of vascular health] and plasma concentration in healthy subjects.

METHOD :
This randomised, placebo controlled, blinded endpoint clinical trial with a parallel design involved 36 male subjects divided into 4 groups. Each group took either placebo or SF-TRE at doses of 50, 100 or 200mg T3 daily for 2 months. The SF-TRE contained 23.54%, 43.16%, 9.83% and 23.5% respectively of _, _, _-T3 and _-tocopherol. Measures of arterial compliance, pulse wave velocity [PWV] and augmentation index [AI], and other parameters that were plasma total antioxidant status, plasma vitamin E concentration, serum total cholesterol and low density lipoprotein were measured before and 2 months after treatment.

RESULTS :
Baseline T3 levels were low, however, after treatment; all treated groups had significantly higher plasma _, _, _-T3 concentrations compared to placebo. There was borderline significance between placebo and 100mg [p=0.076] for their change in AI from baseline to end of treatment; treated groups showed significant improvement in AI after treatment, change for groups placebo, 50, 100 and 200mg being 2.22±1.54, -6.59±2.84, -8.72±3.77 and -6.27±2.67 respectively. Change in PWV with treatment was not significantly different between groups, although groups 100 and 200mg showed improvement from baseline to end of treatment. There was no effect of TRE on other study parameters.

CONCLUSION :
There was a trend towards improvement in arterial compliance with 2 months' of SF-TRE, further studies on patients with vasculopathy is suggested.

PB-19

P16 INK4A EXPRESSION IN CERVICAL PREMALIGNANT AND MALIGNANT LESIONS

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PURPOSE :
To investigate the expression of $p16^{INK4a}$ of normal uterine cervical tissue, pre-cancerous and cancerous lesions.

METHODS :
Immunohistochemical analysis of $p16^{INK4a}$ expression was performed on 3 normal, 36 cervical intraepithelial neoplasia (CIN) [15 CIN 1, 8 CIN 2 and 13 CIN 3], 40 squamous cell carcinoma (SCC), and 7 adenocarcinoma samples. A tumor was regarded as positive when more than 5 % of cells exhibited $p16^{INK4a}$ immunoreactivity. Normal epithelium was negative for $p16^{INK4a}$.

RESULTS :
Immnunoreactivity of $p16^{INK4a}$ was observed in 2/15 CIN 1, 2/8 CIN 2, 7/13 CIN 3, 26/ 40 SCC and 5/7 adenocarcinoma of the cervix. In all p16-positive samples, both nuclear and cyplasmic staining were observed. High expression (> 50 % of cell stained) was found in CIN 3 lesions, SCC and adenocarcinoma. In our study, a statistically significant association was observed between cervical lesion grade and $p16^{INK4a}$ expression (χ^2 = 17.993, P = 0.003).

CONCLUSION :
Our results indicate the $p16^{INK4a}$ expression would be a good marker for cervical dysplastic lesions and cervical cancer. The use of $p16^{INK4a}$ may be useful in difficult diagnostic cases by histological investigation. $p16^{INK4a}$ is also a putative molecular biomarker of cervical adenocarcinoma

PB-20

THE SPECTRUM OF THYROID LESIONS SEEN AMONG PATIENTS ADMITTED TO HUSM OVER AN 11-YEAR PERIOD

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ABSTRACT :

Endemic goiter remains a major concern in many nations including Malaysia. Seven states in the country have been identified by Ministry of Health of Malaysia to have high incidence of goiter and one of these states is Kelantan. Cancer developing from multinodular goitre was noted to be around 34% in our previous study of consecutive thyroidectomy specimens examined. From 1994 to 2004, department of pathology Hospital Universiti Sains Malaysia (HUSM) received a total of 1486 thyroid specimens, either for cytological or histological assessment. The majority (85.8%) of these cases were from female patients and the female to male ratio was 5.6 to 1. The mean age was 40.9 years. Nearly all (91.2%) percent of these cases had fine needle aspiration (FNA) performed. However, of all the FNA cases, only 427 (32.0%) had subsequent histological examination. Diagnosis of neoplastic lesions were made in 12.2% of FNA Cytology and subsequently confirmed in 64 (38.5%) cases. Histopathological examinations showed 296 (76.3%) were non-neoplastic lesions, which comprised of nodular hyperplasia, colloid cysts, Graves disease and various forms of thyroiditis. Of the neoplastic cases, 16.1 % was benign and 83.8% was malignant. Of these, papillary carcinoma makes up 64.4%n of the cases and follicular carcinoma 15.2%. Malignant thyroid lesions were 83.8% of the total thyroid cases.

PB-21

STUDIES TO DETERMINE PLATELET ACTIVATION AS A PREDICTOR OF PRE-ECLAMPTIC TOXAEMIA BY FLOW CYTOMETER

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PURPOSE :
Pre-eclampsia toxaemia is the leading cause of maternal and fetal morbidity and mortality. The search for proximate pathophysiology changes required the identification of early alterations present before the profoundly disordered state that occurs with the overt disease. With this condition, increased platelet activation antedate clinically evident pre-eclampsia by weeks to months in groups of pregnant women destined to develop the disorder. This finding has led to the unifying notion that platelet activation could be an early target for pathophysiological modification in pre-eclampsia. The main objective of the study was to find out whether platelets circulated in the activated state in pre-eclamptic women during the second trimester of pregnancy by means of flow cytometry. The secondary objective was to establish whether platelet activation predicted the onset of pre-eclampsia in the second trimester.

METHODOLOGY :
To achieve these objectives we recruited 65 healthy pregnant women who had an estimated risk of 5% for the development of pre-eclampsia toxaemia. An additional 25 pre-eclampsia women were included as a positive control. Flow cytometry was done using tricolour analysis involving CD61 PerCP, CD62P PE and PAC-1 FITC.

RESULTS :
Our study showed that during the second trimester CD62P expression was significantly high in pre-eclampsia predicted women as compared to healthy pregnant women. Similarly, PAC-1 was significantly increased in pre-eclamptic women as compared to healthy pregnant women.

CONCLUSION :
Women with a potential of developing pre-eclampsia toxaemia had higher CD62P and PAC-1 expressions during the second trimester compared to healthy pregnant women. We concluded that pre-eclampsia was accompanied by platelet activation, particularly perceptible early in the second trimester of pregnancy. Positive platelet activation can therefore be a predictive tool as early at the second trimester of pregnancy in women destined to pre-eclampsia.

PB-22

FLUROSENCE IN SITU HYBRIDIZATION (FISH) DETECTION OF CHROMOSOME 15Q11-Q13 DELETION IN PRADER – WILLI AND ANGELMAN SYNDROME PATIENTS

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PURPOSE :
Prader – Willi syndrome (PWS) and Angelman syndrome (AS) are distinct developmental disorders caused by absence of paternal or maternal contribution of the chromosome region 15q11-q13, resulting from deletion, uniparental disomy or rare imprinting mutations. The diagnosis of these complex disorders may be difficult to establish on clinical grounds. Conventional cytogenetic analysis has been reported to be insufficient for this microdeletion detection. The present study aimed to explore the utility of Flurosence in Situ Hybridization (FISH) technique in order to establish the presence of microdeletion in PWS and AS suspected patients.

METHOD :
Three patients (2girls and 1 boy) suspected of PWS and one boy suspected of AS were studied cytogenetically (employing standard cytogenetic procedures) and also by FISH technique (employing fluorescently labeled probes according to standards and guidelines) to establish the presence of DNA deletion at 15q11-q13.

RESULT :
The microdeletion 15q11-q13 could not be detected by conventional cytogenetic techniques in all the 3 cases. So FISH was employed to confirm the finding.

CONCLUSION :
FISH has been found to be an accurate and reliable method for detection of 15q11-q13 microdeletion in patients suspected of having either PWS or AS.

PB-23

EVALUATION OF NUGENT SCORE AND EACH AMSEL CRITERIA IN THE DIAGNOSIS OF BACTERIAL VAGINOSIS

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PURPOSE :
The aim of this study is to determine the prevalence of bacterial vaginosis (BV) among pregnant women using Nugent's criteria as a standard method of diagnosis. The second objective is to compare the agreement between Amsel criteria and Nugent score in the diagnosis of BV.

METHOD :
A cross sectional study was conducted among consented 270 pregnant women who attended antenatal check-up in Hospital USM, Kelantan (HUSM). Clinical data consisting of vaginal pH, "Amine test," clue cells, and appearance of the vaginal discharge (Amsel criteria) were compared with the vaginal fluid Gram stain (Nugent score) for the diagnosis of bacterial vaginosis. Using Amsel criteria as a gold standard, sensitivity, specificity, positive predictive value and negative predictive value of Nugent score and Amsel criteria were estimated.

RESULT :
The prevalence of bacterial vaginosis among antenatal patients in HUSM was 3%. Nugent score showed a sensitivity of 100%, specificity of 98.5%, positive predictive value (PPV) of 42.9% and negative predictive value (NPV) of 100%. There was an agreement between Amsel criteria and Nugent score (k = 0.594 and p = <1.0) in diagnosing BV.

CONCLUSION :
Bacterial vaginosis is present in a significant proportion of pregnant women in this study and the use of Nugent score is recommended for its diagnosis because of its high sensitivity and specificity.

PB-24

ANALOGY OF BONE MARROW ASPIRATION AND TREPHINE BIOPSY; MARROW INFILTRATION FOR STAGING

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PURPOSE :
It is recommended that bone marrow aspiration should not be used as a standard for staging purpose of the disease including for the assessment of the marrow infiltration by the primary disease. However in practice bone marrow aspiration and trephine biopsy are carried out together. Moreover the recommendation is to review both aspiration biopsy and trephine together and by the same pathologist. In situations where both are not read by the same pathologist; they should be reviewed in close collaboration by two pathologists. In Hospital University Sains Malaysia the trephine biopsy is reviewed by the histopathologist while aspiration is reviewed by a hematopathologist. This might result into two different impressions by two consultants. The aim of our study was to find out the analogy of two reports by two different consultants with respect to marrow infiltration by the primary disease.

METHOD :
To achieve our objectives we did a retrospective study. Bone marrow aspiration reports for staging of the primary disease were identified and the corresponding trephine biopsy reports were traced from histopathology department.

RESULTS :
Twenty three (23) bone marrow aspiration reports over a period of 15 months (Dec 2004-March 2006) were identified which were sent for the staging purpose. Five reports were inconclusive due to poor specimen. Nine out of 18 marrows showed marrow infiltration by the primary disease. The results from trephine showed lower number of infiltration as compared to that reported in aspiration results.

CONCLUSION :
In conclusion we recommend collaborative review of the two investigations in order to avoid difference in opinions. Such an approach shall lead to appropriate staging and treatment protocol for a particular disease.

PB-25

NONRANDOM DICENTRIC (9;12)TRANSLOCATION IN ACUTE LYMPHOBLASTIC LEUKEMIA – A CASE REPORT

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INTRODUCTION :

An important factor in the diagnosis of Acute Lymphoblastic Leukemia (ALL)is that karyotype ia an independent prognostic indicator, with an impact on the choice of treatment. Therefore pretreatment karyotyping on the cells of ALL patient is essential as the identification of the chromosome abnormality remains the best known way to predict how a patient will progress or respond to treatment. Here we present a nonrandom dicentric (9;12) translocation in a case of ALL.

CASE REPORT :

Bone marrow sample of a 16 year old Malay boy was referred to Human Genome Centre, USM, for cytogenetic analysis. He complaint of lethargy for 2 months with low grade fever and poor apetite.Further clinical examination revealed hepatosplenomegaly. Immunohaematopathological studies of the bone marrow confirmed the case as Acute Lymphoblastic Leukemia, L1 of B lineage. Cytogenetic analysis was performed employing short term bone marrow culture, cultures were harvested by standard cytogenetic procedures and karyotype analyzed following International System for Human Cytogenetic Nomenclature (ISCN,1995). GTG-banded karyotype analysis revealed an abnormal clone with the chromosome abnormalities. The abnormal clone was characterized by 45 chromosomes and a dicentric chromosome involving chromosome 9 and 12 with breaks and reunion at bands 9p13 and 12p12, with 45,XY,dic(9;12)(p13;p12) karyotype pattern.The dicentric nature of the chromosome was reconfirmed through C-Banding .

CONCLUSION :

The dic(9;12) translocation has been documented in B-Cell progenitor ALL and the The present case add to the number of cases reported in the literature.The dic(9;12) translocation reported to result in the fusion of the PAX5 and ETV6 genes, is associated with good prognosis and a 5 year survival in greater than 95% of cases.

PB-26

IMMUNOPHENOTYPIC PROFILE OF ACUTE MYELOID LEUKEMIA BY FLOW CYTOMETRY

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INTRODUCTION :
Acute myeloid leukemia (AML) is a group of neoplastic disorders characterized by the proliferation and accumulation of immature hematopoietic cells in the bone marrow and blood. AML accounts for approximately 20% of acute leukemia in children and 80% of acute leukemia in adults. Over the last decade, immunophenotyping has become extremely important not only in determining the lineage of the acute leukemias but also in the detection of mixed lineage leukemias and minimal residual disease. It is also suggested as having a prognostic significance. Our objective was to study the immunophenotypic profile of acute myeloid leukemia at diagnosis.

METHODOLOGY :
This is a retrospective analysis of 53 cases of AML diagnosed at our institution between 2004 and 2005. Diagnosis was based on peripheral blood and bone marrow examination for morphology, cytochemistry and immunophenotypic studies. Immunophenotyping was performed by tricolour flow cytometry analysis using CD45/SSC gating. SPSS version 10 software package was used for statistical analysis.

RESULTS :
AML accounted for 35.2% of all acute leukemias and 87% were adult AML. The commonest FAB subtype was AML-M2 (31.6%). CD13 and CD33 were most commonly present in all AML subtypes. CD14 was most often seen in monocytic and myelomonocytic (75%) leukemias. Lymphoid antigen expression was seen in 41% of cases. CD4 expression was the commonest (23%).

CONCLUSION :
AML accounted for over a third (35.2%) of all leukemias diagnosed at our institution between 2004 and 2005, and adults accounted for a large percentage (87%). The commonest FAB subtype was AML M2. CD13 and CD33 were the most useful markers in the diagnosis of AML. Aberrant lymphoid expression was seen in less than half (41%) of these cases and CD4 was the lymphoid marker most often expressed.

Keywords: immunophenotyping, acute myeloid leukemia

PB-27

THE EFFECT OF SMILAX CALOPHYLLA *ON PLASMA HORMONAL LEVELS IN GLUCOCORTICOID-TREATED RATS*

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INTRODUCTION :
The inhibitory effect of corticosterone (B) on testosterone (T) production seems to be partly attributed by the elevated plasma estradiol (E_2) levels, an effect proposed to be mediated via a glucocorticoid receptor (GR). Estradiol acts centrally by inhibiting luteinizing hormone (LH) secretion and locally on Leydig cells, thus causing a decrease in T production. *Smilax calophylla* (Akar dawai, AD) is an indigenous herb which is consumed by local folks for its aphrodisiac effect. The present study aims to determine whether lyophilized water extract of *Smilax calophylla* counteracts the adverse effects of glucocorticoid (B or Dexamethasone, DEX; a synthetic glucocorticoid which is a potent GR agonist) on plasma T and E_2 levels in normal male Wistar rats.

METHODS :
Control rats were given the vehicle while the treated rats were given AD, B or DEX alone, AD in combination with B (AD+B) or AD in combination with DEX (AD+DEX). All treatments were given for seven consecutive days. Plasma T and E_2 levels were determined using Coat-A-Count Diagnostic Product and the data were expressed as mean ± confidence interval (CI).

RESULTS :
Corticosterone and DEX were found to significantly increase plasma E_2 levels and lower plasma T levels, compared to that of control. Conversely, AD administration in B or DEX treated rats brings all parameters towards control value.

CONCLUSION :
Elevated plasma E_2 levels in glucocorticoid (B or DEX) treated rats could be a contributing factor in reducing plasma T levels, an effect that was counteracted by AD. It seemed that AD and the glucocorticoid (B or DEX) competitively blocked each other, to act on GR in affecting the parameters studied. This further proved that the effect of AD was mediated via GR. In conclusion, AD could counteract the adverse effect of glucocorticoid on testicular function.

PB-28

SCHISTOCYTES WITH AUTOMATED HAEMATOLOGY ANALYZER (SYSMEX XE-2100): COMPARISON WITH MORPHOLOGY

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INTRODUCTION :
Schistocytes are fragments of red blood cells (RBCs) formed in the peripheral blood as a consequence of mechanical damage. Their numbers increase in conditions such as hemolytic anemias, disseminated intravascular coagulation (DIVC), haemolytic uremic syndrome (HUS), and thrombocytopenic purpura (TTP). They are also increased after surgery and in some of the vascular conditions such as vasculitis. Microscopy is the mainstay of the diagnosis of the schistocytes. In normal adults schistocytes number less than 0.1% of RBCs but the numbers are increased in premature babies and infants (0.3 - 5.5%). Our objectives were to evaluate the technical performance of the automated analyzer (Sysmex XE-2100) in evaluating schistocytes, and to compare the results of morphology versus those of the analyzer.

METHODS :
A retrospective study was conducted to achieve the objectives. Altogether, 70 samples with fragments over the past 9 months were identified from within the database of the Sysmex. The full blood pictures of these samples were traced to obtain their respective clinical diagnosis and hematopathological interpretation.

RESULTS :
Results of this study showed a definite agreement between the instrument's results of RBC fragments with the clinical diagnosis and/or with the hematopathological interpretation.

CONCLUSION :
In conclusion the availability of results of RBC fragments by the automated analyzer was very useful due to its high negative predictive value and for the follow up of the cases in which there is definite hemolysis and for routine screening to quickly identify clinically unsuspected positive samples.

PB-29

SEROPREVALENCE OF SYPHILIS INFECTIONS AMONG BLOOD DONORS IN TRANSFUSION MEDICINE UNIT, HOSPITAL UNIVERSITI SAINS MALAYSIA

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PURPOSE :
Blood safety remains an issue of major concern in the transfusion medicine unit and transfusion-transmitted infections are the most commonly encountered complications in transfusion practice. *Treponema pallidum* is screened in blood banks routinely and this test is obligatory for transfusion safety. Our objective was to determine the seroprevalence of syphilis infection and the rate of biological false positive VDRL (Venereal Disease Research Laboratory) test among blood donors in the Transfusion Medicine Unit, Hospital Universiti Sains Malaysia.

METHODS :
A 2-year retrospective study from 2003 to 2004 was conducted at the Transfusion Medicine Unit, Hospital Universiti Sains Malaysia. Serologic screening results and demographic data were analyzed from 14910 donors.

RESULTS
About 0.8% (134) donors were found to have VDRL screening reactive from the pilot tube. Repeat test was done from the body and 5.2% (7) were found to have syphilis infection as determined by a positive TPHA (*Treponema pallidum* haemagglutination assay) test. We noted that biological false positive test was 14.1% (19). The prevalence of VDRL reactivity varied from 8% in 2003 to 1.6% in 2004.

CONCLUSIONS :
The seroprevalence of syphilis infection was higher in 2003 but decreased in 2004. Biological false positive reactions comprised a high proportion of all VDRL reactors and further testing is recommended. Syphilis should be excluded before blood is transfused and the VDRL test should remain the screening test of choice in blood transfusion service. Enhanced counseling and awareness could further reduce the number of cases of syphilis seroconversion among blood donors and improve blood transfusion safety.

Keywords: ***VDRL, blood donors, syphilis seroconversion***

PB-30

IMMUNOHISTOCHEMICAL STAINS (CARCINOEMBRYONIC ANTIGEN, VIMENTIN AND OESTROGEN RECEPTOR) IN THE DIAGNOSIS OF PRIMARY ENDOMETRIAL AND ENDOCERVICAL ADENOCARCINOMA

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PURPOSE :
Primary endocervical adenocarcinoma (ECA) and endometrial adenocarcinoma (EMC), particularly the endometrioid variants, have some overlapping histological features. Endometrioid variant is a major type of ECA, whereas endometrioid EMC is the commonest amongst its histological types. The histological differentiation between ECA and EMC is a common diagnostic problem, but it is important because the treatment for the two conditions is different. We investigated the possibility of distinguishing between the two by using a panel of immunohistochemical (IHC) stains: carcinoembryonic antigen (CEA), vimentin and oestrogen receptor (ER) on paraffin sections of archived blocks.

METHODS :
Archived blocks of histologically diagnosed cases from the files of the Department of Pathology, Hospital Universiti Sains Malaysia (HUSM) and the General Hospital, Kota Bharu (now renamed Hospital Sultanah Zainab II), during January 1994 to September 2004 were utilised. Sections were stained immunohistochemically employing monoclonal anti-human CEA (Dako, clone II-7), monoclonal mouse antivimentin antibody (DAKO, clone Vim 3B4) and monoclonal mouse anti-human ER antibody (DAKO, clone ID5), and Envision +system-HRP (DAB) kit was used for the staining.

RESULTS :
A total of 36 cases each of ECA and EMC were obtained and included in the study. ECA consisted of 12 endometrioid, 14 mucinous, 9 villoglandular and 1 clear cell types. Primary EMC consisted of 33 endometrioid and 3 villoglandular types. CEA was positive in 33 of 36 ECA, both cytoplasmic and membrane, diffusely. It was positive in 6 of 36 cases of EMC with only focal membrane positivity. Vimentin gave diffuse strong positivity in 32 of 36 EMC but weakly in only 1 of ECA. Diffuse positive nuclear staining for ER was seen in 26 EMC, but only in 1 of ECA was positive.

A few other literature reports have produced variable results using the three IHC markers. Our findings are in agreement with those of some other workers. The discrepancy may possibly be attributed to different antibodies used and/or dilution. The use of monoclonal antibody DAKO in our case has probably given more consistent result. The IHC findings along with other clinical evidences will provide a more complete data about the site of origin of the tumour. Study of a bigger sample size can provide a wider variety of histological types and better evaluation of IHC stains.

CONCLUSION :
Our conclusion is that an antibody panel consisting of CEA, vimentin and ER are useful IHC markers in distinguishing between ECA and EMC.

PB-31

INHIBITORY EFFECTS OF MORPHINE ON PERIPHERAL-EVOKED RESPONSES OF VENTRAL POSTEROLATERAL NEURONS IN SPINAL NERVE LIGATED RATS

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PURPOSE :
Systemic administration of morphine inhibits nociceptive activity of ventral posterolateral (VPL) neurons in normal rats. Our objective was to investigate the effect of systemic morphine on the mechanical and thermal evoked responses of VPL neurons in a rat model of neuropathic pain.

METHODS :
Spinal nerve ligation of lumbar L5/6 spinal nerves or sham surgery was carried out in anaesthetised rats weighing 80-90g. Two weeks after the surgery, the rats weighed 220-300g and all were subjected to *in vivo* electrophysiological studies under anaesthesia. Spontaneous and evoked neuronal responses to a range of calibrated monofilaments and thermal stimulus 45˚C were measured. Effects of cumulative dose of intravenous morphine (0.5 mg/kg to 2mg/kg) and naloxone administered following morphine were studied. Data were analysed using NeuroExplorer and Prism v 3.03 and presented as mean maximal % inhibition ± s.e.m.; statistical analysis of drug effects were compared to pre-drug control values using Student's t test.

RESULTS :
Spontaneous activity of the VPL neurons was not altered by morphine, or naloxone in the two groups of rats. There was a significant difference in the 7g evoked response of VPL neurons of sham and SNL rats ($P<0.01$). Morphine significantly inhibited 7g evoked response in SNL rats ($P<0.01$), but not sham rats. Morphine inhibited the noxious evoked responses in sham operated ($p<0.05$) and SNL rats ($P<0.05$). Inhibitory effects of morphine on evoked responses of VPL neurons in SNL rats were significantly reversed by a lower dose of naloxone (0.2mg/kg; $P<0.05$) than in sham rats (0.4mg/kg-1mg/kg; $P<0.05$).

CONCLUSION :
Morphine did not influence spontaneous activity of VPL neurones, but inhibited innocuous-evoked responses of VPL neurones in neuropathic rats, but not sham controls. Inhibitory effects of morphine were blocked by a lower dose of naloxone in neuropathic rats, compared to sham controls. These data are suggestive of a plasticity in opioid receptor modulation of VPL neurones in neuropathic rats.

PB-32

HIV SEROPOSITIVITY IN HUSM BLOOD DONORS

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PURPOSE :
Transfusion of HIV continues to be a threat to safe blood transfusion. Developing countries account for more than 90% of all new HIV cases. In Malaysia the number of infected individuals has increased steadily each year. The objective of this study is to assess the prevalence and trend of HIV infection among blood donors of the Transfusion Medicine Unit, Hospital Universiti Sains Malaysia.

METHODS :
A retrospective study was conducted among blood donors in the Transfusion Medicine Unit, Hospital Universiti Sains Malaysia from 2000 through 2004. Serologic screening results and demographic data were analyzed from 34,259 donors.

RESULTS :
Only 0.196% (67) donors were found to have screening reactive for HIV. However, 30 donors came with repeat samples and 53.3% (16) were found to have ELISA reactive for HIV. Western Blot test showed only one donor was positive, five donors were negative, three donors were indeterminate and seven donors did not turn up for the Western Blot test from second blood sample. The prevalence of HIV reactivity varied from 0.11% to 0.27%. The percentage of screening test reactive was higher in 2002 and 2003.

CONCLUSION :
Enhanced counseling and awareness could in future reduce the number of cases of seroconversion among blood donors and improve their subsequent behaviours. Voluntary donors are urgently required to lower the prevalence of transmissible infection. Additional strategies to exclude donors at very high risk and to attract those at low risk forthe infection should be developed and evaluated to increase blood safety.

Key words: ***Blood donors, HIV infections, seroconversion***

PB-33

COMPARISON OF WOUND HEALING EFFICACY OF MELASTOMA MALABATHRICUM *AND* IXORA COCCINEA *EXTRACTS IN RATS: AN EXPERIMENTAL ANIMAL MODEL*

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PURPOSE :
To determine the effects of *Melastoma malabathricum* and *Ixora coccinea* on wound healing.

METHOD :
Six rats were used in this study. Each rat received two full-thickness wounds. One wound was covered with either test extract (*Melastoma malabathricum* or *Ixora coccinea*) while the other with sham control. The rats were euthanized after periods of 5, 10 and 15 days. Tissue samples were retrieved from the wounds for histological evaluation.

RESULTS :
Initially wounds treated with both extracts elicited chronic inflammation compared to the sham control, which started with mixed inflammatory response. For both extracts, significant exudates with mast cells and rather intense angiogenesis were observed. In terms of inflammation intensity, the wound treated with *Ixora coccinea* extracts showed higher intensity than that of *Melastoma malabathricum*. Eosinophils were present in *Ixora coccinea*-treated wound, an indication of possible allergic reaction. Fibroblast and collagen depositions have also occurred in this initial stage. At day ten, the inflammation level in both wounds has started to decrease. Skin regeneration was observed, with faster rate in *Melastoma malabathricum* compared to that of *Ixora coccinea*. At day fifteen, inflammatory cells have decreased for both and abundant collagen depositions seen. Eosinophils and mast cells has markedly decreased in *Ixora coccinea*-treated wound. Both wounds have been fully covered with keratinized squamous epithelium by this time.

CONCLUSION :
Both *Melastoma malabathricum* and *Ixora coccinea* extracts possess wound healing properties with better efficacy observed in the former extract.

PB-34

COMPARATIVE EVALUATION OF THE EFFECTS OF CHITOSAN DERIVATIVES ON WOUND HEALING: AN EXPERIMENTAL ANIMAL MODEL

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PURPOSE :
To determine the effects of chitosan derivatives on wound healing

METHOD :
Thirty rats were used in this study. Each rat received two subcutaneous pockets of chitosan derivative implant (N-CMC 1%, NO-CMC 5%, Oligo-5 1%, O-C 5%, O-CMC 1% or O-CMC 5%) and sham control. The rats were euthanized after periods of 4, 7, 14, 21 and 28 days. The implants and control with surrounding tissue were retrieved and examined histologically.

RESULT :
Initially, N-CMC 1% elicited acute inflammation while O-CMC 5% started with mixed inflammatory response. The rest of the derivatives began with chronic inflammation. In terms of inflammation intensity, the O-CMC 5% showed the highest intensity followed by O-CMC 1%, NO-CMC 5%, O-C 5%, OLIGO-5 1% and N-CMC 1% respectively. Granuloma formation and multinucleated giant cells can be seen occasionally in all the films. Angiogenesis is rather intense during the first three weeks of all implants except for O-C 5%. Fibroblast and collagen depositions increase with the increase of implantation period for all the implants except N-CMC 1% and OLIGO-5 1%. All films underwent from mild to moderate degradation. These materials are still abundantly present 28 days post-implantation.

CONCLUSION :
All films, except for both O-CMC 1% and O-CMC 5%, are rather biocompatible material when implanted in vivo. Good reaction can be seen in N-CMC 1%, OLIGO-5 1%, O-C 5% and NO-CMC 5% respectively.

PB-35

THE PRELIMINARY STUDY OF INOS EXPRESSION IN NORMAL SKIN AND HYPERTROPHIC SCAR USING REAL-TIME PCR

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INTRODUCTION :
Adequate nitric oxide (NO) level produced by skin tissue plays an important role in normal wound healing and scar formation by maintaining appropriate rate of keratinocyte and fibroblast proliferation. In normal conditions, NO is expressed from constitutive NOS (cNOS). However, during wound healing, NO level is elevated due to the activation of inducible nitric oxide synthase (iNOS). Studies have reported that low concentrations of NO stimulate cell division while high NO concentrations are cytostatic. In hyperthrophic scar (HSc), it was noticed that there was lower expression of iNOS compared to normal skin. Lower level of NO production has been correlated with excessive fibroblast and keratinocyte proliferation and over deposition of collagen in skin abnormalities. To date, the use and information on Real-Time PCR application on iNOS expression analysis in HSc are still inadequate. The objective of this study was to determine the gene expression pattern of HSc and keloid compared to normal skin using Real-Time PCR assay.

METHODS :
Total RNA of tocotrienol (T3)-untreated and treated keratinocytes (normal and HSc) and fibroblasts (normal and HSc) samples were subjected to Real-Time PCR assay. A comparative iNOS expression analysis of One-Step Real-Time PCR was conducted with the inclusion of 18srRNA primer as the endogenous control to normalize sample-to-sample variation. The normalized result was analyzed using the comparative Ct (2^{--Ct}) method to calculate changes in iNOS expression.

RESULTS :
Based on the preliminary assay using the Real-Time PCR, iNOS expression was reduced in the HSc samples. Further studies will need to be conducted to validate the results statistically.

CONCLUSION :
This study will build a better understanding regarding the comparative study of iNOS expression between HSc and normal skin using the Real-Time PCR assay. Therefore, more information on the gene expression pattern can be provided which may contribute to the wound healing research area.

PB-36

EFFECT OF TOCOTRIENOL ON KERATINOCYTES AND FIBROBLASTS IN NORMAL AND HYPERTROPHIC SCAR CULTURES

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INTRODUCTION :
The skin consists of two morphologically different layers that are derived from two different germ layers namely the superficial epidermis and the deeper and thicker dermis. Keratinocytes constitute about 85% of cells in the epidermis. Fibroblasts are the main cells in the dermal layer, and their extracellular matrix (ECM) secretions contribute to the high tensile strength of the skin. Tocotrienols (T3s) are one of the two sub groups of molecules belonging to the vitamin E family. T3s are found to be involved in antioxidant activities and also have a potential beneficial effect on the prevention of hypertrophic scar (HSc). The objective of this study was to evaluate the effect of tocotrienol (T3) on keratinocytes and fibroblasts in normal skin and HSc.

METHODS :
Various concentrations of T3-rich fractions extracted from locally produced palm oil were used in this study. Fibroblast and keratinocyte cultures from both normal and HSc were exposed to different concentrations of T3 for 24, 48 and 72 hours. To further evaluate the effects of T3 on human keratinocytes and skin fibroblasts in normal and HSc cultures, the 3-[4,5-dimethyl-2-thiazolyl]-2,5-diphenyl tetrazolium bromide (MTT) cell viability assay was used.

RESULTS :
As the incubation time was prolonged, the percentage of viable cells from both normal and HSc fibroblasts and keratinocytes decreased. Lower concentrations of T3 enhanced cell growth while higher concentrations of T3 contributed to the reduction of cell numbers. These features were found in both normal and HSc fibroblasts and keratinocytes.

CONCLUSION :
Different cell culture populations showed different responses to T3. This differential response of cultured scar and normal cells provides significant evidence on the potential role of palm oil-based T3 in wound healing and scar management.

PB-37

VALIDATION OF THE OSMOTIC FRAGILITY TEST KIT AS A SCREENING METHOD FOR ALPHA THALASSAEMIA (SEA TYPE)

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INTRODUCTION :
The Southeast Asia (SEA) type deletion of alpha thalassemia 1 is the most common type of alpha thalassemia 1 in SEA countries including Malaysia. Affected heterozygous individuals are asymptomatic and thus are left undiagnosed. These undiagnosed individuals will be potential carriers to more severe forms of alpha thalassemias, particularly hydrops fetalis and hemoglobin H (HbH) disease. Therefore, an effective and simple method of screening is essential for the prevention and control of alpha thalassemia in the population. The objective of this study was to evaluate the validity of a screening method by using the modified single tube red cell osmotic fragility test (OFT) for the detection of alpha thalassemia 1 (SEA type).

METHODS :
This study was conducted on 113 peripheral blood samples taken from antenatal patients in Hospital Universiti Sains Malaysia (HUSM). Preliminary screening was done using OFT, full blood picture, hemoglobin A2 (HbA2) quantitation and serum ferritin assay. Detection of alpha thalassemia 1 (SEA type) was performed by polymerase chain reaction (PCR).

RESULTS :
The sensitivity and specificity of the OFT was 75% and 96.6% respectively. The predictive value of the positive test was 34.6% while that of the negative test was 96.6%. The group with positive OFT showed significantly lower MCV and MCH values as compared to the negative OFT group ($p<0.001$).

CONCLUSION :
The OFT has potential to be a very efficient, simple, reliable and inexpensive screening method for alpha thalassemia 1 (SEA type) in our population as such a prevention and control program is lacking.

Keywords: ***Alpha thalassaemia; screening test; osmotic fragility test (OFT); polymerase chain reaction (PCR)***

PB-38

INCIDENCE OF THROMBOCYTOPENIA IN HEPARINIZED PATIENTS

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INTRODUCTION :
Heparin is widely used for thromboprophylaxis or treatment in many clinical situations, including cardiovascular surgery and invasive procedures, acute coronary syndromes, venous thromboembolism, atrial fibrillation, peripheral occlusive disease, dialysis and during extracorporeal circulation. However, it can cause serious adverse effects, including heparin-induced thrombocytopenia (HIT) which is a common, serious and potentially life threatening condition. Our objective was to obtain the incidence of thrombocytopenia among patients who received heparin treatment.

METHODS :
This retrospective study was conducted in any heparinized patients who developed thrombocytopenia while receiving unfractionated or low molecular weight (LMW) heparin. Altogether 103 patients who received heparin were recruited. The clinical records of these patients were then analyzed for other causes of thrombocytopenia and 89 patients were excluded.

RESULTS :
Only 14 (13.5%) patients were found to be thrombocytopenic during heparin treatment; 10 (9.7%) had received unfractionated heparin while 4 (3.8%) had received LMW heparin.

CONCLUSION :
HIT was a common complication of heparin treatment. Clinicians who are treating patients with heparin should determine the platelet count at baseline and regularly before the commencement and during heparin therapy.

Keywords: ***Heparin, thrombocytopenia***