

PB-1

ANALYSIS OF FLOW CYTOMETRIC IMMUNOPHENOTYPING OF HIV INFECTED SUBJECTS FROM 2005 TO 2007

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Introduction: HIV infection is a global health issue. The typical pattern of HIV primary infection is characterized by high levels of virus in blood followed by a progressive loss of CD4+T cells, elevation of CD8+T cells and progressive impairment of T cell functions.

Objective: To analyze the lymphocyte subsets changes in HIV positive patients.

Materials and method: A retrospective study was conducted on HIV infected patients in HUSM during the period of 2005 till 2007. The patients' records and results were reviewed and analysed in term of percentage and absolute count to determine the lymphocyte changes which will indirectly reflect the degree of severity of the disease.

Results: In HIV infected subjects, the alteration of lymphocytes subsets from 2005 to 2007 mainly occur in CD4+ T cell counts (< 200cells/mm³ (70.8%, 84.6%, 81.1%), CD8+ T cells (<500cells/mm³ (85.4%, 94.8%, 86.5%). The absolute lymphocyte counts also drops down in all CD3, CD19/20, and CD16/56.

Discussion and conclusion: Majority of HIV infected subjects have CD4+ T cells count less than 200 cells/mm³, low CD8+T cells with ratio of less than 1.0 . This may suggest most of the HIV infected subjects in HUSM fall in the late stage of the disease which later might end up with lots of major complications and early death if no prompt treatment action taken.

PB-2

IGG AND IGM ANTICARDIOLIPIN ANTIBODIES IN ANTIPHOSPHOLIPID SYNDROME PATIENTS IN HOSPITAL UNIVERSITI SAINS MALAYSIA

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Introduction: The hallmark of laboratory results that defines APS (antiphospholipid syndrome) is the presence of anticardiolipin antibodies, anti-beta-2-glycoprotein-1 antibodies and lupus anticoagulant on 2 or more occasions at least 12 weeks apart.

Objective: To determine the prevalence of IgG and IgM anticardiolipin (aCL) antibodies in APS patients in HUSM.

Patients and method: A cross sectional study was conducted from January 2004 to December 2007 at Immunology laboratory, Hospital Universiti Sains Malaysia. IgG and IgM anticardiolipin antibody results were analyzed from 365 patients.

Results: For IgG aCL antibodies, in 2004, 7(10.8%) patients were moderately positive while 4(6.2%) were strongly positive. In 2005, 5(7.4%) patients were moderately positive and 4(5.9%) were strongly positive. In 2006, 10(8.2%) patients were moderately positive while 12(9.8%) were strongly positive. In 2007, 10(9.2%) patients were moderately positive and 11(10.1%) were strongly positive. For IgM aCL antibodies, in 2004 and 2005, only one patients (1.5%) each year were moderately positive. In 2006, 3(2.4%) patients were moderately positive. In 2007, 2(1.8%) patients were moderately positive. For strong positivity towards IgM aCL antibodies, in 4 years, only 1 patient in 2007 is strongly positive.

Discussion and conclusion: In this 4 years study, there is increasing trend of moderate and strong positivity towards IgG and IgM aCL antibodies in HUSM. However as aCL antibodies is not the only antibodies found in APS, these finding does not reflect the prevalence of APS in HUSM

PB-3

FOOD ALLERGY IN ALLERGIC DISEASES PATIENTS IN HUSM

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Introduction: The bench mark for food allergy testing is food challenge. However, food challenge is a time consuming and expensive technique not widely practiced

Objective: To use allergen specific-IgE assay (chemiluminescence technique) to determine food allergy in patients attending skin, respiratory and ENT clinics in HUSM.

Patients and method: In this cross sectional study, patients with allergic rhinitis, atopic dermatitis and allergic asthma were included. Results of 109 patients tested for allergen specific IgE assay (chemiluminescence technique) were reviewed. Descriptive analysis was done for 13 types of foods common in everyday diet of Malaysians.

Results: From the total of 109 patients, 96 subjects (88.1%) were positive to at least one allergen tested. Among the food allergen group, clam (56.0%) was the most commonly implicated food, and then followed by shrimp (52.3%), soybean (51.4%), crab (49.5%), tuna (47.7%), cow's milk (45.9%), wheat (45.0), beef and yolk egg (both 44.0%), chicken (43.1%), peanut (41.3%) and egg white (40.4%). The least common food allergen is citrus mix (38.5%)

Discussion and conclusion: The most common food allergy in allergic diseases patients in HUSM is clam and shrimp.

PB-4

LOCALIZATION OF THE MOTOR NEURON SOMATA OF THE STERNOCLEIDOMASTOID MUSCLE IN RAT – A HORSERADISH PEROXIDASE STUDY

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Objective: To localize the motor neuron somata of the sternocleidomastoid muscle in rat.

Subjects and method: Ten Sprague-Dawley rats were used. Under general anaesthesia, the right sternocleidomastoid (SCM) was injected 0.05 ml of 30% horseradish peroxidase. After 48 hours, the animals were perfused with normal saline, then with 1.25% glutaraldehyde and 1% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4), and finally, with 10% sucrose in same buffer. After perfusion, the medulla oblongata and 1st to 6th cervical segments (C-1 to C-6) of spinal cord were placed in above sucrose buffer solution for 24 hours, and their serial transverse sections cut in a cryostat. The sections were treated according to tetramethyl benzidine-HRP method of Mesulam (1978).

Results: The HRP labeled neuron somata were located in the caudal 0.9 to 1.2 mm of medulla oblongata, whole lengths of C-1 and C-2 and rostral three-fourths of C-3. In the medulla oblongata, they were located ventrolateral to the pyramidal fibres that pass dorsolaterally after their decussation. In C-1, they were located in dorsomedial and central columns, in C-2, they were found in dorsomedial, central, and ventrolateral columns and in rostral three-fourths of C-3, they were located in the ventrolateral column.

Discussion and conclusion: The motor neuron somata of the sternocleidomastoid muscle in rat are located in the caudal part of the medulla oblongata, the whole lengths of 1st and 2nd cervical segments and the rostral three-fourths of the 3rd cervical segment of the spinal cord.

PB-5

THE EFFECTS OF AQUEOUS EXTRACT OF LABISIA PUMILA VAR. ALATA (BIOLABISIA) ON REPRODUCTIVE ORGANS IN FEMALE RATS

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Objective: To investigate the effect of commercially prepared standard aqueous extract of *Labisia pumila var. alata* (Biolabisia) on reproductive organs of female rats.

Subjects and method: This study comprised of two experiments. During the first experiment, forty virgin Sprague Dawley rats with regular oestrous cycle were administered with distilled water (control) or Biolabisia at 20, 200 or 1000 mg/kg/day (n=10) daily by gavage. Upon completion of three weeks regimen, they were sacrificed by CO₂ asphyxiation. The same sample size of pregnant rats was used in the second experiment. Treatments with equivalent doses of Biolabisia were initiated during the pre-mating period (two weeks) and continued during the mating and pregnancy periods, up to day 15 post coitus (pc). They were sacrificed and laparotomised on day 16 pc.

Results: Neither significant macroscopic changes nor momentous differences in weights of the reproductive organs were observed. This observation was verified by histopathological investigation. No significant manifestations of Biolabisia -related deformities in the organs examined were evident.

Discussion and conclusion: Results showed that oral doses of Biolabisia , up to a concentration of 1000 mg/kg/day did not pose any significant toxicity risks in the reproductive organs of female rats. The “no observed adverse effect level” (NOAEL) of Biolabisia in this study was 1000 mg/kg/day.

PB-6

THE EFFECTS OF GAMAT EXTRACTS ON PAIN BEHAVIOUR AND C-FOS EXPRESSION IN THE FORMALIN INJECTED RATS

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Objective: To investigate the effects of gamat extracts on pain behaviour and c-fos expression in rats injected with formalin.

Subjects and method: Twelve Sprague-Dawley male rats (220-300 gram) were given intraperitoneal administration of either gamat extracts from *Holothuria* spp. (4 mg/kg) or distilled water after intraplantar injection of 0.05 ml formalin (1%). The rats' behaviour was recorded with a digital camcorder and they were sacrificed two hours after the formalin injection. The brains were removed and examined for c-fos expression. Behaviour and c-fos data were analysed using SPSS, version 13. Behaviour data were analysed using repeated measures analysis of variance (ANOVA) with post hoc Scheffe's test and c-fos data were analysed using independent t-test. Significance level was taken as 0.05.

Results: The pain behaviour in the group receiving gamat was significantly ($P < 0.05$ for each) reduced at 15 minutes (4 mg/kg gamat - 0.7 ± 0.3 ; control- 2.0 ± 0.1) and the differences was maintained until 30 minutes post formalin administration (4 mg/kg gamat - 0.8 ± 0.4 ; control- 2.0 ± 0.2). C-fos expression in the thalamus was also reduced (16 ± 0.87) in the gamat group compared to the control group (85 ± 1.73).

Discussion and conclusion: The gamat extracts from *Holothuria* spp has significantly suppressed the pain behaviour and c-fos expression in the contralateral thalamus in formalin injected rat. Results from this investigation throw some light as to the possible use of gamat extract as an analgesic.

PB-7

IN VITRO ANALYSIS OF THE INTERACTION BETWEEN BIOACTIVE TITANIA FILMS WITH DIFFERENT CELL TYPES

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Objective: To investigate the cellular interaction between various cell types with titanium thin films exhibiting different surface property.

Materials and method: Self-array TiO₂ nanotubes, smooth and porous TiO₂ thin films were produced and characterized by Energy-dispersive X-Ray, field emission scanning electron microscope, Atomic Force Microscope and X-Ray Diffraction (XRD). Mouse bone marrow (BM) and spleen cells, HT-29 established colon carcinoma and mouse 3T3 fibroblast cell lines were prepared using standard cell culture procedures. The cells were cultured on heat sterilized TiO₂ thin films in complete RPMI 1640 growth medium for 48 hrs at 37°C in 5% CO₂-air environment. Complete growth medium and precipitated antibody crystal solutions were used as controls. At the end of the incubation period, the films were washed to remove unbound cells and processed for scanning electron microscopy.

Results: Analysis of the results shows that BM cells grew in linear format along surfaces with pores/tubes while HT-29 cells were able to bind to smooth surface of the thin films. 3T3 fibroblast cells showed preferential binding to areas of the films showing rough surfaces.

Discussion and conclusion: These results suggest that different surface property of the TiO₂ thin film may influence the preferential binding of different cell types. As such, the design of the titanium implants may need to take into account the different intended target tissues in the body.

PB-8

COMET ASSAY FOR CHITOSAN POROUS SKIN REGENERATING TEMPLATES (PSRTs) TREATED ON PRIMARY NORMAL HUMAN EPIDERMAL KERATINOCYTES (PNHEK) CULTURES: AN IN VITRO GENOTOXICITY MODEL

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Objective: To evaluate the genotoxicity of PSRTs treated on pNHEK cultures by using alkaline comet assay.

Materials and method: Four (4) PSRTs (PSRT108, PSRT109, PSRT115 and PSRT116) were assayed on pNHEK cultures via direct contact method. pNHEK cultures were trypsinized, combined with agarose and electrophoresed in electrophoresis solution pH > 13. The DNA from pNHEK was permanently silver stained on slides. The average of comet tail lengths (µm) was scored at 24 and 72 hours post-treatments. pNHEK treated with organotin-polyvinyl chloride (organotin-PVC) served as positive control while with low density polyethylene (LDPE) as the negative control.

Results: The average tail lengths for PSRT108 (11.64 ± 3.86 µm) and PSRT109 (11.78 ± 2.63 µm) was found comparable with negative control (LDPE) (11.66 ± 3.6 µm) at 24 hours while increased 4 µm for PSRT108 and 5 µm for PSRT109 at 72 hours.

Discussion and conclusion: PSRT108 is the most compatible chitosan derivative form of porous skin regenerating template followed by PSRT109, PSRT116 and PSRT115.

PB-9

EFFECT OF LOCAL TUALANG HONEY ON OSTEOPOROSIS IN POSTMENOPAUSAL ANIMAL MODEL

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Objective: To evaluate the effect of local Tualang honey on postmenopausal osteoporosis of ovariectomised rat.

Subjects and method: Three-months-old adult virgin female Sprague-Dawley rats were randomly divided into 5 groups (n=7). Animals of group 1 were subjected to sham operation while groups 2, 3, 4 and 5 had undergone bilateral ovariectomy. The day after postoperative, animals of group 1 (control sham) and group 2 (control ovariectomised (OVX)) were given vehicle treatment (0.5 ml of distill water) whereas those in groups 3, 4 and 5 received Tualang honey at low, medium and high dose (0.2, 1.0 and 2.0 g/kg body weight) respectively by gavage for 14 days. Daily vaginal smears were performed throughout the treatment period. The animals were sacrificed 24 hrs after the last dose of treatment (during diestrous phase). Right posterior tibia was removed, weighed and subjected for histopathological examination (HPE) while blood samples were collected for serum alkaline phosphatase (ALP) assay.

Results: OVX resulted in a significant increase ($P < 0.05$) in serum ALP when compared to sham group, suggesting that OVX increased the bone turnover rate in these animals. Treatment of Tualang honey to ovariectomised rats also showed an increasing in serum ALP (marker of bone formation) and resulted in slightly increased of relative tibia weight. However, there were no significant values were observed for both parameters among all groups. HPE indicated that Tualang honey can increase the trabecular thickness of the tibia in the ovariectomised rats.

Discussion and conclusion: Our results showed that Tualang honey has an important role on bone health in postmenopausal animal model.

PB-10

RENAL ISCHEMIA-REPERFUSION INJURY IN SPRAGUE DAWLEY RATS

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Objective: To investigate the effect of unilateral Renal ischemia-reperfusion injury (RIRI) on urine volume, urine flow rate, kidney index and mean arterial blood pressure (MAP) in anesthetized Sprague Dawley (SD) rats.

Subjects and method: During the acute study, the overnight fasted rats were anesthetized with sodium pentobarbitone, 60mg/kg i.p. after which the rats were maintained on i.v. infusion of normal saline. RIRI was developed by clamping the left renal artery for 30 minutes. A thirty-minute reperfusion period was allowed before starting urine samples collection. Subsequently, six urine collections were taken at twenty-minute intervals for two hours after which the animals were euthanized by an overdose of sodium pentobarbitone.

Results: The data showed a significantly ($p < 0.05$) higher urine volume and urine flow rate in the ischemic rats as compared to the control. Moreover, a significant increase ($p < 0.05$) in the MAP of ischemic rats was noted as compared to the non-ischemic counterparts. However, no significant difference was seen in the kidney index.

Discussion and conclusion: These findings showed that RIRI results in a pattern of compromised renal tubular functions which is characterized by an increase in the urine production. The increase in MAP is thought to be due to renin hypersecretion, which accelerates conversion of angiotensin I to angiotensin II, enhancing adrenal release of aldosterone.

PB-11

LOSARTAN REDUCES SENSITIVITY OF RENAL RESISTANCE ARTERIES' IN SPONTANEOUSLY HYPERTENSIVE RATS (SHR) TO CATECHOLAMINES: CROSS TALK BETWEEN RENIN-ANGIOTENSIN SYSTEM AND SYMPATHETIC NERVOUS SYSTEM

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Objective: To study the possible relationship between renin-angiotensin system and sympathetic nervous system

Subjects and method: Thirty two (32) rats (SHR) body weight ranging from 250-325g were treated for 7 days with losartan, a specific AT1-receptor blocker. Specific involvement of AT1 receptors was confirmed by equivalent actions of losartan. 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 renal nerve stimulation and intrarenal administration of noradrenaline, phenylephrine, methoxamine, dopamine and angiotensin II.

Results: The data showed a significant (all $P < 0.05$) decrease in the vasoconstrictor response to Ang II and all the adrenergic stimuli given after treatment with losartan in SHR rats when compared to the untreated rats.

Discussion and conclusion: Blockade of AT1 receptors caused by losartan lowers the vasoconstrictor response to Ang II markedly and reduces the sensitivity of renal α_1 -adrenoceptors to the neuronal stimuli along with AT1 receptors, indicating a crosstalk relationship between α_1 -adrenoceptors subtype, the major subtype of α -adrenoceptors in renal vasculature and AT1 receptors.

PB-12

THE STUDY IN VITRO OF GAMMA IRRADIATED TUALANG HONEY USING PRIMARY NORMAL HUMAN DERMAL FIBROBLASTS CULTURES

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Introduction: Honey is an important energy food. Honey also has anti-inflammatory, antimicrobial and antioxidant properties. This is favorable to be used in wound management. Fibroblasts (proliferating cells) had been shown to play a vital role during the process of wound healing and are suitable to use for in vitro culture.

Objective: To study the effect of gamma-irradiated Tualang honey on the proliferation of primary normal human dermal fibroblasts (pNHDF) cultures.

Materials and method: pNHDF were tested with serial concentrations (12.5 %, 6.25 %, 3.13 %, 1.56%, 0.78 % and 0.39 %) of Gamma Irradiated (GI) Tualang Honey. The viability of cultured pNHDF was determine with 3-(4, 5-dimethyl-2 thiozoly)-2, 5-diphenyl-2H-tetrazolium bromide (MTT) at 24, 48 and 72 hours and the optical density (OD) was measured by using enzyme-linked immunosorbent assay (ELISA).

Results: The value of GI Tualang honey below 1.56 % was found to be cyto-compatible above 1.56%. The growth of fibroblasts declined due to hyper-osmotic effect. The percentage of fibroblast viability declined from 12.5% to 3.13%.

Discussion and conclusion: The concentration of Gamma Irradiated (GI) Tualang honey below 1.56 % was ideal in proliferation of pNHDF cultures. Therefore, GI Tualang honey below 1.56 % may be used as a baseline to further evaluation of their hidden potential in wound management.

PB-13

EFFECTS OF JUMPING EXERCISE AND SUBSEQUENT SHORT AND LONG TERM CESSATION OF EXERCISE ON BONE IN FEMALE RATS

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Objective: to investigate the effects of 8-week high impact jumping exercise and subsequent short (12 weeks) and long (24 weeks) term cessation of exercise (deconditioning) on bone in female rats.

Subjects and method: Eighty (80), 12 week-old female rats were divided into eight groups, where the groups were given either no exercise for 8, 20 or 32 weeks, or performed 8 weeks of jumping exercise (8E), or 8E followed by 12 or 24 weeks of deconditioning, or 8E followed by 12 or 24 weeks of continuous exercise. The exercise consisted of 40 jumps/day for 5 days/week at a jumping height of 40cm. Tibial fat free dry weight (mass), ultimate bending load (strength), diaphysis maximum diameter, periosteal and endosteal perimeters, cortical and medullary areas were measured. Statistical analysis was performed using one-way ANOVA.

Results: Eight weeks of jumping exercise elicited significant bone gains in all the measured parameters, with the exception of medullary area. After 12 weeks of deconditioning, the 8E-induced gains in ultimate bending load, maximum diameter and cortical area were maintained. However, with the exception of cortical area, all measured 8E-induced gains were lost after 24 weeks of deconditioning.

Discussion and conclusion: The rate of decay of jumping exercise-induced bone gains might differ depending on the measured parameters. The gains in most of the measured parameters could not be maintained after 24 weeks of cessation of exercise implying continued exercise is needed to maintain the beneficial bone effects gained during young age.

PB-14

LASER DOPPLER FLOWMETRY (LDF) & POST-OCCLUSIVE SKIN REACTIVE HYPERAEMIA FOR THE ASSESSMENT OF MICROVASCULAR FUNCTION – EFFECT OF GENDER, MENSTRUAL CYCLE & AGE

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Introduction: Post-occlusive skin reactive hyperaemia (PORH) is increased skin blood flow following release of a brief arterial occlusion. PORH and laser Doppler flowmetry (LDF) is used as a model to assess microvascular function.

Objective: to assess the effects of estrogen changes in the menstrual cycle, age and gender on measurements obtained using this model

Patients and method: This cross sectional prospective study involved 120 healthy females & males between 21 - 50 years, equally distributed to 6 groups according to gender & age group, ie males between 21 - 30, 31 - 40, 41 – 50 years, females between 21 - 30, 31 - 40 and 41 – 50 years. Each group consists of 20 subjects. Males were studied once, females were studied during the low (Days 2-5 of menstrual cycle) and high (Days 10-13) estrogen phases of menstrual cycle. During standardized vascular study session, one minute of baseline skin flux was recorded before forearm blood flow occluded at 200 mmHg for 3 minutes. Occlusion is then released and skin flux was monitored for 2 minutes afterwards. Skin flux was measured continuously by the LDF, primary response was quantified as maximal increase in perfusion after occlusion compared to baseline (PORHmax). Results are presented as mean \pm sem, $p < 0.05$ is set as the statistically significance.

Results: Skin flux increased approximately four folds after occlusion. Males had higher baseline perfusion compared to females (13.53 ± 1.32 vs 10.72 ± 0.51 au), however, PORHmax was not different at 46.04 ± 2.05 and 48.41 ± 2.6 au respectively. Serum estradiol increased approximately 4 folds during high estrogenic compared to low estrogenic phase; however, PORHmax was not different between the two phases. The different age groups did not differ in their PORHmax response.

Discussion and conclusion: Assessment of microvascular function using the model skin post-occlusive reactive hyperemia and LDF is not affected by age, gender and changes in estrogen levels during a regular menstrual cycle in healthy subjects up to the age of 50 years

PB-15

THE EXPRESSION OF CYTOKERATIN 6, INVOLUCRIN, HEAT SHOCK PROTEIN 47 AND FIBROBLASTS SURFACE PROTEIN IN PRIMARY CULTURED HUMAN SKIN IN VITRO: A MODEL FOR SKIN IMMUNOCYTOCHEMISTRY

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Objective: To detect the expression of specific marker HSP 47 and FSP in primary human dermal fibroblasts (pHDF) and the expression of CK 6 with involucrin in primary human epidermal keratinocytes (pHEK).

Materials and method: Confluent monolayer of pHDF, pHEK, commercial available human dermal fibroblasts (cHDF) and commercial available human epidermal keratinocytes (cHEK) culture were trypsinized. pHDF, cHDF, pHEK and cHEK were seeded onto slide chambers to allow the growth at confluent. The entire cells were then fixed with cold methanol for 30 minutes followed by the addition of primary antibodies (HSP47, FSP, CK6 and Involucrin). Slides were rinsed with rinse buffer and incubated with secondary antibody. Color was developed after the addition of streptavidin and chromogen. Control without primary antibody was also included for each cell type.

Results: pHDF and cHDF except pHEK (negative control) cultures were brown coloured with HSP 47 and FSP antibodies. pHEK and cHEK except pHDF (negative control) were also coloured with antibodies CK6 and involucrin.

Discussion and conclusion: The cells that were brownish stained after detection by HSP47 and FSP were identified as skin fibroblasts. Skin keratinocytes were identified with antibodies CK6 and involucrin. Thus, we have successfully established primary skin cells namely fibroblasts and keratinocytes to be used for skin toxicological experiments in future.

PB-16

ALLELIC IMBALANCE IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKAEMIA (ALL): APPLICATION OF SINGLE NUCLEOTIDE POLYMORPHISMS ARRAY (SNPA)

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Introduction: Allelic imbalance is a common genetic event in many types of malignancy. The recent introduction of high-density SNPA for the concurrent analysis of loss of heterozygosity (LOH) and changes in chromosome copy number (CN) in small amounts of DNA has facilitated a comprehensive, genome-wide analysis of tumour cell populations. This method is of value in malignancies where standard cytogenetic analysis is difficult to perform.

Objective: To characterise LOH and CN alterations in leukaemic samples using SNPA.

Patients and method: A cohort of 86 patients presenting with childhood ALL within the Northern Region of the UK (Sept 1986-Jan 2005) were analysed using GeneChip® Human Mapping 10K Array (Affymetrix, Ltd.). Good quality of DNA (250ng) was sent to MRC Geneservice (Cambridge) for sample analysis. Results from SNPA analysis were analysed using proprietary software (Affymetrix). The results spreadsheets obtained from MRC contained information about the identity of each SNP and its chromosomal location were exported to Excel spreadsheets for in house analysis.

Results: Sixty-nine of the 86 samples (80%) showed one or more significant areas of LOH. Consideration of the CN of the regions affected suggested that this was of 2 types: LOH associated with CN reduction (deletion) and LOH associated with no CN change or copy neutral-LOH (acquired isodisomy, AID). Allelic imbalances have been frequently identified on chromosome 9p, 12p and 6q. Loss of 9p has been associated with tumourigenesis and to be progressive in some cases at relapse. A tumor suppressor gene, p16^{INK4a} is always included in 9p deleted region and has been suggested to be involved in leukaemogenesis.

Discussion and conclusion: This study indicated that application of SNPA is very useful to characterize allelic imbalance in childhood ALL. In addition, unlike other whole-genome screening methods, it can readily detect LOH associated with the preservation of the normal CN (AID).

PB-17

OPTICAL MICROSCOPIC CHANGES IN MALE RAT REPRODUCTIVE ORGANS POST-TREATED WITH CARBOFURAN – II

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Objective: To study the effects of carbofuran on the male reproductive organs of the Albino rats.

Subjects and method: The male adult albino rats (n = 24) were grouped into A (control), B and C (experimental). The experimental animals (group B) were administered with 2 mg/ kg body weight/ day carbofuran in saline for 60 days and then sacrificed. The animals of group C were kept without treatment for 30 days and then sacrificed to see the delayed changes. Epididymedes were fixed and processed to see histological changes.

Results: Epithelial degeneration, reduction in spermatozoa in the tubal lumen and increased vascularity were observed in animals treated for 60 days (group B). These structural changes persisted in the organs even after stoppage of carbofuran for 30 days.

Discussion and conclusion: Optical microscopic changes were seen in male rats reproductive organs treated with Carbofuran for 60 days compared to those treated for only 30 days and those not treated.

PB-18

NECROPHAGOUS INFESTATION IN RABBIT CARCASSES DECOMPOSING IN KUBANG KERIAN KELANTAN

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Objective: The influence of habitat, rain and presence of malathion on initial infestation and lifecycle of necrophagous insects in rabbit carcasses decomposing in USM campus, Kelantan was studied for a period of one year, for providing baseline data for estimating postmortem interval.

Subjects and method: Two sets of rabbits (*Oryctolagus cuniculus*), one for sunlit habitat and the other for shaded habitat were decomposed once in a month (July 2006-June 2007). Five rabbits were used in each set, one each for positive and negative controls sacrificed using the carbon dioxide and three killed by varying concentrations of malathion via force-feeding. Rainfall, ambient and carcass temperatures, presence of adult, eggs, maggots, pupae and the duration of lifecycles were documented. Species identification was performed using the standard identification keys.

Results: The dominant species infesting was *Chrysomya megacephala* followed by *C. rufifacies*. Duration of lifecycle was longer in the sunlit habitat compared to the shaded habitat during the rainy months while during the normal months, the above durations were similar. In the presence of rains (> 10.0 mm) initial infestation in the sunlit habitat was delayed, whereas the pupation period was prolonged. Malathion reduced the diversity of infesting species, delayed the initial of infestation by 2-3 days which increased by another day with higher concentrations (50% higher than the lethal dose). However, rains (> 10.0 mm) during the first 2 days of decomposition reduced the delay of initial infestation in the malathion-treated carcasses.

Discussion and conclusion: In general, rain and malathion delayed the initial infestation and prolonged the lifecycle. Such delay and prolongation did not occur when carcasses were decomposed in the shaded habitat even during rains. Furthermore, prolongation of lifecycle attributable from moderate to heavy rains was observed mainly in pupation period.

PB-19

OPTIMIZATION OF PCR TECHNIQUE FOR THE EXONS OF TGF β 3 GENE IN NON-SYNDROMIC CLEFT LIP WITH OR WITHOUT CLEFT PALATE PATIENTS

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Introduction: Orofacial clefts particularly non-syndromic cleft lip with or without cleft palate (CL \pm P) are the most common craniofacial deformities affecting one in every 700 to 1000 newborn worldwide. CL \pm P can be caused by environmental and genetic factors. Many genes are involved in non-syndromic CL \pm P. Several of these genes have been identified and mutations in the *TGF β 3*, encoding a protein known as a cytokine is said to be involved in cell differentiation, embryogenesis and development which may have responsible in a majority of the patients.

Objective: To optimize the PCR amplification in seven exons of *TGF β 3* gene in non-syndromic CL \pm P patients.

Materials and method: Blood samples were collected from the patients and the DNA was extracted using a commercial kit (GENE ALLTM). Primers were designed for all the seven exons and PCR amplification was performed for optimization using the Gradient PCR machine. The amplified PCR products were run on agarose gel to confirm the size of the PCR products.

Results: The PCR method for all the seven exons of *TGF β 3* gene has been optimized and the correct product sizes have been obtained. The best annealing temperature for amplification of the exons was 60°C for exon 1 and 2, 54°C for exon 3, 59.2°C for exon 4, 55.4°C for exon 5, 50°C for exon and 58°C for exon 7.

Discussion and conclusion: In this preliminary study, the amplification of the seven exons of *TGF β _3* gene has been successfully optimized, which will be subjected to mutation analysis.

PB-20

PREVALENCE OF HERBAL MEDICINE USE AMONG CANCER PATIENTS IN KELANTAN

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Objective: To determine the prevalence and determinants of herbal medicine use among Kelantanese cancer patients.

Patients and method: This is a cross-sectional descriptive study conducted among 79 cancer patients from the Oncology Ward (3 Selatan) and the Oncology Clinic, HUSM using structured open and close-ended questionnaires. Microsoft Statistical Package for the Social Sciences (SPSS) version 12 was utilized to assess demographic data, prevalence and determinants of herbal medicine usage, types and knowledge of herbal medicine usage amongst those patients.

Results: The use of different herbal medicine for cancer treatment is profound amongst cancer patients in Kelantan (41.8% of respondents). The aged respondents (40 years and above versus 40 years and below) show significant differences ($p < 0.05$). Majority (60.6%) believed herbal medicine had been beneficial in returning them to physical fitness and to relieve cancer symptoms. No association was indicated between cancer type or gender and use of herbal medicine. However, level of education was influential in determining usage of individual herbal medicine. Further, non uniformity in patterns was observed for the different herbal medicine preparation mentioned by the respondents.

Discussion and conclusion: Herbal medicine usage is substantial in this population. There is less differentiation between social groups in relation to herbal medicines usage. Kelantanese cancer patients' characterization is different when compared to those reported in studies of western cancer patients. Different level of usage of herbal medicines reported has highlighted the importance of understanding patterns for specific indigenous practices in and at the national level. Information gathered in this study can help to establish representative characterization of cancer patients in Malaysia.

PB-21

PREVALENCE OF HERBAL USAGE AMONG THE KELANTANESE MALAY WOMEN OVER THE PREGNANCY PERIOD

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Objectives: To determine the prevalence of herbal usage over the pregnancy period among the Kelantanese Malay women and to determine the association between level of knowledge about the herbs and practice of consuming herbs over the pregnancy period.

Patients and method: This is a cross sectional descriptive study conducted among 456 Kelantanese Malay women at the Antenatal Ward, HUSM from September 2007 to December 2007 using structured close-ended questionnaires adapted from studies by Soon and Hasni, Nordengand Havnen and Azriani with some modifications. Microsoft Statistical Package for the Social Sciences (SPSS) version 12 was utilized to assess demographic data, prevalence of herbal usage and types of herbs used during the pregnancy period.

Results: Majority (55.7%) of respondents were housewives. 57.2% were Gravida 2 to 5. 61% had attended secondary education. 85% were aged between 21 to 40 years. Herbal usage during pregnancy period was 34.3% while 73% utilized herbal medicines in labour. 141 women mentioned that they consumed herbs due to their beneficial effects in the childbirth process. 77% agreed upon its efficacy and safety. Independent t-test on the association of knowledge about herbs and practice was low. The primary herbs consumed were 'Sanggul Siti Fatimah' (*Euphorbia haterophylla* L.), 'Serai' (*Cymbopogon citrates*), 'Bawang Merah' (*Allium ascalonicum*) and 'Bawang Putih' (*Allium sativum*) and 'Misai Kucing' (*Orthosiphon stamineus*).

Discussion and conclusion: Herbal medicine is still being held in an important position among the Malay Kelantanese women communities. The usage of these herbs during the pregnancy period has not been previously reported amongst Kelantanese Malay women. A detailed study is therefore needed to establish among others the efficacy and safety of these herbs where the well-being of mother and fetuses are crucial.

PB-22

OPTIMIZATION OF ANTIBODIES FROM LYMPHOCYTES SECRETION (ALS) ASSAY METHOD FOR DETECTION OF ACTIVE TUBERCULOSIS

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Objective: To determine the immunological responses by measuring the antibody levels against the Culture Filtrate Proteins of Mycobacterium tuberculosis in lymphocytes culture supernatants from tuberculosis and non-tuberculosis patients by using ALS assay method.

Patients and method: Ethical clearance was obtained from Human Ethical Committee. Informed written consent was obtained from every patient recruited in this study. A total of 10 mL heparinised peripheral bloods of smear positive pulmonary tuberculosis patient were taken. The peripheral blood mononuclear cells were separated using routine density gradient centrifugation method by Ficoll-Histopaque. The cells were suspended in 24 well-tissue culture plate in RPMI 1640 containing 10% fetal calf serum and incubated for 72 hours in 37°C with 5% CO₂ supplement. The supernatant were harvested and assayed for the presence of anti-CFP antibodies by ELISA method.

Results: The ALS assay method was successfully optimized. The best lymphocytes cell counts to get the best result determined at the counts of 106 cell/ ML. A number of 25 patients confirmed (smear and culture positive) to have pulmonary tuberculosis and 9 healthy controls were included in this study. The measured IgG antibodies level for patient varied from 0.730 to 2.931 giving mean value of 1.52 whereas the mean value for healthy control was 0.75.

Discussion and conclusion: This new serological method gives a promising result for the diagnosis of pulmonary tuberculosis. It has the value as a supplementary test or to replace currently available diagnostic method. Larger sample size is required for a better statistical analysis.

PB-23

LOW INCIDENCE OF FLT3 GENE MUTATION IN MALAY PATIENTS WITH ACUTE MYELOID LEUKEMIA

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Objective: To detect the fms-like tyrosine kinase 3 (FLT3) gene mutations in acute myeloid leukemia (AML) patients.

Patients and method: Thirty samples of whole blood were obtained from Malay patients diagnosed as AML, Hospital Universiti Sains Malaysia (HUSM). Genomic DNA was extracted using a commercial kit (GENE ALL™), with the protocol provided by manufacturer. DNA concentration was determined by performing qualitative measurement using NanoDrop Spectrophotometer. The PCR amplification was carried out according to the method suggested by the manufacturer (InVivoScribed Technologies, USA). The PCR products were digested with EcoRV endonuclease for FLT3 D835 mutation detection. The amplified product of FLT3 D835 and ITD were electrophoresed through 1.5% agarose gel stained with ethidium bromide and then were visualized under UV light.

Results: Out of 30 patients, mutation of FLT3 ITD was detected only in 2 patients (6.67%) and none for D835 mutation.

Discussion and conclusion: This study may suggest the low incidence of FLT3 gene mutation in Malay patients with AML and may not implicate as a useful prognostic marker in Malaysian population.

PB-24

DOES FORCED SWIMMING EXERCISE AFFECT THE NEUTROPHIL/ LYMPHOCYTE RATIO AND BODY WEIGHT OF MALE RATS?

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Objective: To investigate whether swimming exercise at the early age has any effect to the body system especially on the body weight and blood parameters of male rats.

Subjects and method: Twenty male Sprague Dawley pups were divided into two groups as control and forced swimming test (FST) groups. Each group consists of ten pups. At the age of 7, 8 and 9 days, the pups were forced to swim in the warm water (30 ± 0.5 °C) for 60 sec and 90 sec, respectively. The pups were then removed from the water, dried with a towel and placed in a warm enclosure. They were returned to their mother after regained a pink color and able to move on their own. Blood smear was taken from the tail vein and their body weight was measured weekly.

Results: There was no mortality rate occurs after the FST. Neutrophil/lymphocyte ratio was significantly increased in FST group ($p < 0.05$). The body weight of control and the FST groups were increased with the pups' age. However, the body weight for FST group was significantly decreased starting on days 14, 21, 28, 35 and 42 and returned to normal on days 49 and 56.

Discussion and conclusion: This study shows that neonatal FST can cause the stress to the male rat pups but not up to kill them. For the future experimental works, we will evaluate any changes in the hippocampus on days 9, 16, 30 and 51 after the FST via neurogenesis. Analysis of the stress hormone level at the respective ages will be conducted and its relationship with the changes of hippocampus will be determined.

PB-25

AGE-RELATED CHANGES IN SUPEROXIDE DISMUTASE IN THE KIDNEY OF SPONTANEOUSLY HYPERTENSIVE RATS (SHR) AND NORMOTENSIVE (WKY) RATS

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Introduction: Hypertension has been increasingly associated with oxidative stress. Superoxide dismutase (SOD) is the primary antioxidant enzyme that protects against oxidative stress.

Objective: To determine the total SOD activity and its mRNA expression in the kidney of spontaneously hypertensive rats.

Subjects and method: Systolic blood pressure was measured in SHR and age-matched WKY rats at the age of 5,7,8,10,12 and 16 weeks. At the end of each age period animals were sacrificed and the left kidney was removed for the estimation of total SOD activity (spectrophotometrically) and mRNA expression of CuZn-SOD and Mn-SOD using Real-time PCR.

Results: Systolic pressure was significantly higher in SHR from the age of 5 weeks onwards. No significant differences were evident either in the total SOD activities or mRNA levels of Cu-Zn-SOD and Mn-SOD between SHR and age-matched WKY rats.

Discussion and conclusion: It appears that the SOD activity and its mRNA expression are not significantly altered in SHR till the age of 16 weeks.

PB-26

DENTURE SET-UP MOULD – A SIMPLE INNOVATION WITH MANY BENEFITS

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Introduction: Complete denture construction is a multistage process. The teeth are set-up in the laboratory on to the working model mounted on an articulator as per the recorded bite registration. One takes about 90 minutes to complete an upper and lower teeth set-up. This restricts the number of denture appointment on a given day. Denture Set-up Mould fabrication was taken up as a project to overcome this problem.

Objective: To reduce the working time in setting up the teeth so that more cases can be taken by the technicians.

Materials and method: The moulds are of different sizes according to the teeth and jaw size. The teeth are set according to the principles of teeth setting. The mould is reusable and can be disinfected.

Results: A six-months study before and after use of denture set-up mould showed that by using the mould, time for teeth setting time was reduced to 30 minutes. Denture appointments could be increased from 7 to 12 patients per day. The waiting time for new denture patients was reduced from 5-6 months to less than 2 months. The number of dentures fabricated by each technician monthly increased from 20 to 36 units. This led to increase in government revenue from issue of dentures from 77%. However, some limitations observed were the mould could not be used in situations where the vertical dimension is reduced and there is no provision for edge-to-edge or cross bite conditions

Discussion and conclusion: Despite certain limitations, this innovation benefits all its stake holders including the dental service providers, its customers as well as the government.

PB-27

PRODUCTION AND CHARACTERIZATION OF POLYCLONAL ANTIBODY AGAINST 35 KDA PROTEIN OF SHIGELLA FLEXNERI

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Introduction: Antibodies are important tools used by many investigators in their research and have led to many medical advances. Polyclonal antibodies are antibodies that are derived from B-cell. It consists of mixture of immunoglobulin molecules secreted against a specific antigen, each recognising a different epitope. In a previous study, a specific antigen of 35 kDa in size for *Shigella flexneri* (*S. flexneri*) was demonstrated against IgA.

Objective: To study the production of polyclonal antibodies in mice and rabbit against the specific 35 kDa protein of *S. flexneri*.

Materials and method: Surface associate proteins of *S. flexneri* 2a were separated via SDS-PAGE and 35 kDa protein was purified by standard electro-elution technique. The purified protein was emulsified with Complete Freund's Adjuvant (CFA) and was injected into five New Zealand White rabbits and ten C57/BL6 mice. This is followed by two booster injections at two weeks interval. Sera were collected at day seven and day fourteen post-injection. ELISA and Western blotting techniques were used to determine the titer and specificity of the polyclonal antibodies obtained.

Results: The specific antibody against 35 kDa was detected at day seven after injection in mice whereas in rabbit it was only detected at day fourteen.

Discussion and conclusion: High titer of antibodies against the 35 kDa protein of *S. flexneri* was obtained that could be used in the development of antibody-based diagnostic test for shigellosis.

PB-28

IMMUNOBLOTTING ANALYSIS OF THE EXPRESSION OF IBMR3 ANTIGEN IN MOUSE AND RAT TISSUES

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Objective: To investigate the expression profile of the IBMR3 antigens in different mouse and rat tissues by using immunoblotting technique.

Materials and method: IBMR3 hybridoma cells were cultured *in vitro* in RPMI-1640 complete growth medium using standard cell culture technique. The cell culture supernatant was collected, aliquoted and stored at 20°C freezer. Various mouse and rat tissues were snap-frozen and stored in LN2 nitrogen. For immunoblotting, 6 micron frozen sections of the various tissues were prepared and collected in Eppendorf tubes. The sections were then lysed in lysis buffer, and equal concentration of lysates was run on 7.5% SDS-PAGE. The separated protein bands were transferred to nitrocellulose paper for immunoblotting using neat IBMR3 antibody supernatant. Isotype-matched irrelevant mouse antibody was used as control. The immunoblot were subsequently subjected to densitometric analysis.

Results: IBMR3 Ag was differentially expressed in the mouse tissues with the highest number of bands detected in muscle and the lowest in heart and liver. In rat, more bands were detected in brain and kidney with less in spleen. In both mouse and rat, highest expression of IBMR3 Ag was found in the lung and the lowest in the brain.

Discussion and conclusion: The results from this study suggest that the IBMR3 antigens were differentially expressed in mouse and rat tissues. Future study will be designed to identify and investigate the nature of the IBMR3 antigen and its potential role in tissue development.

PB-29

FUNCTIONAL ANALYSIS OF ZNS AND ZNSE NANOPARTICLES ON MOUSE SPLEEN CELLS

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Objective: To investigate the ability of the chemically synthesized nanoparticles Zinc Selenide (ZnSe) and Zinc Sulfide (ZnS) to interact with the spleen cells from the Balb/c mouse.

Subjects and method: ZnSe and ZnS in different concentration were prepared by mixed up the powder form of the nanoparticles with RPMI-1640 growth medium. Female balb/c mouse was sacrificed by cervical dislocation technique. Spleen cells were aseptically obtained by teasing, and plated in 96-well plates in complete growth medium containing different concentration of the nanoparticles. Viable cell numbers at 0, 24, 48 and 72 hours exposure were estimated using MTS technique. Morphological analysis of the nanoparticles was done by using UV-light microscope.

Results: By using UV-light microscope, the emission spectra of both compounds were found to be highest at 360 nm (blue). Viable cell analysis using MTS assay showed that ZnS, but not ZnSe, nanoparticles at 1.9 and 7.8 mg/ml were able to significantly ($p < 0.05$) induce the proliferation of mouse spleen cells after 72 hrs co-cultures. However, the IC_{50} of both nanoparticles were greater than 1000 mg/ml.

Discussion and conclusion: The findings from this study indicate that ZnS, but not ZnSe, at low concentration may interact with immune cells. Taken together, the emission spectrum and the biological property of both nanoparticles may indicate their potential for finding applications in medicine.

PB-30

N-NITROSO-N-METHYLUREA (NMU) INDUCED BREAST CARCINOMA IN RAT MODEL: USM EXPERIENCE

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Introduction: N-Nitroso-N-methylurea (NMU) synonymy known as MNU is a carcinogen that widely used in rat experimental breast cancer research.

Objective: To study anti-angiogenesis in rat model that has been grown with breast tumour.

Subjects and method: NMU (Sigma Aldrich, N4766) was diluted in 0.9% NaCl at 70mg/kg bodyweight then injected into 60 female Sprague Dawley rats age between 21 to 28 days old. Twice injection was given to each rat intraperitoneally and tumour growth was observed

Results: Out of 60 rats, 44 (73%) were observed to develop breast carcinoma with mean latent period is 3.5 month with minimum and maximum latent periods are 1.5 and 8 month, respectively. Topographic location showed the ratio of 2:1 observed at axillary and inguinal regions. Instead, NMU injection produces various side effects where 85% of the rats developed retinopathy within a week post-injection, 30% possessed generalized alopecia within 2 month post-injection. Some rats also exhibited multiple carcinomas proven by H&E evaluation such as malignant fibrohistiocytoma, verrucous carcinoma, and bony metastasis.

Discussion and conclusion: It was also observed that short latent period was achieved in less extreme climate fluctuation and laboratory disturbances. This study showed that the animal model of NMU-induced breast cancer was successfully reproduced. These required optimum external factors to accomplish desirable tumor latency and yield.

PB-31

STABILITY OF RECOMBINANT BCG (RBCG) EXPRESSING MALARIAL AND TUBERCULOSIS EPITOPES IN MICE

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Introduction: An attenuated strain of *Mycobacterium bovis* bacille Calmette-Guérin (BCG) is considered a promising candidate for the development of live vector systems to deliver foreign antigens to the immune system.

Objective: To determine the stability of a recombinant BCG (rBCG) clone expressing malarial and tuberculosis epitopes in immunized mice.

Materials and method: In this study, we constructed a recombinant BCG strain which express the synthetic genes encode a malarial epitopes namely the F2R(II)EBA and (NANP)3 as well as two T cell epitopes of the *M. tuberculosis* ESAT-6 antigen. Balb/c mice (n= 5) were immunized intraperitoneally (i.p.) with 10^6 cfu of the rBCG in 200 ml PBS containing 0.1% Tween 80 (PBS-T80). After 30 and 60 days, the same amount of rBCG was injected i.p. as boosters. The stability of the construct was evaluated in the selected organs of the immunized mice 28 days after the last immunization by PCR. Bacterial counts in these organs were also determined.

Results: PCR analysis showed that the rBCG clone was detected in the peritoneal wash out, spleen, lung and liver of the immunized mice. Bacterial counts from these organs showed that the highest bacterial were detected in the spleen (5.2×10^4 cfu) followed by liver (2.8×10^4 cfu), lung (2.7×10^4 cfu) and peritoneal (2.2×10^4 cfu).

Discussion and conclusion: PCR and bacterial dissemination analyses showed that the rBCG was stable and no significant effect to the organs of the immunized mice.

PB-32

USING XGRID TO IMPROVE FASTA EFFICIENCY FOR ALIGNMENT OF MULTIPLE DNA AND PROTEIN SEQUENCES

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Introduction: Xgrid is a distributed computing technology introduced by Apple™ Inc. to create low-cost supercomputers. Large-scale searching of protein or DNA sequence databases and multiple sequence alignments take considerable computing power and time.

Objective: To use Xgrid technology to speed up the alignment process in FASTA.

Materials and method: FASTA version 34 was downloaded from University of Virginia website (<http://www.fasta.bioch.virginia.edu>), compiled and installed on 10 Apple iMac computers running Mac OS 10.5.2 in a local cluster using Apple™ Xserve™ as controller. Nucleotide and protein databases were downloaded from NCBI, and 100 DNA and 100 protein Salmonella Typhi sequences were aligned using Xgrid. Results were recorded in seconds from the time of execution of search to completion.

Results: The time taken to align 100 Salmonella Typhi protein sequences with 25,746 protein sequences in the NCBI database took 1395 seconds, whereas Xgrid took only 346 seconds (25%). The time taken to align 100 Salmonella Typhi DNA sequences with 30,246 DNA sequences in the NCBI database took 2860 seconds, whereas Xgrid took only 886 seconds (31%).

Discussion and conclusion: Xgrid technology represents a low-cost alternative to harnessing the power of unused computers in a local network to accelerate the speed of sequence alignment of FASTA, and can be used to increase efficiency, save time and cost in computational analysis of DNA and protein databases.

PB-33

N-NITROSO-N-METHYLUREA (NMU) PREPARATION FOR THE INDUCTION OF MAMMARY CANCER IN SPRAGUE DAWLEY RAT MODEL

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Introduction: NMU-induced breast tumour in rat model is commonly used in mimicking the histology and pathogenesis of human breast tumour. Nevertheless, NMU preparation for the optimum induction becomes a critical point as commercially available NMU are variable.

Objective: To compare and observe NMU preparation of two commercial NMU products.

Subjects and method: A total of 129 female Sprague Dawley rats were obtained from Laboratory Animal Research USM (LARUSM) at 21 days of age. Rats were housed three per cage and fed with 702P mouse pellet and distilled water ad libitum throughout experiment. At 26 to 28 days of age, rats were randomized into three groups and injected intraperitoneally with 70mg NMU/kg body weight. Group 1 and Group 2 injected with NMU (lot no. 083K1076 & 075K0686, SIGMA ALDRICH, respectively) dissolved with 0.9% NaCl solution acidified to pH 5 with acetic acid. Group 3 injected with the same NMU as Group 2 but dissolved with 0.9% NaCl solution without acidification. All mixture was vortexed vigorously till completely dissolved.

Results: For Group 1, 76.36% out of 55 rats died within a week post-injection whereas Group 2 showed 80% mortality rate with main clinical sign was watery diarrhea. In contrast, Group 3 showed 75.93% survival rate out of 54 injected rats produced 41 rats with high yield mammary carcinoma.

Discussion and conclusion: These indicated that acidification of NMU preparation as used in the conventional method is not necessary and in fact harmful to the rats used for induction of breast cancer.

PB-34

ELEVATED LEVEL OF ALPHA-FETOPROTEIN IN INFANT SERUM WITH CYSTIC HYGROMA

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Introduction : Cystic Hygroma (CH) are non-malignant malformations of the lymphatic system characterized by single or multiple fluid-filled lesions that occur at sites where the lymphatic system connects to the venous system, most commonly the back of the neck. CH can be visualized using abdominal ultrasonography by 10 weeks' gestation. Elevated alpha fetoprotein levels in amniotic fluid have been reported in pregnancies with CH but not in infant serum.

Case Report : A term baby boy with huge cystic neck mass was delivered by elective caesarean section. Birth weight was 3.9 kg with good Apgar score and respiratory effort. There was no sign of upper airway obstruction. Huge mass with the size of 10 x 20cm was located at left sided of the neck, extended upwards and displaced the left ear forward. The mass was multilobulated, cystic with positive transillumination test. CT scan identified a large loculated and multiseptated cystic mass which was consistent with CH. No extension of mass into the thoracic cavity and the thyroid was normal. AFP was markedly elevated with the level of 178 744.3 ng/ml. Repeated AFP at 2 weeks interval was 8277.7 ng/ml. Patient was treated by sclerotherapy using OK-432 injection 6 weekly.

Discussion and Conclusion: AFP remains at relatively high levels during early infancy period, hence AFP needs to be monitored serially and interpreted cautiously in combination with other relevant tests. Therefore the guideline normally take the level of AFP < 72000ng/ml in newborn and it reduced by half at 2nd-3rd week and the level is < 10ng/ml from 2 years onward.

PB-35

DENSITY AND DIVERSITY OF INDOOR ASPERGILLUS AT THE MAIN CAMPUS OF UNIVERSITY SCIENCE MALAYSIA

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Introduction: Indoor Air Quality (IAQ) is being used to describe the characteristics of inner conditions of a building that determine the health and comfort of the inhabitants.

Objective: To investigate the identity, diversity and density of *Aspergillus* in the rooms at the Main Campus of USM.

Materials and method: Sampling of *Aspergillus* in the air of 16 randomly selected main rooms, including hostels, lecture halls, tutorial rooms, schools and laboratories, at the main campus of USM were carried out by using Andersen spore sampler and swab technique. Macroscopic and microscopic characteristics of the cultures were investigated and identified to the species level. The density of the life spores of *Aspergillus* indoor and outdoor (for comparison) were presented in (colony forming unit = CFU/m³ air). By using an international guideline, an office room is classified as a danger zone when the CFU/m³ is more than 200. Other air parameters noted were temperature and relative humidity (RH).

Results: The identity and frequency (%) of detection of 39 isolates of indoor *Aspergillus* were *A. niger* (51.3%), *A. flavus* (15.4%), *A. japonicus* (7.7%), *A. niger* # 1 (7.7%), *A. fumigatus* (7.7%), *A. terreus* (2.6%), *A. oryzae* (2.6%), *A. glaucus* (2.6%) and *A. sclerotiumniger* (2.6%). The range of temperatures recorded was 19-31°C and relative humidity (RH) was 39-97%. One room was considered as a danger zone with 283.69 CFU/m³. The lowest CFU/m³ was a room at the School of Industrial Technology (30.40 CFU/m³). The temperature, humidity and types of materials used were identified as factors that enhanced the proliferation of the fungi. Proper maintenance of A/C system is hereby recommended to reduce the indoor air humidity.

Discussion and conclusion: Thirty nine isolates of *Aspergillus* collected in the air of selected main rooms of the Main campus of USM were identified into nine species namely *A. niger* (51.3%), *A. flavus* (15.4%), *A. fumigatus* (7.7%), *A. japonicus* (7.7%), *A. terreus* (2.6%), *A. niger* # 1 (7.7%), *A. oryzae* (2.6%), *A. glaucus* (2.6%) and *A. sclerotiumniger* (2.6%).

PB-36

SYNTHESIS OF FERULIC ACID DERIVATIVES

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Introduction: 4-hydroxy-3-methoxycinnamic acid which is better known as ferulic acid is a cinnamic acid derivatives existing ubiquitously in the plant kingdom. It is easily absorbed and metabolized in the human body (Ou et.al, 2004). It has become an effective component of Chinese medicine herbs such as *Angelica sinensis* and *Cimicifuga heracleifolia* (Fang, 1998, Sakai et.al, 1999). Its good effect on human has brought ferulic acid to the many physiological functions, including anti-oxidant, anti-microbial, anti-inflammatory, anti-thrombosis, and anti-cancer activities (Ou et.al, 2007). It is also used for treatment in sperm viability (Zhang et.al, 1996) and protects against coronary disease and lower cholesterol in liver (Ou et.al, 2004).

Objective: To study on the synthesis of three ferulic acid derivatives which is known as methyl ferulate, allyl ferulate and epoxy ferulate. These derivatives are believed to have better medicinal properties than ferulic acid.

Materials and method: The first step of this work is the protection of the carboxylic group of ferulic acid by the methyl group through the esterification process. The mixture of ferulic acid, dichloromethane (DCM), dimethylaminopyridine (DMAP), methanol, dichlorocarbamide (DCC), and glycerol was stirred for 3 hours using the magnetic stirrer under the constant temperature of 0° C. The product formed was filtered and washed using DCM. This product was then reacted separately with allyl and epoxyde at its hydroxyl group. In this step, the mixture of the first product with tetrahydrofuran (THF), sodium hydroxide (KOH) and tetrabutylammonium iodide (TBAI) was stirred for 6 hours under the room temperature. All the products were purified by column chromatography and characterized by FTIR and ¹HNMR spectroscopy.

Results: 88.70% yield of the first product was formed, while 48.89% and 1.53% yield of the second and third product were formed. The FTIR spectroscopy of the three products shows all the suspected peaks in the range of 4000cm⁻¹ to 400cm⁻¹. Some of the important peaks such as OH peak and C=O peak which is slightly sharp can be seen clearly through the spectroscopy. ¹HNMR spectroscopy proved that the three products are pure after column chromatography since all the peaks of the protons appeared, suit the protons in the structure. In other words, the experimental result is similar to the theoretical result of the work.

Discussion and conclusion: Three ferulic acid derivatives has been synthesized and after characterization through FTIR and HNMR, it was conformed that the products are methyl ferulate ((*E*)-2-metoksi-4-(3-metoksibuta-1,3-dienil)fenol), allyl ferulate (3-aliloksi-4-hidroksifenil-3-metoksiferulat) and epoxy ferulate (4-hidroksi-3-(oksiran-2-iloksifenil)-3-metoksi ferulat). It is hope that these derivatives will be able to help the researchers in studying its beneficial properties especially in medical field.

PB-37

BRAIN'S STRIATAL TISSUE AND BONE MARROW AS A SOURCE OF NEURAL STEM CELLS: A PLEMINARY STUDY IN USM, MALAYSIA.

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Introduction: It has been proposed that CNS stem cells are the original multipotent cell type, with extended self-renewing capacity and the ability to give rise to progenitor cells. So, whether stem cells can be manipulated to replace cells in diseased tissue depends on knowing more about their basic properties.

Objective: To study on whether stem cells can be manipulated to replace cells in diseased tissue.

Subjects and method: After undergo the dissection procedure, the embryo's striatal brain tissue and the bone marrow (from femur of mother) of Sprague-Dawley rats were isolated. The tissue is then cultured in a serum-free medium: Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12 (DMEM/F-12; 1:1) with supplements, EGF (20ng/ml) and FGF-2 (20ng/ml). The isolated striatal tissue and bone marrow were dissociated with Pasteur pipette and cultured. Primary cultures were maintain at 37°C, 5% CO₂ and are fed every 2 days. The primary culture is maintained until typical cell clusters (neurospheres) have been obtained. The cells were allowed to differentiate by removing the growth factor.

Results: The nerve cells were obtained from cell culture after 7 days from both striatal brain tissue and bone marrow. The nerve cells been characterized based on the basic morphology observed by light microscope, which we observed the cell body, nucleus, axon, and dendrite.

Discussion and conclusion: Neural stem cells were generated from embryonic rat's brain tissue and adult rat's bone marrow which were isolated and culture in the neural stem cells basal medium, basic FGF-2, EGF and heparin.

PB-38

PHARMACOLOGICAL PROPERTIES OF GABA (A) RECEPTORS BY THE ESSENTIAL OIL OF MYRISTICA FRAGRANS USING THE ELECTROPHYSIOLOGICAL TECHNIQUE ON XENOPUS SP. OOCYTES

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Introduction: GABA (A) receptors are member of the superfamily of ligand-gated ion channels and it is the most widely distributed inhibitory receptor in higher regions of the mammalian CNS. These inhibitory receptors are pentameric transmembrane protein complexes that form an ion channel permeable to chloride ions (Cl⁻) and can be modulated by variety of pharmacologically and clinically important drugs, such as benzodiazepines, barbiturates, steroids, anesthetics and convulsants. These drugs produce at least part of their clinically relevant effects by interacting with distinct allosteric binding sites on GABA (A) receptors. Scientists continue to study the compounds from variety of plant species in order to test with these receptors. *Myristica fragrans* or nutmeg, whose seed is widely used as a spice, is a tropical, dioeciously evergreen tree native to the Moluccas or Spice Island of Indonesia and Malaysia. Previous reports have indicated that the pharmacological activity of *Myristica fragrans* mainly exist in its essential oil fraction.

Objective: To determine the effects of essential oil from *Myristica fragrans* on GABA (A) receptor.

Materials and method: The fruits of *Myristica fragrans* were extracted to get its essential oil to further investigation. *Xenopus laevis* oocytes were prepared and injected for next experiment. Enhancement of chloride currents by modulators of GABA (A) receptors were measured at a GABA concentration eliciting between 5 and 10 % of the maximal current amplitude (ranging between 3 and 8 μ M) was determined at the beginning of each experiment. A modulation of chloride currents through GABA (A) receptors composed of 1 2 2 subunits was analyzed and the expressions of GABA (A) receptors were calculated to get expression percentage.

Results: The modulation of GABA current by essential oil of *Myristica fragrans* at different GABA concentrations contributed some expression when this essential oil was tested at precise concentration.

Discussion and conclusion: The essential oil from *Myristica fragrans* enhanced the maximum chloride current of 1 2 2 receptors.

PB-39

EXPERIMENTAL GENOMICS DETECTS 36 NOVEL SNORNAS IN PLASMODIUM FALCIPARUM

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Introduction: Small nucleolar RNAs (snoRNAs) are one of the numerous and well-studied class of non-protein-coding RNAs (npcRNAs). SnoRNAs are involved in rRNA biogenesis. Based on structural motives, snoRNAs are divided into two subclasses: C/D-box snoRNAs and H/ACA-box snoRNAs and function via complementarity to rRNA targets. Most of eukarial snoRNAs are expressed ubiquitously. However, there is a number of orphan snoRNAs that lack significant complementarities to rRNA. Although functions for those npcRNAs are largely unknown, some of them were linked to human diseases such as Prader-Willi syndrome.

Objective: To identify the snoRNAs from malaria parasite, *Plasmodium falciparum*.

Materials and method: Total RNA from the erythrocyte stage of the parasite was extracted and size-fractionated (from 10 to 60 and from 60 to 500 nt) and constructed into two separate cDNA libraries. Around 35,000 cDNA clones were randomly selected, sequenced and assembled into contigs by DNASTar's Seqman program. Contigs were submitted to GeneBank (BlastN) analysis. Secondary structures of these RNAs were determined by Mfold programme. Northern blot hybridization was carried out to determine their expression patterns.

Results: 36 snoRNAs from *Plasmodium falciparum* was identified. Based on conserved sequence and structural motifs, they could be categorized as C/D (29) or H/ACA (7) box subclasses of snoRNAs. About half of snoRNA genes are localized in intron of protein-coding genes. Others are located in the intergenic region, raising the possibility that they may be independently transcribed. Interestingly, most of the identified snoRNAs have orthologs in the human and yeast RNome, targeting the corresponding regions on the respective 18S and 28S rRNAs (25S rRNA in yeast). This conservation showed that the predicted modifications could be important for rRNA function.

Discussion and conclusion: Post-transcriptional modifications guided by box C/D and/or H/ACA snoRNAs are important for rRNA functions. Since rRNAs are major targets for antimalarials, it is tempted to speculate that by intervening the rRNA modification there is a possibility to disrupt growth development of *Plasmodium falciparum*.

PB-40

ANTIPROLIFERATIVE ACTIVITY OF BRUCEA JAVANICA FRUIT EXTRACT TOWARDS HELA CANCER CELL LINES

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Introduction: *Brucea javanica* is a deciduous shrub which reported to have fruit with some therapeutic properties. Fruits of *Brucea javanica* (BJF) has been used as a folk remedy in Chinese medicine, and it has been shown to exhibit antiamoebic, antimalarial, antiplasmodial, antileukaemic and anticancer.

Objective: To determine the antiproliferative activity of *Brucea javanica* fruits extracts towards cervical adenoma cell line, HeLa and breast cancer cell line, MCF-7 and to reveal apoptotic morphology of treated cell with comparison to control.

Materials and method: *B.javanica* fruits were extracted using petroleum ether, methanol and distilled water. All three crude extract were treated on HeLa and MCF-7 to test for their antiproliferative activity by means of methylene blue assay. From the result, the best inhibitory concentration at 50 % living cells (IC_{50}) was chosen to undergo cellular and nuclear morphology by means of Hematoxylin and Eosin (H&E) staining and Hoechst 33258 staining, respectively.

Results: All the extract exhibited IC_{50} value less than 100mg/ml and methanol crude extract towards HeLa cells shown the best IC_{50} value with 2.8 mg/ml. Thus, HeLa cells treated with 2.8 mg/ml BJF methanol extract were selected to undergo microscopic analysis. The analysis of cellular morphology and nuclear morphology on treated HeLa cells at 24, 48 and 72 h were done by Hematoxylin and Eosin (H&E) staining and Hoechst 33258 staining, respectively. The typical apoptosis characteristics were observed in H&E stain. The apoptotic indexes of BJF-treated cells were revealed almost similar degree of apoptosis at time dependent manner compare with positive control, tamoxifen.

Discussion and conclusion: BJF methanol extract exhibited antiproliferative effect on HeLa cells via inducing-apoptosis. Therefore, it may be a potential candidate as a natural anticancer agent from plant resources.

PB-41

ANTI-PROLIFERATIVE ACTIVITY OF FOUR SPECIES OF MALAYSIAN 'ULAMS' TOWARDS SELECTED CANCER CELL LINES

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Introduction: *Vitex pinnata* (pucuk leban), *Barringtonia racemosa* (pucuk putat), *Oroxylum indicum* (pucuk beko) and *Syzygium polyanthum* (pucuk serai kayu) have been used as 'ulams' or fresh salad in Malaysia. These plants have been reported to have antioxidant properties. Plants with antioxidant properties may have anticancer properties.

Objective: To determine the inhibitory concentration of 'ulams' extract at 50% of cancer cells proliferation from whole cells population (IC_{50}).

Materials and method: Each plant has subjected to extraction procedure using petroleum-ether and methanol to produce petroleum-ether extract and methanol extract respectively. Only methanol extracts of 'ulams' were used to treat the cancer cells.

Results: Result showed that IC_{50} value of *V. pinnata* was 17.38 mg/ml, *E. polyanthum* was 5.50 mg/ml and *B. racemosa* was 3.47 mg/ml. These values considered extinction because it was less than 20 mg/ml, which is the indicator of a good anti-proliferative agent. Meanwhile, *O. indicum* did not show any IC_{50} value. Thus, it is not a potent extract for anti-proliferation agent.

Discussion and conclusion: *V. pinnata*, *S. polyanthum* and *B. racemosa* have a good potential to become an anti-proliferative agents. Taken together, these three plants might contain promising natural anti-cancer agents.