

The 11TH Annual Scientific Meeting of the College of Pathologists, Academy of Medicine Malaysia was held at the Crowne Plaza Mutiara Hotel, Kuala Lumpur from 8-10 June 2012. Abstracts of papers presented follow:

OP-AP-01. Protein expression profile of DNA mismatch repair genes and its association with clinicopathological characteristics in Malaysian Lynch Syndrome patients

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Introduction: Lynch syndrome also known as hereditary nonpolyposis colorectal cancer accounts for approximately 1-5% of all colorectal cancers. Germline mutations in a group of DNA mismatch repair (MMR) genes (MLH1, MSH2, MSH6, PMS1 and PMS2) have been found to be responsible for Lynch syndrome cases. **Objectives:** This study focuses on the determination of MMR (MLH1, MSH2, MSH6 and PMS2) protein expression profile by immunohistochemical analysis and its association with clinicopathological characteristics in clinically suspected Malaysian Lynch syndrome patients. **Methods:** Forty six (46) patients who fulfilled any of the revised Bethesda Guidelines criteria were recruited from four collaborating centres in Malaysia. Clinicopathological information of suspected Lynch syndrome cases that underwent bowel resection from collaborating centres was reviewed. Immunohistochemical analysis for MLH1, MSH2, MSH6 and PMS2 proteins were performed on paraffin embedded carcinomatous tissues. **Results:** Colorectal cancer protein expression analysis for MLH1, MSH2, MSH6 and PMS2 antigens showed absent expression of any MMR proteins in 14 out of 46 suspected Lynch syndrome patients (30.4%). There was a significant association between abnormal MMR protein expression with tumor site ($p=0.006$), tumor location ($p=0.012$), tumor size ($p=0.018$), histological differentiation of cancers ($p=0.004$) and growth pattern of tumor ($p=0.003$). **Conclusion:** In conclusion, the abnormal expression of MMR protein in colorectal cancers in suspected Lynch syndrome patients was associated with specific clinicopathological characteristics. However, further evaluation of germline mutation analysis of MMR genes has to be performed to characterize the mutations.

OP-AP-02. Low-affinity nerve growth factor receptor (NGFR) p75 expression, but not S100, is significantly associated with post-surgical complication in Hirschprung's disease.

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Introduction: Hirschsprung's disease (HD) is characterised by absence of ganglion in the large bowel,

leading to chronic bowel obstruction. The incidence is approximately 1 in 5000 live births. (NGFR) p75 is one of the neurotrophin factor families, known to regulate and induce differentiation of central and peripheral neurons. S-100 protein is a structural protein having multiple functions expressed by enteric Glial cells (EGC). *Objectives:* To evaluate the association of post-surgical outcome of HD patients and the expressions of low affinity NGFR p75 as well as s-100 proteins in resected bowel specimens. *Methods:* Forty paraffin embedded tissue blocks from HD resected bowel were retrieved from the archive of the Histopathology department, Hospital Kuala Lumpur. Post-surgical outcomes were obtained from patients medical record. Expressions of NGFR p75 and s-100 proteins were detected by immunohistochemistry staining. Image analysis software was used to calculate the degree of NGFR p75 and s-100 expressions. *Results:* Post-surgical complications were seen in seven of 40 HD cases. p75 expression was significantly elevated in HD patients with post-surgical complication compared to patients without complications (Mean \pm SEM of post-surgical complication and no complication group: 2.02 ± 0.52 and 1.39 ± 0.24 ; $p = 0.04$). S-100 protein expressions show no significant difference between the groups (Mean \pm SEM of post-surgical complication and no complication group: 2.27 ± 0.70 and 1.61 ± 0.24 ; $p = 0.52$). *Conclusion:* In conclusion, HD patients with post-surgical complication exhibit high expression of NGFR p75 marker.

OP-AP-03. ‘Pathology in cloud’

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Introduction: Digital pathology is making its way from academic medical centers and research institutions to reference labs and hospitals, and is being used today in a variety of specific clinical applications where it provides value. One of the most valuable uses has been facilitating consultations, internally within pathology groups, and externally between groups, either for informal consultations or for formal secondary interpretations. For these applications the capability to share information quickly and securely over wide-area networks (WANs) and ‘in the cloud’ is essential. Pathology in Cloud, such as SecondSlide (www.secondslide.com), enables pathology cases to be shared online in a secure manner without requiring any changes to an institution firewalls or network configuration. *Result:* Reviews from renowned pathologist will be shown in the results. *Conclusion:* Cloud technology provides fast turnaround time, overcome IT hurdles and enhances the efficiency with reduced costs.

OP-AP-04. The effect of thymoquinone on pathogenesis of type 1 diabetes mellitus.histological and immunological experimental study

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Introduction: Type I diabetes mellitus (T1DM) results when the immune system attacks and destroys the insulin-producing beta cells of the pancreas resulting in an absolute lack of insulin. To our best of knowledge the effect of Thymoquinone TQ (the major component of Nigella sativa) on pathogenesis of T1DM has never been explained. *Objectives:* The aim of this study was to determine the effect of TQ on the levels of main immunological cell markers, in addition to evaluate the possible protective effects of TQ against beta cell damage from T1DM in pancreas. *Methods:* TQ doses (5 mg/kg and 10mg/kg) were administrated once a day for 30 days to male Sprague-Dawley rats with Streptozocin (STZ)-induced T1DM. Levels of autoimmune anti islet cell antibodies (ICA), Pan T cell marker

(CD90), Pan B cell marker (CD19), and Pan innate cell marker (CD11b) were evaluated by ELISA method in addition to assessment of glucose and serum insulin level. Histological examination of rat pancreas from each study group was carried out to evaluate the severity of insulitis at day 30. *Results:* TQ treatment (in both doses) has been shown to provide a protective effect by significant increase ($P < 0.001$) serum insulin and decrease level of glucose compared with the untreated diabetic group, and significant decreased the levels of all immunological markers specially anti islet cell antibodies. Histological analysis of pancreatic tissue indicated that STZ caused beta-cell destruction and severe insulitis, while prevention of insulitis in TQ-treated rats was significant. *Conclusions:* The results of this study demonstrate the immunomodulatory effect of TQ against autoimmune reactions and immune defense occurs in T1DM can be significantly improved by the administration of TQ. Moreover, histological observations demonstrate that the degree of inflammation was ameliorated by TQ administration in pancreatic tissue.

OP-AP-05. In vivo imaging of human mesenchymal stem cells with microcomputed tomography

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Introduction: Mesenchymal stem cells (MSC) can differentiate into multiple cell types, and therefore can be used for cellular therapies, including tissue regeneration. However, the participation of stem cells in tissue repair is not well understood as our knowledge on cell migration and engraftment are limited. Therefore, implementing a non-destructive temporal imaging technique to track stem cells *in vivo* is essential to obtain a better understanding of the mechanism of stem cells in tissue regeneration. *Objectives:* To assess the effects of loading MSC with gold nanoparticles on cell proliferation and viability, and to track labeled cells following subretinal injection in an *in vivo* rat model with microcomputed tomography (microCT). *Methods:* In this study, we utilized various gold nanoparticle concentrations and assessed the efficiency of gold labeling on MSC using phase contrast microscopy. The cells used were obtained from human umbilical cord Wharton's Jelly. The effect of nanoparticle loading on cell proliferation/viability and cytotoxicity was analyzed using a CellTiter-Glo luminescent cell viability assay and LIVE/DEAD stain. The uptake of gold nanoparticles was also visualized by a transmission electron microscope. In addition, nanoparticle uptake and retention over time was assessed using inductively coupled plasma mass spectrometry (ICP-MS). The gold loaded cells were then injected into the subretinal layer of a royal college of surgeon (RCS) rat and assessed with microCT. *Results:* Our results demonstrated that loading MSC with gold nanoparticles did not alter the viability and capability of the cells to proliferate and, based on the ICP-MS results, imaging and tracking of MSC up to two weeks was feasible. The TEM images showed the encapsulation of gold nanoparticles in membrane-bound organelles in the cytoplasm. When injected into the subretinal layer of a RCS rat, loaded cells could be traced with microCT. *Conclusion:* Gold nanoparticles could be used to label human umbilical cord mesenchymal stem cells without affecting its viability and capability to proliferate. Local injection of cells into the eye of a rat model was successfully tracked *in vivo* with microcomputed tomography.

OP-AP-07. Expression of p27 in normal squamous epithelium, cervical intraepithelial neoplasia and invasive squamous cell carcinoma of the uterine cervix.

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Introduction: The development of cervical squamous cell carcinoma is a multistep process which involves deregulation of the cell cycle. p27Kip1 (p27) is a member of the cyclin-dependent kinase inhibitor family of the cell cycle protein. **Objectives:** The aim of this study was to evaluate the expression of p27 in normal squamous epithelium, cervical intraepithelial neoplasia (CIN) and invasive squamous cell carcinoma (SCC) of the uterine cervix and its association with prognostic markers of invasive cervical SCC. **Methods:** This is a retrospective study on 189 samples comprising of 45 normal cervical tissues, 46 low grade CIN (CIN I), 38 high grade CIN (CIN II and III) and 60 invasive cervical SCC. The expression of p27 was studied in all samples by immunohistochemistry using a monoclonal antibody specific for the protein. The p27 expression was evaluated by the percentage of positive cells (nuclear staining) and rated as low when 50% of cells were positive for p27 and high when > 50% of cells were positive for p27. **Results:** High p27 expression was identified in all samples (100.0%, 45/45) of normal cervical squamous epithelium, 80.4% (37/46) of low grade CIN, 76.3% (29/38) of high grade CIN and 55.0% (33/60) of invasive SCC. There was significant progressive decrease of p27 expression in agreement with progression from normal squamous epithelium to CIN to invasive SCC ($p<0.001$). However, there was no significant correlation found between p27 expression and prognostic markers (tumour size, tumour grade and clinical stage) of invasive cervical SCC. **Conclusions:** Our study confirmed the potential important role of decrease p27 expression in the development and progression of cervical squamous cell neoplasm. This result suggests that p27 expression level may serve as a suitable marker of risk progression for dysplastic lesions of the cervix.

OP-AP-08. DNA methylation of PAX1 and SOX1 genes as a molecular biomarker for detection of cervical cancer

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Introduction: DNA methylation is an early event in carcinogenesis. Testing for DNA methylation has potential in cervical cancer screening. **Objective:** The aim of this study is to determine the sensitivity and specificity of paired box gene 1 (PAX1) and sex-determining region Y, box 1 (SOX1) genes as a biomarker for detection of cervical cancer. **Methods:** DNA was extracted from 194 liquid-based cytology (LBC) samples collected from Universiti Kebangsaan Malaysia Medical Centre (UKMMC) and Hospital Kuala Lumpur (HKL), which include normal cytology samples ($n=51$), Atypical Cells of Undetermined Significance (ASC-US) ($n=34$), Low Grade Squamous Intraepithelial Lesion (LSIL) ($n=50$), High Grade Squamous Intraepithelial Lesion (HSIL) ($n=52$) and Squamous Cell Carcinoma (SCC) ($n=7$). DNA methylation was determined using real-time methylation-specific polymerase chain reaction (MS-PCR) amplification, which can identify PAX1 and SOX1 genes. **Results:** The methylation frequency in normal, LSIL, HSIL, and SCC was significantly different ($p<0.01$) for the two genes (SOX1 and PAX1). There was a trend increase in methylation of these genes with increasing severity of cervical squamous lesions. The percentage of methylated genes increased with the severity of cervical squamous lesions ($p<0.001$). These two gene combination

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(SOX1/PAX1) showed the best performance to distinguish SCC. The specificity of these two genes for detecting SCC was 94.1%, and its sensitivity was 98%. *Conclusion:* DNA methylation of SOX1 and PAX1 genes are potential molecular biomarkers in the triage of abnormal smears. Both genes are new generation biomarkers for cervical cancer screening and can be used as an adjunct to HPV testing for primary screening.

OP-AP-09. Preliminary study of VCAM-1 and ICAM-3 expressions in pre and post-chemotherapy osteosarcoma patients in relation to its clinical progression.

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Introduction: Osteosarcoma is a very aggressive bone malignancy that if it is left untreated will lead to fatality. Most patients presented with metastasis at time of diagnosis have a low survival rate compared to non-metastasized. Current treatment offered is not promising and the survival rate is unsatisfactory. Cellular adhesion molecules are protein found on cell surface which help cells to bind with other cells or with extracellular matrix which surrounded it has been shown to play a major role in modulating osteosarcoma mechanism. VCAM and ICAM are cell adhesion molecules that are known to involve in cell signaling, metastasis and angiogenesis. Little study has been done to compare VCAM and ICAM in pre and post-chemotherapy. Understanding the expression of both VCAM-1 and ICAM-3 will lead to a better understanding towards its role in the severity of tumor progression. *Objective:* VCAM-1 and ICAM-3 tissue protein expression in pre and post-chemotherapy of osteosarcoma patient was systematically assessed and compared in relation to its clinical progression. *Method:* 2 positive osteosarcoma cases of which both have lung metastasis at time of diagnosis are obtained. Their samples are then prepared for Hematoxylin and Eosin staining for reviews and subsequently preceded with immunohistochemistry staining against VCAM-1 and ICAM-3 for antibody detection. The assessment was carried out on both pre and post-chemotherapy samples and normal fractured bone were used as the experimental control. Follow-up was carried out to observe the patient progression. *Results:* From the study, expression of vcam-1 in both pre and post-chemotherapy are very weak and comparable to the normal bone sample. However, patient 2 significantly express vcam-1 protein in both pre and post-chemotherapy sample. ICAM-3 protein expression was not observed in both patients at pre and post-chemotherapy sample as well as normal bone. *Conclusion:* VCAM-1 could be involved in prognosis of osteosarcoma severity. However further elucidation need to be carried out to confirm the role of VCAM-1 in osteosarcoma progression.

OP-H-10. Interferon gamma expression from transfected bone marrow-derived mesenchymal stem cells inhibits proliferation of chronic myeloid leukemia cells in vitro.

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Introduction: Chronic myeloid leukaemia (CML) is a haemopoietic stem cell disorder caused by the BCR/ABL gene rearrangement. To date, the only curative therapy for CML is allogeneic stem cell transplantation. However, significant morbidity and mortality associated with the procedure and the need for a matched donor exclude this as the best treatment option. Currently, various studies are carried out to develop an alternative approach for CML treatment for example targeted gene delivery

of therapeutic cytokines such as interferon gamma (IFN γ) using bone marrow-derived mesenchymal stem cells (BM-MSC). *Objective:* In this study, we investigated the proliferation of K562 cells in vitro after co-culture with transfected BM-MSC expressing IFN γ . *Methods:* BM-MSC were isolated from bone marrow aspirate by Ficoll-Paque gradient centrifugation. The expanded BM-MSC were then characterised by cell surface markers and differentiation assays. BM-MSC were subsequently transfected with pORF-hIFN γ plasmid by nucleofection. Transfection efficiency of pORF-hIFN γ into BM-MSC was determined from intracellular hIFN γ expression via flow cytometry. ELISA was used to measure the levels of hIFN γ protein expressed in vitro, while the mRNA expression of hIFN γ in BM-MSC was determined by real time PCR. The proliferation of K562 cells was evaluated by luminescent-based viability assay after seven days of culture with/without nucleofected BM-MSC. *Results:* BM-MSC were successfully isolated and displayed the biological properties consistent with MSC. Flow cytometry analysis showed that the transfection efficiency of BM-MSC was at 56%. BM-MSC also expressed IFN γ mRNA and protein 24 h post nucleofection. A preliminary in vitro study showed 54% K562 growth inhibition when co-cultured with nucleofected BM-MSC after seven days. *Conclusion:* Local production of IFN γ by BM-MSC via nucleofection successfully inhibited the proliferation of K562 cells in vitro. MSC as vehicle platform in IFN γ delivery could be further explored as a promising treatment option for CML patients.

OP-H-11. Induction of tumour immunity in murine acute myeloid leukaemia model using bone marrow-derived dendritic cells vaccine

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Introduction: Acute myeloid leukaemia (AML) is a malignant disorder of progenitor cells in myeloid haematopoiesis. Eighty percent of AML patients are able to achieve complete remission with the current therapeutic treatment. However, about half will relapse despite stem cell transplantation. Dendritic cell (DC)-based vaccine may be an attractive alternative therapeutic candidate for AML patients with residual disease as the DCs were shown to be capable of inducing tumour antigen-specific T cell immunity in vitro and in vivo. *Objectives:* To generate functional murine bone marrow-derived DCs in vitro & To determine efficacy of induced antitumour immunity in murine acute myeloid leukaemia model using bone marrow-derived DC compared chemotherapy. *Methods:* DCs were generated in vitro by culturing murine bone marrow cells in the presence of granulocytes-macrophages colony-stimulating factor (GM-CSF), interleukin-4 (IL-4) and tumour necrosis factor- $\hat{\pm}$ (TNF- $\hat{\pm}$) and subsequently pulsed with murine AML cell line (C1498) at the ratio of 3:1 (tumour lysate: DC). The DC-based vaccine was evaluated for its ability to activate T cell proliferation and induce antileukaemic cytotoxicity in vitro. An AML murine model was created by implanting the same cell line subcutaneously into the midflank of female C57BL/6 mouse. Tumour bearing mice were subjected to local intratumoural injection with DCs vaccine or chemotherapy treatment with cytosine arabinoside (AraC). *Results:* The current study showed that generated DC-based vaccine was able to induce proliferative response in naive T cells. In vitro functional study showed that tumour specific T cells were able to lyse murine C1498 AML cells. In vivo study showed that chemotherapy (100mg/kg AraC) only marginally increased the survival of mice and the majority had tumour regrowth after treatment ended. Intratumoural injection of DC vaccine alone provided relatively better antitumor effect and longer survival although tumour regrowth was also observed. *Conclusions:* Bone marrow derived dendritic cell-based vaccine against acute myeloid leukaemia was successfully generated in vitro and exhibited better antitumour effect compared to chemotherapy in mice.

OP-H-12. Study on the reported acute transfusion reaction among paediatric patients of Universiti Kebangsaan Malaysia Medical Centre

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Introduction: Hospitalized paediatric patients often receive blood transfusion as part of their medical treatment and it is found that transfusion reactions are common in these patients. University Kebangsaan Malaysia Medical Centre is a referral hospital with sub-specialty services where number of transfusion is expected to be high. *Objectives:* The objective of this study was to determine the incidence of transfusion reaction among the paediatric patients and also to find out the blood products involved and the associated disease pattern of the patients. *Methods:* This was a retrospective analysis of all reported transfusion reactions among paediatric patients aged day-1 of life to 18 years who received transfusion in year 2010. The data were retrieved from the hospital laboratory information system as well as the hard copies of the reports. *Results:* A total of 49 transfusion reaction events were reported out of the total 2948 blood transfusions. The overall incidence of transfusion reactions was 1.7% of total blood transfused. There were 24 cases (0.8%) of allergic reactions, 13 cases (0.4%) of unexplained passing of red coloured urine (haematuria), 9 cases (0.3%) of febrile non haemolytic transfusion reaction (FNHTR), one case (0.03%) of anaphylactic reaction, one case (0.03%) of FNHTR and haematuria and another one case (0.03%) of increased temperature of less than 10C. RBC were most commonly involved (86%), followed by Platelets (12%) and FFP (2%). 64% patients were from paediatric haematology unit, 16% from paediatric oncology unit and surgical and other medical patients represents 12% and 8% respectively. *Conclusion:* The overall incidence of acute transfusion reaction among paediatric patients was 1.7%. Allergic reaction was the most frequent transfusion reaction followed by haematuria and FNHTR. We found a unique reaction of isolated haematuria in our paediatric patients. Further study to look into the cause of this haematuria will be done in near future.

OP-H-13. Interventional study to improve full blood picture (FBP) requests for adult patients in Hospital Kuala Lumpur

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Introduction: There is an increase number of test request for full blood picture (FBP) in Haematology Unit, Pathology Department, Hospital Kuala Lumpur (HKL) without corresponding increase in manpower, leading to prolonged laboratory turnaround time and delay in the issuance of the test results. *Method:* Multifaceted interventional study was conducted in November 2010 until November 2011 in order to reduce the number of unnecessary FBP test request and implement remedial measures to improve the turnaround time of the FBP results. *Results:* After analysis of the baseline data, FBP Requests Guideline based on The International Consensus Group for Haematology Review criteria was proposed for general use in HKL with the aim to reduce unnecessary and redundant FBP as well as FBP request with incomplete clinical history. With the implementation of this FBP Request Guideline, there was a reduction of 23.6% in unnecessary FBP and 3.6% reduction in redundant FBP request. There was also reduction in 8.1% of FBP requests without clinical history received during the post-intervention study. *Conclusions:* This study showed that FBP Request Guideline is effective in reducing the FBP workload by reducing unnecessary and redundant FBP request. The reduction in workload will improve the LTAT, thus, greatly improved the quality of patient care with regards to hematological assessment as well as reducing unnecessary expenditure.

OP-H-15. The prevalence study of causes of deferral blood donor in blood donation service of Hospital Tengku Ampuan Afzan (HTAA) and its correlation with thalassemia.

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Introduction: Mobile blood donation aims to recruit the blood donor and collect the blood as much as possible. Only healthy person with certain criteria can be selected as the potential blood donor during mobile blood bank. The criterias including age, weight, level of hemoglobin, medical illness, high risk behavior etc. Anaemia is one of the commonest causes of rejection among potential blood donor. Since thalassemia is a high prevalence disease in Malaysia, this might be one of the causes of low haemoglobin level among deferred blood donor. **Objective:** The aim of this study is to investigate the causes of deferral blood donor in Blood Bank Services of HTAA and causes of anaemia among the deferral donor. From these, we can determine the type of thalassemia among the anaemic deferral donor. **Method:** A cross sectional study was done from January 2011 until September 2011 at Hospital Tengku Ampuan Afzan, Kuantan, Pahang. 83 blood samples were collected from consented deferred donor due to low hemoglobin level. The samples then tested for full blood count, full blood picture, serum ferritin levels and hemoglobin analysis. **Result:** The commonest cause of deferred blood donor in this study is low haemoglobin level. From 83 blood samples, about 12% having thalassemia trait/haemoglobinopathy whereby beta thalassemia trait (4.8%), alpha thalassemia trait (3.6%), Hb E trait (2.4%) and Hb H disease (1.2%). (This is a preliminary result). **Conclusion:** Thalassemia trait can be detected among low hemoglobin level deferred donor. Thus mobile blood service can be one of the screening tools for detecting thalassemia trait in the healthy population.

OP-H-16. Hypercoagulable state among thalassemia patients.

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Introduction: Increase incidence of thromboembolism among thalassemia patient had triggered various studies done to determine hypercoagulable state among thalassemia patient. Several factors for the hypercoagulable state had been identified such as RBC membrane disruption, chronic platelet activation and defect in coagulation pathway. **Objectives:** To determine the level of Protein C, Protein S, Free Protein S and Antithrombin III among thalassemia patients and to compare the level of Protein C, Protein S, Free Protein S and Antithrombin III level between thalassemia patients and healthy control. **Methods:** A case control study done at Hospital Universiti Sains Malaysia. Thirty six thalassemia patients who came for regular blood transfusion and 20 healthy blood donors for normal control were recruited. Blood samples were collected and analyzed for Protein C, Protein S, Free Protein S and Antithrombin III using ACL Elite Pro. **Results:** The result showed mean Protein C, Protein S and Free Protein S levels were significantly lower in thalassemia patients (54.5 \pm 13.2%, 94.4 \pm 18.7% and 70.1 \pm 12.3% respectively) compared to normal control (94.1 \pm 16.3%, 105.1 \pm 16.8% and 99.7 \pm 17.3% respectively), whereas mean Antithrombin III showed no significant difference (116.1 \pm 27.3% and 124.4 \pm 12.5%; respectively). **Conclusion:** There were a significantly decreased Protein C, Protein S and Free Protein S in thalassemia patients which might suggests hypercoagulable state in thalassemia patients. Since there are a lot of similarities in finding from other studies, we believe that many more study to look for other parameters contributing to hypercoagulable state in thalassemia patients is needed.

OP-MM-01. The use of conventional methods in identification of Brucella isolates.

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Introduction: Brucellosis is a zoonotic disease of world-wide distribution and still imposes a major impact on the public health issues. The infection was thought to be uncommon in Malaysia however the truth is we are seeing only the tip of the iceberg. Many cases were either undiagnosed or were wrongly diagnosed as other infection. This is partly due to the difficulty in identification of the organisms as neither biochemical nor serological method alone can be considered as the reliable guide to the identification of Brucella species. *Objective:* To speciate the Brucella isolates obtained in our laboratory. A total 10 isolates were collected during an outbreak that occurred in Penang in May 2011 and identified up to species level using conventional methods and the ability of the isolates to resist dye. The serotype of each Brucella isolates were also tested using monospecific antisera that were obtained commercially. *Results:* The majority of the isolates 50% (n=5) were identified as *Brucella melitensis* Biotype 3. One isolate was demonstrated to be *Brucella melitensis* Biotype 1 with another isolate displaying conflicting results with possibility of *Brucella melitensis* either Biotype 2 or 3. Two isolates (20%) were noted to be of *Brucella suis* Biotype 4 and one isolate (10%) was *Brucella abortus* Biotype 1. *Conclusion:* This study demonstrated that, despite being outbreak specimen isolates, diversity was observed amongst the isolates. Hence, an assumption that isolates collected during an outbreak are the same is erroneous, warranting detail examination and identification of each isolate. In order to further confirm the identification of the organisms other methods such as PCR as well as high resolution melt curve analysis (HRM) may be useful. Conventional methods have many confounding factors that may contribute to the inaccuracy of results as well as time consuming. Thorough clinical data of patients should also be looked into in depth as to ascertain the risk factor for the infection.

OP-MM-02. Antibacterial and antiulcer activities of diospyros argentea ethanolic leaf extract.

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Introduction: *Diospyros* species, from ebenaceae family is quite famous among researches who involves in natural product. However, there is not much study has been conducted on one of the species, which is *Diospyros argentea*. In this study, antibacterial and antiulcer properties of *Diospyros argentea* ethanolic leaf extract (DAELE) were evaluated. *Method:* Antibacterial activities were determined by using disc diffusion method and the zones of inhibition were measured. Minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC) were also tested. In antiulcer study, four groups of rats were pretreated with carboxymethyl cellulose (ulcer control group), omeprazole (positive control group) and two concentrations of 250 and 500 mg/kg of DAELE. Absolute ethanol was administered an hour later and the rats were sacrificed on the subsequent hour. Ulcer area, pH, mucus and histological evaluation were done. *Results:* The in-vitro antimicrobial screening revealed that the extract has antibacterial activity against different bacteria with zone of inhibition ranges from 8-11mm, MIC (18.75-37.5 mg/ml) and MBC (37.5 – 75.00 mg/ml). The gastric mucosa of DAELE pretreatment group showed milder injury compared to control groups, as well as increase in acidity and mucus production. *Conclusion:* The results suggest that DAELE contains antibacterial and antiulcer properties and may be helpful as an alternative treatment.

OP-MM-03. The ecology of decomposition on rabbit carcasses in Cameron Highland, Pahang

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Introduction: Forensic entomology is the use of insects in legal investigations. Through insect's activities, a biological clock begins allowing an estimation of time of death. **Objective:** The stages of decomposition and the faunal succession on rabbit carcasses in highland were studied in Cameron Highland, Pahang (4.491N, 101.39E, 1517.3 m) since forensic entomological data for that particular region is still lacking. **Method:** Three New Zealand White rabbit (*Oryctolagus cuniculus*) carcasses weighing approximately 2 kg were sampled daily until the decomposition process completed. Representative specimens of adult flies, larvae, pupa and mites were collected from the carcasses and processed in the laboratory. **Results:** No significant difference in the rate of decomposition and faunal succession between the carcasses. One carcass decomposed completely in 22 days, however, mummification was observed in the other two. The biodiversity of flies associated with decomposition in the area includes *Lucilia porphyrina*, *Chrysomya megacephala*, *Chrysomya rufifacies*, *Chrysomya villeneuvei*, *Chrysomya pinguis*, *Hemipyrellia ligurriens*, *Ophyra spinigera*, *Ophyra chalcogaster*, *Fannia* sp. and *Boettcherisca highlandica* represented by both adults and larvae, meanwhile *Chrysomya nigripes*, *Chrysomya thanomthini*, *Musca domestica*, *Atherigona* sp., *Parasarcophaga albiceps*, *Sarcophaga* sp., *Sepsidae*, *Phoridae* and *Psychodidae* were represented by adults only. Faunal succession followed the sequence of dominant flies i.e. *Calliphoridae*, *Sarcophagidae*, *Muscidae* and lastly *Sepsidae*. We also recovered *Mesostigmata*, *Prostigmata*, *Astigmata* and *Oribatida* mites throughout decomposition which could be used for future implementation in forensic investigations. **Conclusion:** The data obtained from this study provides more accurate indicators for local forensic scientists in solving criminal cases especially on determination of time and primary location of death.

OP-MM-05. Typing of non-typeable *Haemophilus Influenzae* isolates by molecular epidemiological methods.¹Nurul Hamirah Kamsani, ²Norazah Ahmad, ¹Zaini Mohd Zain*¹Institute of Medical Molecular Biotechnology, Faculty of Medicine, Universiti Teknologi MARA, 47000 Sungai Buloh Campus, Selangor, ²Institute for Medical Research, Jalan Pahang, 50588 Kuala Lumpur*

Introduction: Non-typeable *Haemophilus influenzae* (NTHI) is known to cause illnesses such as chronic respiratory tract diseases, otitis media, pneumonia, meningitis and sinusitis. Several molecular methods have been established to determine the genetic diversity of many bacteria but reports on the typing of NTHI isolates are lacking. Over the years, the Institute for Medical Research, Kuala Lumpur has collected many strains of NTHI but the genetic relationship of these isolates has yet to be analysed. **Objective:** To determine the most appropriate method for the study on the relatedness of NTHI strains. **Methods:** Twenty-eight NTHI isolates were subjected to pulsed-field gel electrophoresis (PFGE), multilocus sequence typing (MLST) and restriction fragment length polymorphism (RFLP). The reliability of these methods was compared. **Results:** A total of 25 PFGE patterns were produced by *Sma*I digestion, which represented a genetically assorted population. It was observed that the isolates fell into four major clusters with genetic distances of >40% similarity. There were three branches at F value of =0.9, representing at a genetic distance of >90% similarity (clonal groups). The PFGE revealed that 3 of 28 (10.7%) NTHI strains that were isolated from three distant locations had 100% similarity in their PFGE patterns and thus to be genetically related. By using MLST method, new combinations of allele numbers were found and a number of new unique sequence types (STs) were detected. Moreover, MLST demonstrated that strains which have been assigned as identical or closely related by PFGE were clustered with the same STs, and strains with

different allelic profile were separated into different branches in PFGE. Digestion with Hin1II in RFLP however, produced only two to three bands for each isolates. *Conclusion:* Both PFGE and MLST produced comparable results, unlike RFLP which was less discriminating. In comparison to PFGE, MLST was a more appropriate method of typing of NTHI isolates.

OP-CP-21. Immunomodulatory effect of Nigella Sativa oil in the disease process of type 1 diabetic rats

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Introduction: Type 1 diabetes mellitus (IDDM) is considered to be an autoimmune disease. It is characterized by the presence of antibody (humoral) and T-cell (cellular) responses to islet proteins (antigens). Nigella sativa oil (NSO) has been documented to have hypoglycemic properties for type1 & type2 of diabetes, but to our knowledge the effect of this plant on immunopathology of IDDM has never been examined. *Objectives:* This study was carried out to investigate the potential immunomodulatory effect of administration of NSO in the disease process of IDDM. *Methods:* We have evaluated with the help of ELISA kits the levels of anti- islet cell antibodies (ICA), Pan T and B-lymphocytes markers (CD90, CD19), and innate cell marker (CD11b)] in male Sprague-Dawley rats with Streptozocin-induced IDDM; in addition to assessment of glucose and serum insulin through the experiment. The four groups (6 rats each) under study received or not different doses of NSO. The results have been compared to the ones obtained from healthy and non treated diabetic rats. *Results:* IDDM increased the levels of serum glucose, levels of T, B lymphocytes markers, innate cells marker, and ICA and decreased serum insulin level, meanwhile treatment of diabetic rats with NSO (especially at high doses) significantly decreased the levels of all immunological parameters($p<0.001$) beside it significantly resulted in elevation of serum insulin level ($p<0.001$). *Conclusions:* These experimental results indicate the immunomodulatory effect of this plant against autoimmune reactions occurs in IDDM and immune defense in IDDM can be significantly improved by the administration of NSO. The data may provide new strategies for using Nigella sativa oil to be recommended in the clinical management, control, and prevention of IDDM. Moreover, NSO may be suggested to be used in management of other autoimmune diseases.

OP-CP-22. Reporting of HbA1C result with Hb variant in a tertiary centre in Malaysia

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Introduction: HbA1c is widely used for evaluating long term glycaemic control in patients with diabetes and is directly related with risk of chronic diabetic complications. Presence of Hb variant may interfere with the accuracy of HbA1c results. Hb variant can be detected during HbA1c analysis. *Objective:* To determine the percentage of Hb variant reported during routine HbA1c analysis of samples received by Chemical Pathology Unit Hospital Kuala Lumpur. *Methods:* A cross sectional study using retrospective data of HbA1c results over five months period analysed on Biorad Variant II Turbo, a high performance liquid chromatography (HPLC) assay. Hb variant is reported in the HbA1c result if they were detected in the chromatogram during HbA1c analysis. *Results:* A total of 11,904 patients with HbA1c results were included; 53.2% were females. Only 273 patients (2.3%) were reported to have had possible Hb variant, in which Hb analysis was suggested to confirm the diagnosis. *Conclusion:* Presence of Hb variant was notified during HbA1c reporting. Although the percentage of Hb variant was low, it is nevertheless important to be reported.

OP-CP-23. Urinary NGAL in multiple myeloma- a preliminary report

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Introduction: Renal impairment is a common feature of multiple myeloma (MM). Depending on the definition of renal insufficiency; this complication is reported in 15% to 40% of MM patients. 10% of patients present with severe renal failure. Renal impairment can also develop over time, and an estimated 25% to 50% of patients are affected during the course of their disease. Neutrophil gelatinase-associated lipocalin, or NGAL an early marker of kidney injury, its synthesis undergoes a dramatic up-regulation in renal tubular cells after kidney injury. **Objective:** to study urinary NGAL levels in MM patients and correlate with renal impairment. **Methods:** Thirty three multiple myeloma patients were included (15 newly diagnosed and 18 patients who are on treatment for MM) for the study purpose. Urinary NGAL was measured by a chemiluminescent microparticle assay using the ARCHITECT platform (Abbott Diagnostics Inc., Abbott Park, IL). GFR was calculated using CKD-EPI formula. **Results:** GFR was <60ml/min in 15 MM patients and the remaining had GFR >60ml/min. Urinary NGAL levels in these two groups of patients were 247.32 ± 94.16 ng/ml (mean \pm SD) and 40.33 ± 15.80 ng/ml respectively. NGAL results were significantly higher in group with GFR <60 ml/min ($p = 0.027$). There was a negative correlation between GFR and urinary NGAL levels. ($r = -0.5475$ $p = 0.001$). Manufacturer reference value was verified in our laboratory. Urinary NGAL levels were > 131.7 ng/mL in six newly diagnosed MM patients and all of them had GFR less than 60 ml/min except one. **Conclusion:** Our preliminary report suggests that increased urinary NGAL levels may indicate renal damage in multiple myeloma patients. Increased level of urinary NGAL in whom GFR is >60 ml/min whether it indicates an impending renal damage in multiple myeloma patients needs to be proved by studying in a larger cohort of patients.

POSTER PRESENTATIONS**P-AP-01. The role of CD10 immunohistochemistry in the grading of phyllodes tumour of the breast**

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Introduction: It is important to properly diagnose phyllodes tumours of the breast since all grades may recur and the borderline and malignant ones may also metastasize. In general, no single histological criterion can be used in the grading and instead, it is based on several histological parameters. CD10 has recently been reported to be expressed in spindle cell neoplasia and has been used to differentiate endometrial stromal sarcoma from leiomyoma and leiomyosarcoma. **Objective:** To determine the relationship between the degree of CD10 expression in the stromal cells of phyllodes tumour and tumour grading. **Methods:** A total of 61 cases of mammary phyllodes tumours over the past 11 years were searched from histopathology files, University Malaya Medical Centre. The paraffin blocks were retrieved and 4mm thick slides were prepared and stained using an antibody against CD10 with the envision method. Fibroadenoma case was used as a control slide and breast myoepithelium as the internal control. Each stained slides was independently and semiquantitatively analysed for the intensity and percentage of the stromal cells stained. The staining intensity was graded as negative (no staining), mild, moderate and strong if the staining was much weaker, slightly weaker and same intensity as that of the myoepithelium, respectively. The tumour was considered positive for CD10 if the staining intensity is moderate to strong in 20% or more of the stromal cells. **Results:** 21 (44.7%) of 47 benign phyllodes tumour, 5 (83.3%) of 6 borderline phyllodes tumour and all 8

cases (100%) of malignant phyllodes tumour showed positive expression for CD10 immunostain. **Conclusion:** There was a significant increase in CD10 expression in the stromal cells as the lesions progressed from benign to borderline and malignant phyllodes tumour.

P-AP-02. Clinicopathological characteristics of ovarian cysts received in centre for Pathology Diagnostic and Research Laboratory, Faculty Of Medicine, Universiti Teknologi Mara: A six month study.

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Introduction: Ovarian cysts are common condition affecting women of all ages, while ovarian cancer comprised 5% of all cancers in Malaysia. The prevalence of ovarian cancer among ovarian cysts received in histopathology laboratory is unknown. This data is important in order to create awareness on the types of lesion and also in terms of training of histopathologists and students.

Objective: To investigate the types of ovarian cancer prevalent in ovarian cysts specimens received by the Histopathology Laboratory, Center for Pathology Diagnostic & Research Laboratory (CPDRL) UiTM and correlate them with clinical and pathological characteristics. **Method:** All ovarian cysts received in CPDRL from August 2011 to January 2012 were studied. The cases were analyzed for patient demography, clinical presentation, size of lesion and histopathological diagnosis. The data were analyzed using SPSS 17.0, employing student t- test method, statistically significance was taken as $p < 0.05$.

Results: Within this period, 57 cases were received. The age of the patient was 34.09 ± 10 years (mean \pm SD). The most prevalent ethnic group was Malay (83.9%) followed by Chinese (5.4%) and Indian (5.4%). Abdominal pain and incidental finding during other abdominal surgeries were the common presentations. The mean \pm SD of the ovarian cysts diameter were 64.22 ± 49.58 mm. There was no correlation between age of the patients and diameter of the cysts. Histopathological diagnoses includes benign/hemorrhagic simple cyst 38.6%, cystic teratoma 19.3%, serous cystadenoma 12.3%, endometriotic cyst 8.8%, mucinous cystadenoma 3.5% and one case each of yolk sac tumor, endometrial carcinoma and germ cell tumor (1.8%). **Conclusion:** The majority (94.3%) of the ovarian cysts received are benign. Majority of the patient are Malays and of young age. The most common cyst is simple/ hemorrhagic cyst followed by mature cystic teratoma.

P-AP-03. Grading ovarian serous carcinoma using a two tier grading

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Introduction: Ovarian cancers cause most of the gynaecological deaths. Recently, it has been proposed that the 3-tier (well, moderately, and poorly differentiated) grading system for ovarian serous carcinomas be replaced by a 2-tier (low grade and high grade) system which is shown to be useful in predicting outcome. **Objectives:** To evaluate a two tier system for grading of ovarian serous carcinoma as compared to a three tier system & to determine whether a predictive relationship exists between grade and survival. **Methods:** Cases of ovarian serous carcinomas diagnosed between 2005 to 2010 (5 years duration) in the Department of Pathology, Kasturba Medical College, Manipal, were retrieved from the archives and analysed based on the assessment of nuclear atypia with the mitotic rate used as a secondary feature and classified into low grade and high grade, i.e. in a two tier classification. For comparison tumours were also graded using the Shimizu and Silverberg system as grade 1, 2 or 3. Median survival was calculated using the Kaplan- Meier method and

the curves were compared using the log rank tests. Multivariate analysis was performed using Cox proportional hazard method. *Results:* A total of 45 cases of ovarian serous adenocarcinomas were assessed as per the 2 tier and the Shimizu/Silverberg grading system. Majority of the low grade category were placed in grade 1 of Shimizu Silverberg system while majority of high grade cases serous carcinomas were placed in grade 2 of Shimizu/Silverberg system. The predictive ability of both, the two tier grading system and Shimizu/Silverberg grading system over survival was found to be insignificant ($p > 0.5$). *Conclusion:* The prognostic utility of the two tier grading system was statistically not supported by the present study though there was good overall correlation between the present system and the Shimizu/Silverberg grading system.

P-AP-04. Preliminary analyses on detection of SYT-SSX fusion-transcripts in synovial sarcoma

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Introduction: Synovial Sarcoma is a rare cancer and account for 5-10% of adult soft tissue sarcomas. The tumour exhibits unspecified histogenesis composed primarily of spindle cells with variable epithelial components. Despite establishment of some immunohistochemistry staining, making a definitive diagnosis of synovial sarcoma remains a challenging task. This is due to the histo-morphology and immunophenotypes similarities of this tumour to other types of soft tissue sarcoma. *Objective:* The current study aims to apply a molecular method for detection of SYT-SSX fusion transcript, a common molecular defect (>90% of the cases) in Synovial Sarcoma irrespective of the histologic subtypes. *Method:* Paraffin-embedded fixed-tissue (PEFT) blocks of 3 confirmed and 15 possible cases of Synovial Sarcoma were retrieved from Department of Pathology, Tengku Ampuan-Afzan Hospital, Kuantan and subjected to RNA purification using the standard spin column protocol. A one step direct RT-PCR was performed using SYT-SSX and PBGD primer sets for detection of SYT-SSX fusion gene and the reference gene PBGD respectively. *Results:* Our preliminary molecular analyses showed positive SYT-SSX fusion transcript in all 3 confirmed cases and 5 possible cases of synovial sarcoma. Further analysis is still on going for the remaining samples. *Conclusion:* Molecular detection of SYT-SSX fusion-transcript is useful in establishing the diagnosis of Synovial Sarcoma.

P-AP-05. Sensitivity and specificity of endomysium antibodies by using two histological substrates in the diagnosis of coeliac disease

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Introduction: Coeliac Disease (CD), also known gluten-sensitive enteropathy, is an intestinal disease occurs in both children and adults, characterized by malabsorption, diarrhoea, weight loss, abnormal stools and abdominal distention. One of the known effective tests for the diagnosis of active CD is the assessment of antiendomysium antibodies (EMA) by using monkey oesophagus as a substrate. *Objectives:* A case-control study was carried out to elucidate prospectively the sensitivity and specificity of EMA by using two substrates in the diagnosis of coeliac disease with comparison with histological findings. *Methods:* The study consisted of 314 potential coeliac patients and 100

normal controls. Oesophago-gastroduodenoscopy (OGD) was done to each patient and three biopsies from distal duodenum were obtained. *Results:* Duodenal biopsies revealed histopathological changes of CD (Marsh III; villous atrophy) in 226 cases from 314 patients, (155 children and 71 adults). The remaining 88 were labeled as non-coeliac patients. The sensitivity and specificity of EMA (monkey oesophagus) test among both children and adults coeliac patients were 93.8% and 100% respectively, while the sensitivity and specificity of EMA (umbilical cord) test among children and adults were 96.6% and 100% respectively. *Conclusions:* The human umbilical cord tissue is better for using as a substrate than the monkey esophagus tissue among our coeliac patients. This simple immunohistochemical method permits unlimited testing and characterization of EMA in an easily and commonly available and inexpensive human tissue, which is a perfectly adequate substitute than the distal oesophagus of animals (monkey) since monkey's esophagus is ethically questionable for large scale investigations.

P-AP-06. Endoscopic ultrasound guided (EUS) fine needle aspiration (FNA) biopsy of solid pancreatic lesions: A review of 111 cases

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Introduction: EUS- guided FNA biopsy of pancreas is a standard practise for diagnosis & staging of pancreatic malignancy. Material obtained by EUS guided FNA forms the basis of therapeutic decisions. Samples can be obtained in small lesions< 25 mm in size by EUS-guided FNA. *Objectives:* 1.To know diagnostic accuracy of on site assessment at endoscopy suit on air dried smears using Diff Quik Stain {Rapid Onsite Evaluation (ROSE)}. 2. To find out diagnostic accuracy, sensitivity & specificity of EUS- guided FNA of pancreatic Malignancy & co -relation with histology.3.To compare cytodiagnosis on Diff Quik with conventional PAP Stain smears, Cytospin Preparation & Liquid Based Media (Thin Prep). *Methods:* 111 cases of EUS guided FNA biopsy of Pancreas performed during the year January 2008 to December 2009 having solid pancreatic mass/lesion on USG/CT & suspicious for malignancy or malignant on clinical & radiological investigations were reviewed. Material was obtained for cytological examination & processed for Diff Quik stain, PAP stain, Thin Prep, Cytospin & Cell Block. *Results:* There was 83.9% correlation between onsite diagnosis & Final cytological diagnosis. The Overall diagnostic accuracy for Malignancy was 89.7%, sensitivity 90.6% & specificity 100%.False Negative diagnosis was encountered in 10.3% cases where cytological diagnosis was correlated with histology & other investigations. 51% of cases showed intranuclear inclusions, in malignant cases on Diff Quik Stain. Thin prep was found to be superior to conventional PAP Stain in atypical & suspicious for malignancy cases. *Conclusions:* On site assessment permits a highly accurate preliminary diagnosis of malignancy & increases diagnostic yield of the samples. EUS- guided FNA is a sensitive modality that enables specific & accurate diagnosis of pancreatic malignancy. We recommend EUS guided FNA biopsy of pancreas for all cases suspicious or malignant on clinical & radiological grounds.

P-AP-07. Diagnostic flex-rigid pleuroscopic biopsy of parietal pleura for exudative pleural effusions in suspected malignant/tuberculosis cases: our experience in 219 cases.

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Introduction: Flex-rigid pleuroscopic biopsies provide a diagnosis in exudative pleural effusion when other less invasive procedures fails to do so. It is well tolerated with minimal discomfort & risk. It replaces thoracocentesis & close needle biopsy for patients with exudative pleural effusions of unknown origin. **Objectives:** Present study was carried out to know the exact aetiology of exudative pleural effusion when other diagnostic investigations fail to give correct diagnosis & to differentiate between Tuberculosis & Malignancy in cases suspicious of malignancy on clinical grounds. **Method:** Present study was carried out from 1st January 2010 to 31st December 2011. Pleuroscopic biopsies were obtained in 219 cases by Chest Physicians in endoscopy suit with patient under conscious sedation & Local Anaesthesia using flexi rigid fibre optic pleuroscope. Tissue obtained was placed in 10% formalin & processed in histopathology lab with standard technique. Sections were stained by H & E Stain & microscopic examination was performed. Z.N. Stain was done in all Tuberculosis cases & IHC for TTF1 stain & other markers were carried out in all malignant cases. **Results:** Adequate biopsy material for interpretation was obtained in 210 cases (95.9%) out of 219 cases. On histopathology examinations 79 cases (37.6%) were Tuberculosis, 64(30.5%) were Malignant (Primary from Lung & other sites), 5 cases (2.4%) were Empyema & 62 cases (29.5%) were Nonspecific inflammation. **Conclusions:** Adequate sample was obtained in 95.9% cases & correct diagnosis of Tuberculosis & Malignancy was obtained in 68.1% of cases in undiagnosed exudative pleural effusions. Nonspecific pleuritis was encountered in 29.5% cases {Mostly Para pneumonic effusions (Infections), Collagen Vascular diseases, Pulmonary infarct, Drug reactions etc. were needed to be ruled out}. Pleuroscopic biopsy using a semi rigid instrument is a safe & effective procedure for the diagnosis & management of pleural diseases in suitable patients.

P-AP-08. Screening for coeliac disease among paediatric and adult type 1 diabetes mellitus patients by using histological, serological and HLA typing

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Introduction: Coeliac disease (CD) is an autoimmune disease characterized by small intestinal malabsorption of nutrients after the ingestion of wheat gluten or related proteins from rye and barley. An association between CD and many other autoimmune diseases including type 1 diabetes mellitus (T1DM) has been recognized for a long time in many different populations. **Objectives:** This study was carried out in Iraq to (a) estimate the prevalence of CD among pediatric and adult T1DM, (b) describe HLA class II genotypes predictive of CD in both CD and T1DM patients. **Methods:** A total of 118 (74 children and 44 adults) T1DM Iraqi patients underwent serologic screening of CD by using 2 tests: IgA anti endomysial antibodies (EMA) by indirect immunofluorescence technique (IIF) and IgA anti tissue transglutaminase antibodies (t-TG) by enzyme-linked immunosorbent assay (ELISA). All positive sera patients for either one test or both underwent duodenoscopy and at least four biopsies were taken from the second part of the duodenum to confirm histological diagnosis of CD according to Marsh criteria. **Results:** From all T1DM, there were 19 (16.1%) confirmed CD cases

(14 children 11.8 % and 5 adults 4.2%) depending on positive serological results and histological findings of Marsh III criteria used to confirm the diagnosis of CD. There was highly significant association between both DQ2 and DQ8 antigens with both T1DM and CD patients, while DR3 and DR5/7 antigens frequencies yielded no evident association. *Conclusion:* The study confirmed the association between T1DM and CD but more than expected ratio among Iraqi population.

P-AP-09. Dermatitis herpetiformis among coeliac and non-coeliac disease patients

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Introduction: Dermatitis Herpetiformis (DH) is an autoimmune condition related to eating gluten characterized by extremely itchy, burning, stinging skin rash. DH is currently considered to be coeliac disease of the skin. *Objectives:* This study was carried out for the first time in Iraq to estimate the prevalence of DH among CD and non CD patients. *Methods:* 72 patients (48 children and 24 adults) were recruited in the study. All of them were suspected to have DH disease on clinical grounds divided into 2 groups; group 1 was consisted of 46 CD patients were previously diagnosed as coeliac patients according to Marsh histological findings, and group 2 was consisted of 26 non CD patients (suspected to have CD on clinical ground with negative CD histological findings). All patients underwent (1) serologic tests of coeliac disease with IgA antiendomysial antibodies (EMA) and IgA anti-tissue transglutaminase antibodies (t-TG) by using indirect immunofluorescence technique (IIF) and enzyme-linked immunosorbent assay (ELISA) respectively and (2) skin biopsies from skin lesions with direct immunofluorescent technique (DIF) to confirm DH diagnosis. *Results:* Among 46 CD patients there were only 3 diagnosed as DH (1 child 2.3% and 2 adults 4.7%), while 1 case adult (3.8%) was confirmed as DH among non coeliac patients. DH patients shared with CD the same serological and histological findings; moreover, they have granular IgA deposits in the dermal papillae by DIF in the perilesional skin, which is the hallmark of the DH. *Conclusion:* The study confirmed that the direct immunofluorescence of perilesional skin is the gold standard for establishing the diagnosis of dermatitis herpetiformis.

P-AP-10. Inflammatory myofibroblastic tumour in genitourinary tract: A case series

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Introduction: Inflammatory myofibroblastic tumour (IMT) is a rare disease and uncommonly found in the genitourinary organ. It is also known as pseudosarcomatous myofibroblastic proliferation, inflammatory pseudotumour, pseudosarcoma, atypical myofibroblastic tumour, atypical fibromyxoid tumor, and plasma cell granuloma. *Method:* We describe three cases of IMT of which their histopathological slides were examined in Hospital Kuala Lumpur. All cases presented with urinary symptom and mass in the bladder. Physical examination, radiological investigations and histological examination on biopsy and resection of the mass were performed. *Result:* Case 1: A 22-year-old Malay girl presented with history of massive haematuria with anaemia. No other associated urinary symptoms. Subsequent cystoscopy examination revealed there is a large mass occupying the bladder. The patient underwent transurethral resection of bladder tumour. Case 2: A 46-year-old Indonesian man presented with acute urinary retention and perineal pain. Cystoscopy examination revealed

there is a huge mass occupying the bladder. The mass was protruding from the prostate. The patient underwent transurethral resection of bladder and prostate tumour. Case 3: A 57-year-old Chinese gentlemen presented with painless haematuria. The cystoscopy examination revealed there is a mass occupying the bladder. The patient underwent transurethral resection of bladder tumour and subsequently cystoprostatectomy. The histological examinations of the mass of all the cases showed IMT which was positive for vimentin, smooth muscle actin and ALK-1. *Conclusion:* IMT is a rare disease and having similar features as sarcomatous tumours may cause difficulty in diagnosing it. Strong clinical correlation, good understanding of the disease and thorough examination with support of immunohistochemistry is mandatory in the process of diagnosis.

P-AP-11. Agreement between endometrial histopathology findings of outpatient pipelle sampling and the conventional curettage: A three year study

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Introduction: Blind endometrial sampling is among the commonest procedures to diagnose endometrial pathology. Outpatient Pipelle sampling has replaced conventional diagnostic dilatation and curettage (D&C) as the first line of investigating abnormal uterine bleeding. Is Pipelle sampling able to replace D&C as a reliable and safer means of investigation? *Objective:* To establish the accuracy of Pipelle endometrial sampling as a diagnostic tool; and agreement between histological results of Pipelle and D&C. *Methods:* A retrospective analysis was performed on women who undergone D&C at University of Malaya Medical Centre between January 2008 and August 2011, whereby 127 subjects who had complete histopathology records of D&C and Pipelle sampling were included. Demographic data, hormonal usage and indication for Pipelle sampling were recorded. Histological results were cross tabulated and analysed. *Results:* Mean age was 50.2 ($SD \pm 10.0$). Mean parity was 2.8 while 69.3% had experienced menopause. On overall, 74% of Pipelle and D&C findings were concordant. Of the 6 cases of malignancy, only a third was diagnosed accurately on Pipelle and three were diagnosed as endometrial hyperplasia. Over 70% of endometrial hyperplasia cases were picked up by Pipelle (15 of 21 cases). Diagnostic accuracy was 90.1% for normal endometrium and 59.1% for benign polyp. All leiomyomata and other malignancies were not detected by Pipelle. *Conclusion:* Pipelle sampling is reliable in distinguishing between normal and abnormal endometrium despite limitations in cases of focal pathology. Pre-malignant and malignant findings warrant further investigation for validation and severity assessment.

P-AP-12. Androgen receptor gene amplification & protein expression in prostate cancer cases detected by array CGH and immunohistochemistry techniques.

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Introduction: AR gene amplification is involved in prostate tumor progression and leads to increase protein expression of the AR gene. *Methods:* Twenty samples of formalin-fixed paraffin embedded (FFPE) tissue blocks from patients (mean age at diagnosis 72.9 years; range 60-88) diagnosed with prostatic adenocarcinoma at UKM Medical Centre (UKMMC) between year 2005-2008 were used in this study. Four of these cases were recurrent prostatic adenocarcinoma. All specimens were obtained from transurethral resections (TURP) or orchietomies. AR gene amplification was detected using array CGH while AR protein overexpression was evaluated by immunohistochemistry technique. Amplified AR cases on array CGH either with or without AR protein expression on

immunohistochemistry were compared with clinicopathological data. *Results:* Array CGH revealed 6 (6/20; 30%) cases with AR gene amplification at Xq11-12 and 14 (14/20; 70%) did not. Of the four recurrent cases, two showed AR gene amplification and two showed non amplified AR. All cases with AR amplification showed moderate to strong AR protein expression in patients with advanced stage ($p>0.05$) while weak expression was found in two cases of patients in early stage, although these findings were not significant ($p>0.05$). AR expression was observed in all 20 cases, 5 (25%) were weakly staining, 5 (25%) moderate and 10 (50%) strongly positive. Statistical analyses were insignificant between amplified AR and non-amplified AR when comparing with age, tumor stage, Gleason grade and PSA level. *Conclusions:* AR amplification and protein expression do not show significant association with stages of prostate adenocarcinoma.

P-AP-13. A case of CMV infection diagnosed from placenta: Refreshing the classic owl's eye

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Introduction: CMV (Cytomegalovirus) infection of the placenta may demonstrate the diagnostic viral inclusions and/or placental vilitis. Today, by immunohistochemistry, the CMV infected cells are easily identified without having to scrutinise every villous to look for the inclusions. We might have even forgotten the beatiful nature of the owl's eye inclusions. We would like to share an ordinary case of congenital CMV infection with these characteristic inclusions. *Case:* A 25 year old lady, in her second pregnancy presented with an intrauterine fetal death at 27 weeks gestation. There was no history of trauma, abdominal pain or pervaginal bleeding. Her first son was born a year ago and is currently alive and well. The fetal scan shows IUGR (intrauterine growth restriction) with parameters of about 20 weeks gestation. With Prostin induction, she delivered a macerated stillborn. On histology, the placenta shows hydropic villi with scattered large cytomegalic cells with eosinophilic inclusions within the stroma. Some of them display the characteristic owl's eye inclusions. On immunohistochemical staining, these inclusions are reactive to CMV antibody. *Conclusion:* CMV infection is one of the commonly occurring intrauterine infections. Recognizing the histopathological features may aid in identifying the cause of intrauterine death as many of these women are asymptomatic.

P-AP-14. Carcinoid tumours: A 5-year retrospective study in University of Malaya Medical Centre

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Introduction: Carcinoid tumours are uncommon neuroendocrine tumours thought to arise from the enterochromaffin (Kulchitsky) cells found throughout the crypts of Lieberkuhn of the gut(1). In a recent Surveillance, Epidemiology, and End Results (SEER) database review of over 35,600 patients, carcinoid tumours were found to be significantly more common than cancers of the oesophagus, stomach, pancreas and hepatobiliary system(2). *Objective:* We aim to study the prevalence and histomorphology of carcinoid tumours in University of Malaya Medical Centre. *Methods:* A total of 51 patients with histopathologically diagnosed carcinoid tumour were recruited in this 5-year retrospective study from 2005 to 2009 conducted at University of Malaya Medical Centre, Malaysia. Carcinoid tumours were classified based on the histomorphological characteristics of these tumours. *Results:* Of all the types of carcinoid tumours, malignant neuroendocrine tumours were most prevalent (54.9%), followed by benign carcinoid tumours (41.2%) and atypical carcinoid tumours (4%). Of all the patients, the Chinese (41.2%) formed the largest group, followed by Indians (29.4%) and

Malays (23.5%). Female population (56.9%) was affected more than males (43.1%). The incidence peaks in middle-aged (55-64 years) patients. The most frequently affected site is the gastrointestinal tract (31.6%). Of all the data studied, ethnicity appears to be the only significant factor ($p<0.05$) associated with prevalence of carcinoid tumour irrespective of histologic classification. *Conclusion:* The study shows that malignant neuroendocrine tumours were not uncommon, and the group most at risk is middle-aged Chinese women.

P-AP-15. Gastroprotective effects of dicranopteris linearis leaf extract against ethanol-induced gastric mucosal injury in rats

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Introduction: Dicranopteris linearis is a medicinal plant commonly used traditionally in the treatment of many ailments. *Objective:* To evaluate the gastroprotective effect of ethanolic extracts of Dicranopteris linearis leaf extract (DLELE) against ethanol-induced gastric mucosal injury in experimental rats. *Methods:* The rats were divided into four groups respectively pre-treated orally with carboxymethyl cellulose (CMC) solution (ulcer control groups), omeprazole 20 mg/kg (reference group), 250 and 500 mg/kg of DLELE (experimental groups) one hour before oral administration of absolute ethanol to generate gastric mucosal damage. After an additional hour, the rats were sacrificed and the ulcer areas of the gastric walls were determined. The ulcer control group exhibited severe mucosal injury, whereas groups pre-treated with DLELE exhibited significant protection of gastric mucosa. These findings were also confirmed by histology of gastric wall. *Results:* Significant increases in gastric mucus production and decrease in acidity of gastric content were observed in treated groups with DLELE compare to ulcer control group. In conclusion, treatment with DLELE prior to absolute alcohol has significantly protect gastric mucosa as ascertained grossly by significant reduction of ulcer area, increases in gastric mucus production and decrease the acidity of gastric content and histology by comparatively decreases in gastric mucosal injury, reduction or absence of edema and leucocytes infiltration of submucosal layer compared to ulcer control group. *Conclusion:* DLELE was able to decrease the acidity and increase the mucosal defense in the gastric area, thereby justifying its use as an antiulcerogenic agent.

P-AP-16. Trends in tuberculosis and TB/HIV related mortality in Hospital Kuala Lumpur from year 2001 to 2010.

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Introduction: Tuberculosis (TB) is a chronic communicable disease caused by Mycobacterium tuberculosis. TB remains a major global health problem despite the availability of highly efficacious treatment. In 2010, there were 8.5-9.2 million cases and 1.2-1.5 million deaths (including deaths from TB/HIV co-infection). Currently, TB is the second leading cause of death from infectious disease worldwide (after HIV) and is a leading cause of death among people who are HIV-positive. *Objectives:* To determine the trends in TB and TB/HIV related mortality registered at Forensic Department Hospital Kuala Lumpur. *Methods:* The study was conducted at the Forensic Department Hospital Kuala Lumpur. All the mortality cases related to TB and TB/HIV co-infection registered

from 2001 to 2010 were included. All the cases were confirmed by post-mortem examination and laboratory investigations. *Results:* There were 35 733 mortality cases registered between January 2001 to December 2010. Of these, 637 (1.78%) cases were related to TB. In 2001, Proportional Mortality Ratio (PMR) was 16.81. The highest PMR was 22.38 (in 2005) and the lowest was 12.83 (in 2003). From 2008 to 2010, PMR showed downward trends (2008: 22.37, 2009: 17.45, 2010: 14.68). Of 637 cases, there were 43 (6.75%) TB/HIV co-infection cases. TB/HIV co-infection cases were ranging from 1.56% in 2001 to 12.07% in 2009. From 2008 to 2010, TB/HIV co-infection showed inconsistent trends (2008: 9.46%, 2009: 12.07%, 2010: 6.38%). Majority of TB cases were males (79.43%), Malaysian (85.09%), Malays (50.74%), and age group between 20 to 39 years old (45.2%). For types of TB, majority were pulmonary TB (64%) followed by extra pulmonary TB (32.5%) and reactivated TB (3.15%). *Conclusion:* TB related mortality remains high in Hospital Kuala Lumpur though the trends have substantially decreased in the last three years. Over the past 10 years, the mortality rate of TB/HIV co-infection has increased. Sex, age and race are important non-modifiable risk factors for TB related mortality. In view of the study outcome, effective and comprehensive measures are essential to reduce burden of disease caused by TB.

P-AP-17. A review of atypical glandular cells on pap smears in University Malaya Medical Centre from January 2005 until December 2009

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Introduction: Atypical glandular cell is an uncommon cytological finding on Pap smears. However it has been quoted by various literatures that it has a significant association with premalignant and malignant conditions. *Objectives:* To look on the prevalence of atypical glandular cells in our population, the correlation with the histological findings and the clinical outcome of the patients. *Methods:* This is a retrospective study. All Pap smears with the findings of atypical glandular cells between January 2005 and December 2009 were retrieved from the Department of Pathology, University Malaya Medical Centre. The smears were classified according to the report either atypical glandular cell of undetermined significance favour neoplasm (AGUS-FN), favour reactive (AGUS-FR) or not otherwise specified (AGUS-NOS). Each patient's age, parity, menopausal status, further investigations performed, diagnosis, treatment and follow up history were reviewed. *Result:* There were 129 out of 31733 (0.004%) Pap smears reported as atypical glandular cells of undetermined significance. Out of this, 57.4% had colposcopic assessment, 58.9% had Pipelle endometrial sampling and 25.6% had hysteroscopic assessment done, with 32.5% of these patients did not have any further investigations due to loss from follow up. There were 13.2% (17/129) cases diagnosed with cancer; 28.6% (10/35 cases) of AGUS-FN and 8% (7/88 cases) of AGUS-FR. All cases of AGUS-NOS were normal. There is significant association between type of AGUS and histopathological outcome ($p<0.05$). The types of malignancy were: for AGUS-FN (4 endometrial cancer, 3 cervical cancer, 3 ovarian cancer) and for AGUS-FR (4 endometrial cancer, 2 ovarian cancer and 1 cervical cancer). The clinical outcomes for patients with AGUS were: under cytosurveillance (31.8%), hysterectomy (16.3%) discharged from follow up (17.8%), defaulted follow-up (41.1%) and passed away (6.2%). *Conclusion:* AGUS is a significant finding on Pap smears as it is associated with risk of malignancy. Therefore it requires further investigations and follow-up.

P-AP-18. Determination of TOP2A gene status using chromogenic in situ hybridisation (CISH)

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Introduction: Topoisomerase II alpha (Top2A) protein is a direct molecular target of Top2A inhibitors such as anthracycline. Coamplification and overexpression of Top2A was potentially useful as a predictive marker for response to anthracycline therapy for breast cancer. **Objectives:** The aim of this study was to correlate Top2A gene amplification with Top2A protein expression and clinicopathological variables. **Methods:** Tissue microarray (TMA) including 23 cases of breast carcinoma was performed by immunohistochemistry (IHC) for Top2A protein. Top2A gene status was determined by chromogenic in situ hybridization (CISH) using Zytodot 2C SPEC TOP2A/CEN17 probe (Zytovision, Germany). IHC staining for Top2A was interpreted as positive when more than 10% staining of the tumour nuclei were detected. CISH was interpreted as amplified (TOP2A/CEN17 ≥ 2.0) and non-amplified (TOP2A/CEN17 < 0.8). **Results:** Two of 23 (8.7%) cases showed positive staining of Top2A and 21 (91.3%) cases were negative. Three (13%) cases were Top2A amplified and 20 (86%) cases were non-amplified. Top2A gene amplification was found to be correlated with Top2A protein overexpression ($p=0.012$). Top2A amplification was also significantly associated with HER2 positive ($p=0.015$). Top2A amplification was not significantly associated with large tumour size ($>2\text{cm}$), Grade 2, negative lymph node and positive ER and PR. **Conclusions:** CISH can be used to identify gene amplification but further study with bigger sample size is needed in predicting correlation between Top2A protein overexpression and gene amplification.

P-AP-19. Proposed new comprehensive histological cataract severity assessment using multiple parameters with a five-tiered scoring system.

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Introduction: Cataract is defined as the clouding of lens resulting in reduced visual acuity. There are many clinical grading systems used in assessing the severity of cataract such as Lens Opacification Classification System (LOCS II) grading system. However, histological grading/ scoring system are not well developed. **Objectives:** To develop a new comprehensive histological scoring system for cataract and to evaluate the reproducibility and clinical correlation of the scoring system. **Methods:** Forty lenses from galactose fed rats ($n=30$) and normal diet fed rats ($n=10$) were examined under light microscope for defined histopathological criteria. The criteria are (1) presence of intact anterior epithelium cells (2) normal lens fibers (3) vacuoles and (4) amorphous hyaline area. The criteria are further divided into a 5-tiered scoring system according to severity change. Scoring was done by 3 independent observers. The scores were tabulated and correlated with the slit lamp assessment results. Statistical analysis was performed using Pearson's Correlation test. **Results:** The mean histological score in the criteria correlates with the clinical grading with R value of 0.919. The higher the clinical grading, the higher the histological score with score 0 vs 0 in normal lenses and 4 vs 4 in severe cataract; $R=0.919$. Sensitivity and specificity of the scoring are 0.71 and 0.74 respectively. **Conclusion:** This proposed scoring system could be a useful tool in assessment of cataract severity in research.

P-AP-20. Mediastinal lipoblastoma: a rare childhood tumour

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Introduction: Lipoblastoma is a rare, benign mesenchymal tumour arising from the embryonic fat tissue. The tumour is found most commonly in the extremities. Many cases are incidental findings of a growing subcutaneous mass. Male infant and children younger than age 3 years are most commonly affected. Thus, this type of primary tumour located in mediastinum and chest wall is extremely rare. So far, 8 series over 100 lipoblastoma patients have been reported through literature review. **Method:** We report a case of mediastinal lipoblastoma occurring in a 2 year old Malay boy. It was an incidental finding of mediastinal mass seen on chest X-Ray during admission for recurrent pneumonia. **Result:** Radiological findings of the chest show homogenous opacity behind the heart shadow with ill-defined cardiac silhouette. The CT scan thorax shows a large posterior mediastinal mass with solid and fatty component displacing the heart anteriorly and splaying the carinal angle. A pre-operative ultrasound guided biopsy showed mainly adipocytes at varying stages of maturation suggesting a diagnosis of mediastinal lipoblastoma, which was latter histologically confirmed on the completely resected tumour. **Discussion:** Lipoblastoma is a rare tumour which follows a benign course with no report of metastasis. Complete surgical resection is the best treatment for this condition and favours long term prognosis. Among the mediastinal tumours of childhood, the most common diagnosis in descending order are germ cell tumours, haematological malignancies and neurogenic tumours such as neuroblastoma, ganglioneuroblastoma and primitive neuroectodermal tumour (PNET). Mediastinum is an uncommon site of lipoblastoma and hence, tissue biopsy findings may be easily dismissed as inadequate or unassessable. **Conclusion:** We would like to emphasize that mediastinal lipoblastoma should be considered in the differential diagnosis in a rapidly growing mediastinal mass in children.

P-AP-21. Unusual clinical presentation of lung adenocarcinoma

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Introduction: Ocular metastases can precede the diagnoses of the primary malignancy. However, intraocular metastasis from lung carcinoma as an initial presentation is very rare. Here, we present a patient with intraocular metastasis as the first symptom of lung adenocarcinoma and a short review of the relevant literatures. **Case Report:** A 51-year-old Malay lady presented with loss of vision in her right eye which started with painless progressive blurring of vision. Her initial medical history, apart from having on and off constipation was negative for any lung symptom. Ophthalmologic examination showed retinal detachment and orbital magnetic resonance imaging (MRI) confirmed retinal detachment with presence of right ocular mass. The patient then subjected to the removal of her right eye. Macroscopic examination of the eye ball showed an intraorbital greyish mass. Microscopic examination confirmed an intraorbital tumour with papillary growth pattern. Immunohistochemical studies showed the tumour cells are strongly positive with CK7 and TTF1; positive with S100 protein (moderate intensity) while negative with HMB45, Melan A and thyroglobulin. On follow up, the patient complained of productive cough and on review of the chest X-ray, showed a left mid zone lung mass. Bronchoscopic examination did not show any endobronchial lesion seen. No biopsy was taken as the scope could not pass through the upper lobe due to external compression. **Conclusion:** Loss of vision due to intraocular metastasis as the primary symptom of lung cancer is very uncommon. Tumour cells positivity for CK7 and TTF1 with negative HMB45, Melan A and thyroglobulin support the tumour is of lung origin. Therefore, a great index of suspicion for a secondary metastasis is essential when an intraocular lesion appears.

P-AP-22. Identification and validation of novel aberrant gene promoter hypermethylation in oral squamous cell carcinoma

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Introduction: Aberrant methylation, is one of the hallmarks of carcinogenesis, occurs early in cancer development and is associated with transcriptional genes silencing. Therefore, identification of genes with aberrant promoter hypermethylation can provide clues for the elucidation of cancer pathways and provide attractive biomarker candidates for the detection of early neoplastic events. **Methods:** In our study, whole-genome methylation microarray was applied to identify novel genes with tumour specific DNA methylation of promoter CpG islands in Oral Squamous Cell Carcinoma (OSCC). Using a combination of an Illumina Infinium HumanMethylation450 bead array and a sensitive, fluorescence-based real-time PCR technique, Methylation-Sensitive High Resolution Melting (MS-HRM), the methylation levels was investigated in 3 normal mucosa and 20 primary OSCC tumour samples. **Results:** Ninety one genes with tumor-specific hypermethylation profiles were identified by Partek's Genomics software ($p < 0.05$). The identified hypermethylation profiles of 3 selected genes, previously not known to be affected by OSCC, were further validated using MS-HRM. The aberrant genes were confirmed by showing frequent promoter region hypermethylation with 55% of CELRS3, 50% of DDAH2, and 40% of PIK3R5 in OSCC samples. Vice-versa normal epithelium revealed a significantly lower methylation level of the same promoter regions. In our study, pathological stages were statistically significantly associated with tumour hypermethylation of CELRS3 ($P=0.049$), DDAH2 ($P=0.01$), and PIK3R5 ($P=0.037$) in one way ANOVA statistical analysis. **Conclusions:** These genes revealed altered hypermethylation patterns with a profound transcriptional association, indicating that hypermethylation of these genes may play a direct regulatory role. The hypermethylation changes of three selected genes frequently detected in OSCC samples, indicating that they may be used as biomarkers for early oral cancer detection. Moreover, the identification of the novel candidates epigenetically inactivated TSGs provide new insights into oral tumourigenesis.

P-AP-23. Diagnosis of pilomatrixoma in the elderly: Role of fine needle aspiration (a case series)

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Introduction: Pilomatricoma is an uncommon neoplasm in middle-aged and old patients. It is however more common in children. Careful clinical examination, thorough cytological and histological investigations and a high index of suspicion results in an accurate diagnosis, in 1961, Forbis and Helwig found the cell of origin to be the outer root sheath cell of the hair follicle and proposed the name, pilomatricoma, now called pilomatrixoma. **Case Report:** We report on 4 patients aged 55, 59, 68 and 70 years, with uncommon clinical and histological presentation including localization, absence of ghost cells and malignant features of pilomatrixoma in the Pathology Department of B.P.Koirala Institute of Health sciences, Dharan, Nepal. Fine needle aspiration was performed pre-operatively to confirm the diagnosis. **Discussions:** The results from these cases show that smears

from FNA can help make a conclusive diagnosis of pilomatrixoma, even with uncommon clinical presentations and potential malignant transformation. The finding of a smear with clusters of tightly arranged basaloid cells surrounded by delicate fibrillar material, squamous nucleated, shadow and giant cells, calcium deposits and numerous naked nuclei with inflammatory cells in the background should lead to a diagnosis of pilomatrixoma. *Conclusion:* FNA provided an adequate investigation for the diagnosis of pilomatrixoma in case with aberrant clinical presentation even in the absence of ghost cells.

P-AP-24. Analysis of heat shock protein 27 in chronic atrophic gastritis, helicobacter pylori-associated chronic gastritis and gastric cancer by immunohistochemistry.

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Introduction: Gastric cancer is the 7th most common cancer in Malaysia. It is usually diagnosed at advanced stage thus causing high mortality. Unfortunately, until recently there is no useful biomarker for gastric cancer that has been identified. Therefore, there is a need to find a diagnostic marker especially at its early stage. Recent reports showed that Heat Shock Protein 27 (Hsp27) was over-expressed in gastric cancer and increased in the sera of gastric cancer patients. However, there are very few studies reported on the Hsp27 expression in chronic gastritis, the precursors of gastric cancer. *Objectives:* In this study, immunohistochemistry was used to determine the expression of Hsp27 in gastric cancer and its precursor lesions. It should suggest whether the alteration in the expression of Hsp27 occurs prior to the development of gastric cancer and can be detected even in chronic atrophic gastritis and Helicobacter pylori-associated chronic gastritis. It will also disclose whether these alterations may correlate and predict early stage of gastric cancer. *Methods:* Immunohistochemical staining of Hsp27 was performed on 54 chronic atrophic gastritis, 53 Helicobacter p/ylori-associated chronic gastritis and 46 gastric cancer samples to determine the expression of Hsp27. The immunohistochemical staining was scored semi-quantitatively. Wilcoxon Signed-Rank Test and Mann Whitney U Test were used for statistical analysis. *Results:* Analysis showed that there are significant differences between the expressions in both types of gastritis with gastric cancer, with higher expression in gastric cancer ($P<0.05$). There are also significant differences in the Hsp27 expressions in chronic atrophic gastritis and gastric cancer with their normal adjacent mucosa ($P<0.05$). *Conclusions:* Overexpression of Hsp27 was found in the samples of gastric cancer and both types of gastritis. Further studies need to be carried out to ascertain the association of Hsp27 expression in gastric cancer and its precursor lesions.

P-AP-25. Case report: Melanosis ilei

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Introduction: Macrophages are normal constituent of colonic lamina propria as well as in the small intestinal tract. When pigmented macrophages are found, this is referred to as melanosis. Melanosis is a misnomer. In many of the cases, brown pigment that accumulates in the macrophages in this condition is hemosiderin and/or lipofuscin not melanin. *Case presentation:* A 44 year-old lady presented with chronic watery, non-bloody diarrhea for the past one year. She was diagnosed chronic renal failure and had renal transplant in 1995. Colonoscopy examination reported hemorrhagic terminal ileum mucosa. Histologic examination of terminal ileum biopsy showed abundant collections of

hemosiderin-containing macrophages within the edematous lamina propria of the villi. Multiple biopsies from colon were unremarkable. The diagnosis of melanosis ilei was made. *Discussion:* Melanosis of the colon (melanosis coli) is not uncommon while melanosis of the small intestine is extremely rare. The reported cases of melanosis ilei were either described in association with melanosis coli or as ileal pigmentation alone. The pigments in melanosis ilei include hemosiderin, lipofuscin, charcoal and silicates of aluminium and magnesium (antacid usage). Among common causes of melanosis include chronic use of laxative, chronic ingestion of oral iron, gastrointestinal bleeding in long-term use of salicylates, chronic renal failure and drugs ingestion (hydralazine, propanolol, hydrochlorothiazide). Chronic renal failure usually causes melanosis duodeni and one case of melanosis ilei associated with chronic renal failure is attributed to chronic ingestion of oral iron [3]. The association of chronic renal failure in our case with melanosis ilei is not clear.

P-H-30. A preview of impaired platelet aggregation to ADP agonist in National Blood Centre, Malaysia.

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Introduction: Bleeding symptoms are common in population. However mild bleeding disorder is difficult to assess its clinical relevance. One of the cause is due to platelet function defect (PFD) either related to abnormality in the membrane receptor on platelet or its signal transduction pathway. ADP receptor, namely P2Y12 played a central role in platelet activation. Its importance lies as itâ€™s the therapeutic target for cardiovascular disease. *Objective:* The objective of this study is to acquire information on patients with impaired platelet aggregation to ADP agonist in National Blood Centre and determine its clinical and sociodemographic characteristics. *Methods/Results:* The data recorded were from 1st January 2009 to 31st May 2011. The results showed out of 251 platelet aggregation test (PAT) performed, 33 were found to have impaired platelet aggregation to ADP agonist, 2uM (prevalence of 14%) using local cut off point of 65%. Out of those, 16 (6.4%) possibly have inherited/familial ADP receptor dysfunction, and 17 (7.6%) patients had secondary causes of impaired platelet aggregation to ADP either associated with von Willebrand disease (vWD), glycogen storage disease (GSD) and acquired platelet dysfunction with eosinophilia (APDE). As for sociodemographic characteristics, majority of patients were Malay, with slight male preponderance and age ranges from 8 months to 62 years old. *Conclusion:* This is a pilot study looking into percentage of platelet function defects related to ADP receptor. Further study can be done to look into prevalence of other platelet receptor defects to build a national registry on mild bleeding disorder related to platelet function defects. Eventhough the importance may not be as significant as other well known bleeding disorder; the knowledge related to platelet function defects may be of importance in the future in research related to platelets as biomarkers or potential therapeutic application in various diseases.

P-H-31. Performance evaluation of STA-RÂ® evolution at Hospital Kuala Lumpur

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Hospital Kuala Lumpur

Introduction: STA-RÂ® Evolution is a fully-automated, stand-alone workstation with robotic capabilities for total laboratory automation system. Clotting, chromogenic and immunological assays can be performed simultaneously in random access mode producing fast, accurate and reliable results. The goal of the evaluation is to determine the performance of STA-RÂ® Evolution analyzer based on several parameters. *Method:* Between and within run precision study of PT, APTT,

fibrinogen and D-Dimer assay were performed with 30 replicates of normal and pathological samples. Correlation study of PT, APTT, fibrinogen and D-Dimer assays were evaluated between STA-R® Evolution and the reference analyser, STA-Compact using 50 patient specimens spanning the whole physiological range. Reagent carryover study was evaluated by comparing the mean of APTT run in two series. Linearity of fibrinogen and D-Dimer assay was accessed with dilution ratio from 1/5 to 1/160 and its results were compared with theoretical values at different concentration using linear regression. *Results:* Between and within run precision study for PT, APTT and fibrinogen showed good precision which satisfied the manufacturer's specification. Significant correlation was observed between STA-R Evolution and STA-Compact for PT, APTT, fibrinogen and D-Dimer assay with $R > 0.97$ ($P < 0.05$). Carryover study also showed excellent performance with no significant carryover of reagent. Fibrinogen assay showed a good linear regression graph with $r > 0.99$ in the studied range from 0.9 g/l whereas D-Dimer assay showed a good linear regression graph with $r > 0.99$ in the studied range from 0.2 ug/ml to 1.8 ug/ml . *Conclusion:* Our evaluation of STA-R® Evolution showed a good performance in terms of precision, linearity, accuracy and carry over with meet the International Standard and the standard claimed by the manufacturer. The STA-R® Evolution also showed a good correlation and comparable with STA-Compact is currently using in our lab.

P-H-32. Latent iron deficiency among blood donors in Universiti Putra Malaysia

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Introduction: Iron deficiency is one of the most common nutritional deficiencies worldwide and has several causes. Blood donation is recognised as the most common iatrogenic cause for iron deficiency among healthy adults with loss of about 225 mg of iron per donation. *Objectives:* To screen for the prevalence of anaemia, latent iron deficiency and iron deficiency anaemia among blood donors in our local population. *Methods:* The lowest acceptable entry levels of Hb were $>12.5\text{g/dl}$ for males and $>11.5\text{g/dl}$ for females, estimated by copper sulphate method from finger prick. Three ml each of venous blood was taken into EDTA and plain tubes from the diversion pouches during blood donation from each eligible blood donors. Their blood samples were subjected for full blood count (FBC) analysis using automated haematology analyser within 4 hours of blood collection. Their serum was extracted and subjected for serum ferritin estimation by chemiluminescence method. *Results:* A total number of 87 females and 71 males, aged 19-55 years old blood donors were recruited. Majority of them were Malays (64.5%) followed by Chinese (31.1%) and Indian (4.4%). Their mean Hb level was 13.8 g/dl, the lowest and highest value were 9.3 g/dl and 20.4 g/dl respectively. 8% (12/158) of them were anaemic for their gender with the Hb value range from (9.2 -11.4) g/dl for female (93%). A male donor was anaemic with a Hb of 11.8g/dl. Three (2%) were suffering from iron deficiency anaemia and eight (5%) were in latent stage of iron deficiency. Five of the donors with latent iron deficiency were new donors. None of these donors gave history of chronic blood loss or known to have chronic medical illness. *Conclusion:* Iron deficiency occurs in several stages with anaemia as a late manifestation. In this study, iron deficiency was found among new and regular blood donors of both sexes. An accurate portable device for objective Hb measurement pre donation is recommended. Apart from public health education on the importance of iron supplementation post donation, an alternative approach should include serum ferritin measurement at first donation and subsequently once every year for voluntary blood donors who are regular donors so as to avoid iron deficiency.

P-H-33. National thalassaemia laboratory database 2010

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Introduction: Thalassaemia is one of the most common genetic blood disorders in the world and pose an important public health problem in Malaysia. In 2005, Ministry of Health has started national screening programme for thalassaemia in order to identify thalassaemia carriers and provide counseling to the individuals and their family members. Full blood count and high performance liquid chromatography (HPLC) are used for this purpose. **Results:** A total number of 45811 samples were received by all the state hospitals in 2010 in which 14057 (30.7%) cases were reported as normal while other 7562 (16.5%) were beta thalassaemia, 12374 (27%) alpha thalassaemia/to rule out alpha thalassaemia, 4999 (10.9%) haemoglobinopathies/thalassaemia haemoglobinopathies and 6819 (14.9%) iron deficiency/suspected iron deficiency anaemia. Beta thalassaemia was the commonest type of thalassaemia detected (n=7562) with 7090 (15.5%) cases of beta thalassaemia trait, 64 (0.14%) beta thalassaemia intermedia and 87 (0.19%) beta thalassaemia major. Majority of beta thalassaemia major was diagnosed in Sabah ((n=87 (74%)). A total of 363 cases of Hb E-Beta thalassaemia (0.79%) and 210 cases of Hb E homozygous (0.46%) was detected. 486 cases (1.1%) of Hb H disease were diagnosed in which 21% and 15% were from Kuala Lumpur and Kedah respectively. Only 4 cases of hydrop fetalis reported (2 cases from Negeri Sembilan and Perlis each). Another 11884 cases (25.9%) were suggestive of alpha thalassaemia but confirmation with molecular technique is required. Other haemoglobinopathies detected include Hb S (n=57), Hb C (n=27), Hb D Punjab (n=10), Hb Lepore (n=18) and Hb J (n=4).

P-H-34. A case series of HB Adanainteraction with beta thalassaemia

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Introduction: Codon 59 mutation (haemoglobin Adana) is a rare non-deletional form of Beta-thalassaemia that was first described in two Turkish patients in 1993. It was reported to have caused severe clinical phenotypes when inherited in a homozygous or compound heterozygous manner with other Beta-thalassaemia mutations. We presented two sets of siblings from two families who presented with thalassaemia intermedia. The siblings from family 1, II-1/1 and II-4/1 were young adults who gave a history of intermittent jaundice and anaemia during acute infective events but never required blood transfusions. The siblings II-1/2 and II-2/2 from family 2 were children where II-2/2 required a couple of blood transfusions to date. Clinical examination revealed hepatosplenomegaly for all patients. Their haemoglobin levels ranged from 7-9 g/dl, with borderline MCV and MCH. DNA analysis confirmed compound heterozygosity for codon 59 mutation and alpha 3.7 gene mutation for II-1/1 and II-4/1 and haemoglobin Constant Spring and codon 59 mutation for II-1/2 and II-2/2. Codon 59 mutation could affect either the Beta-1 or Beta-2 gene. The heterozygous carriers had normal to mild reduction in haemoglobin levels with borderline MCV and MCH indices. However, in homozygous state, codon 59 mutations were shown to cause hydrop fetalis even in the presence of other two normal genes. In our cases, the patients were compound heterozygous for codon 59 mutation and betathalassaemia. The resultant phenotypes were that of thalassaemia intermedia due to the hyperunstable state of the haemoglobin. It was also shown that codon 59 mutation affecting beta1 gene gave a less severe phenotype than beta2 gene because beta2 gene encodes for 2-3 times more beta globin protein than beta1 gene. Although the incidence is rare, the correct identification of codon 59 mutation was necessary to help with genetic counseling.

P-H-35. Micro-mapping of beta thalassemia carrier in Sabah

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Introduction: Thalassemia is a public health problem in Malaysia and causes a significant burden on public health care system. In Malaysia, Sabah had the most number of registered patients (transfusion-dependent beta thalassemia major and HbE/beta) at 1,272 with Kadazan/Dusun ethnic group accounting for about 50% of cases. The peninsular states showed a distribution pattern that followed the population density but with a distinct pattern of HbE/beta thalassemia being more pronounced in the northern states bordering Thailand, however there were no clear micro-mapping of Sabah. **Objective:** This data analysis is an attempt at providing an overall picture of the distribution of beta thalassemia in Sabah for a better allocation of resources in the management of thalassemia disease as a whole. **Method:** Data are collected and analyzed based on the number of FBC samples that were screened at primary care level (e.g. klinik kesihatan) and samples that were subsequently forwarded for Hb analysis. **Results:** In 2011, 63,937 FBC were screened and 13,469 (21%) samples were sent for Hb analysis from all over Sabah, including W.P Labuan. 2871 beta thalassemia carrier (4.49%) are identified. 71 cases of new thalassemia major birth are detected in 2011. Further analysis shows that beta thalassemia carrier rate is higher in the west coast and northern districts of Sabah whilst central area and east coast of Sabah have a lower incidence of <1%. 52% of all beta carrier detected are of Kadazan/Dusun ethnicity. **Conclusion:** This micro-mapping demonstrate that beta thalassemia carrier is a major burden mostly affecting the west coast and northern part of Sabah. With increased risk of new thalassemia (major) birth, screening and counselling should be step up in these areas along with better re-distribution of resources either financial, facilities or man-power to ensure the success of The National Thalassemia Prevention and Control Program.

P-H-36. Clinical utility of immature platelet fraction percentage in thrombocytopenic patients

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Introduction: Thrombocytopenia is a common haematological abnormality and no simple diagnostic test is available to diagnose thrombocytopenia pathogenesis. A new rapid automated method to assess reticulated platelets, the immature platelet fraction (IPF) using XE-2100 blood cell counter with upgraded software (Sysmex, Japan) is described and its clinical utility assessed. **Methods:** Peripheral blood samples collected into K2EDTA (Beckton Dickinson, USA) were analyzed at University Malaya Medical Centre. One hundred samples from apparently healthy blood donors (all routine full blood count parameters including platelets within the healthy reference range) were used to establish a normal reference range for IPF%. The study sample was divided into 2 groups; thrombocytopenic patients with normal or decreased thrombopoietic activity (21 patients with dengue fever, 5 with acute leukaemia, 5 postchemotherapy for peripheral blood stem cell transplantation, 1 with Non Hodgkins Lymphoma and 1 with aplastic anemia), and thrombocytopenic patients with increased thrombopoietic activity (9 with immune thrombocytopenia, 2 with thrombotic thrombocytopenic purpura, 5 with disseminated intravascular coagulopathy and 1 with Evans' syndrome) groups. **Results:** IPF% was measured in 100 healthy blood donors and in 50 thrombocytopenic patients. An IPF% reference range in healthy individuals was established as 0.0-3.6%, with a mean of 1.8%. The mean for IPF% in thrombocytopenic patients with normal/decreased thrombopoiesis was 6.8% and the mean of IPF% in thrombocytopenic patients with increased thrombopoiesis was 13.9%. The highest values were found in the immune thrombocytopenia patients. **Conclusions:** A rapid, inexpensive automated method for measuring IPF is feasible and should become a standard parameter in evaluating thrombocytopenic patients.

P-H-37. Correlation study and establishing reference ranges for esr using roller 20 PN

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Introduction: Length of Sedimentation Reaction in Blood (LSRB) which is commonly but incorrectly termed the erythrocyte sedimentation rate (ESR) is traditionally measured by Westergren Method (WM). LSRB is widely used as a screening test for inflammatory diseases. WM has its own disadvantages although it is a reference method. A new automated LSRB measurement such as Alifax Roller 20 PN is able to produce results in 20 seconds. **Objective:** To correlate results from Roller 20 PN with Mixrate X-20 and WM. Another objective was to establish local reference range for ESR tests for both genders using Roller 20 PN. **Methods:** For correlation, 90 blood samples from healthy subjects were chosen and run on Roller 20 PN (Alifax, Italy), Mixrate X-20 (Vital Diagnostics, Italy) and WM. All the procedures followed manufacturer's instructions and ICSH guidelines for ESR test. **Results:** The results showed that no significant differences between Roller 20 PN and WM and also with Mixrate X-20. Local reference range for ESR using Roller 20 PN for male is 4+20 mm/hr and female is 10+28 mm/hr. **Conclusion:** ESR measurement with Roller 20 PN is reliable, convenient and correlates well with reference WM and Mixrate X-20. The universal reference range is expected to differ between population and analysers.

P-H-38. Variations of schistocytes count on the blood film: Report of three cases of thrombotic thrombocytopenic purpura

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Introduction: Thrombotic thrombocytopenic purpura (TTP) is an uncommon severe hematological syndrome characterized by occlusive microangiopathy due to intravascular platelet aggregation. Diagnosis is based on the pentad of clinical features: Coomb's negative microangiopathic hemolytic anemia, thrombocytopenia, fluctuating neurological symptoms, renal abnormalities and fever. Presence of schistocytes on blood smear is a characteristic finding of TTP which carries diagnostic implications. **Case report:** Here we report three unrelated young women presented with different clinical manifestation during peripartum period. They were diagnosed as TTP based on clinical suspicion and laboratory findings. All patients had low platelet count but none of them had clear classical PENTAD presentations. Full blood picture (FBP) for 2 of them showed only occasional schistocytes during presentation but slightly increase after a serial blood film investigation. The other patient showed numerous schistocytes on admission and on repeated film. Two of them were successfully treated with plasma exchange but unfortunately the other (presented as scanty schistocytes on FBP) died most likely due to thrombotic complications. **Discussion:** Plasma exchange is an effective treatment for TTP. Rapid diagnosis is crucial. Diagnostic criteria of TTP is based mainly on the finding of thrombocytopenia and microangiopathic hemolytic anemia in the absence of disseminated intravascular coagulation (DIC) and other known causes of thrombotic microangiopathy. The presence of schistocytes on a blood smear is the morphologic hallmark of the disease. The Schistocyte Working Group of the International Council for Standardization in Haematology (ICSH) has prepared specific recommendations to standardize schistocyte identification, enumeration, and reporting in peripheral blood film. They deal with the type of smear, method of counting and morphological description based on positive criteria (helmet cells, small, irregular triangular, or crescent-shaped cells, pointed projections, and lack of central pallor). The presence of schistocytes on blood film should be reported and diagnosis is not excluded until proven otherwise.

Conclusion: In summary, we report 3 cases of TTP with different morphological presentations. The presence of schistocytes (even only occasionally found) as a dominant RBC abnormality on smear must lead to high suspicion of index for rapid diagnosis and early treatment.

P-H-39. Red cell alloimmunization in post transfusion patients with hematoooncologic disease.

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Introduction: Alloimmunization is common in transfused patients. Alloimmunization to red cell (RBC) antigens resulting from the genetic disparities between donor and recipient is one of the risks of blood transfusion. Because of intensive marrow depression and improved survival, patients with hematoooncologic disease are transfusion dependent. It has been advocated that these patients should receive blood that is matched for blood group antigens other than ABO and D. The patients may experience such severe anemia that red blood cell (RBC) transfusions are required for symptomatic palliation. It is often exacerbated by myelosuppressive chemotherapy or radiotherapy. From the study we can produce data registry which are important for better transfusion management of the transfusion dependent patients. **Objective:** The aim of the study is to estimate the prevalence of red cell antibody among hematoooncologic diseases patients and to correlate with the diagnosis. **Methods:** This cross-sectional study was conducted in Transfusion Medicine Unit, Hospital Universiti Sains Malaysia between January to June 2011. Blood samples from 132 hematoooncologic patients who fulfilled the inclusion and exclusion criteria were screened for the red cell antibody using Diamed Techno Twin Station. Antibody identification was performed using Diamed ID-DiaPanel-P and CSL Phenocell C. Clinical transfusion records of the patients were reviewed and analysed. All data were analyzed with computer software using Statistical Package for the Social Science (SPSS) software Version 18. **Results:** Red cell antibodies were detected in seven patients. The overall immunization rate was 5.3%. Four patients developed single antibody, two patients developed 3 types of antibodies and one patient developed 2 types of antibodies. Three patients were diagnosed as Non Hodgkin Lymphoma while one with Acute Lymphoblastic Leukaemia and one with Multiple Myeloma antibodies. **Conclusion:** Our data showed low alloimmunization rate in post transfusion hematoooncologic patients. Antibody formation was comparable to that in other diseases requiring multiple blood transfusions. However, patients who underwent intensive chemotherapy formed antibodies at a much lower rate than other patients.

P-H-40. Detection of JAK2V617F in myeloproliferative disorders by RFLP analysis.

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Introduction: JAK2V617F mutation was found mainly in myeloproliferative group of disorder (MPDs) with a high frequency in polycythaemia vera (PV). JAK2V617F mutation results from a G → T transversion at nucleotide 1849 in exon 14 of the JAK2 gene, this result in an amino acid substitute of valine by phenylalanine at codon 617. **Objective:** This study is to confirm the diagnosis and to determine the frequency of JAK2 mutation in myeloproliferative disorders. **Methods:** We performed DNA extraction for routine bone marrow and peripheral blood samples. Polymerase chain reaction (PCR) amplification of JAK2 was performed with a final reaction mixture of 25 µL containing in-house designed primers for detection of the single hotspot mutation in JAK2V617F. The JAK2 PCR product was incubated with the restriction enzyme BsaXI for 16 hours at 37°C. Samples analyzed by restriction size were accompanied by a positive (known homozygous JAK2 mutation) and a negative control (known wild type of JAK2 mutation). PCR analysis for BCR-ABL1 transcript was carried out for all CML cases. **Results:** Majority of patient in PV are homozygous (42.3%), for the

JAK2V617F allele, 9.6% were heterozygous and 48.1% were wild-type JAK2V617F. Heterozygous (35.6%) for the JAK2V617F was predominantly observed in ET, 13.3% was homozygous and 51.1% was wild-type of the total ET case. High frequency of JAK2V617 homozygous (36.6%) was observed in MPD compared to 16.9% of heterozygous mutation and 46.5% were wild-type JAK2V617F. *Conclusions:* The JAK2V617 mutation appears to arise in myeloid lineage proliferation. Restriction site analysis can offer as screening tool of JAK2 genes mutation in MPDs.

P-H-41. FISH analysis approach for detection of MLL rearrangement in acute myeloid leukemia.

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Introduction: Structural abnormality of the 11q23 band bearing MLL (mixed lineage leukemia or myeloid-lymphoid leukemia) genes are among the most common cytogenetic aberrations in acute leukemia. In acute myeloid leukemia (AML), 11q23 chromosomal abnormalities are important prognostic factors, which the prognosis is intermediate regardless of age. At least 50 partner genes of 11q23 translocation at different chromosome loci have been described, however, in some cases the presence of 11q23 abnormalities does not always correlate with that of MLL gene rearrangement. *Objective:* To detect MLL gene rearrangements by fluorescence in situ hybridization (FISH). *Methods:* Bone marrow or blood cells from patients with AML were harvested after overnight or synchronized culture of unstimulated culture. 72-hour culture was performed to determine constitutional cytogenetics. Metaphase chromosomes were banded by the conventional G-banding technique and karyotyped according to ISCN 2009. Vysis MLL probe (Vysis, Downers Grove, IL, USA) covering the MLL locus on chromosome 11q23 was used for FISH analysis. *Results:* MLL gene rearrangements were studied on 3 with newly diagnosed patients and one with post-treatment AML. A total of 3 patients were found to have chromosomal changes with MLL aberrations. One patient showed no MLL rearrangement and t(11;22) familial rearrangement was diagnosed. *Conclusion:* FISH analysis is sensitive as compare to conventional cytogenetic to detect cryptic rearrangement of MLL gene rearrangement. It is recommended that karyotypic analysis always be complemented by FISH methods to unravel MLL rearrangements.

P-H-42. Flow cytometric analysis of nuclear DNA ploidy and proliferative activity in acute leukaemia

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Introduction: DNA analysis using flowcytometry got prognostic implication. By using DNA index (ratio of DNA content between leukaemic and normal cells) to evaluate the ploidy status and analysis of cell cycle give helpful information about proliferative activity of cell population of interest. In acute lymphoblastic leukaemia, children with higher ploidy (>50 chromosomes) carry best prognosis while children with hyperdiploidy (47-50 chromosomes) have intermediate prognosis and those with hypodiploidy have relatively poor prognosis. However, the prognosis for other ploidy groups shows variation between different studies and the probability of treatment failure was found to be greater for patient with percentage of S-phase below the median value. *Objectives:* To study the correlation of nuclear DNA ploidy and proliferative activity with respond to induction chemotherapy in acute leukaemia. *Methodology:* This is a cross sectional analysis of 30 cases of newly diagnosed acute leukaemia at our institution in 2009 and 2011. The diagnosis was based on peripheral blood and bone marrow examination for morphology, cytochemistry and immunophenotypic studies. DNA

analysis using flowcytometry were performed to determine the presence of aneuploidy cells and the percentage of cells in S-phase using BD FASCANTO system. SPSS software package, version 17, was used for statistical analysis. *Results:* Aneuploidy identified in 23 (74.2%) patients, 17 (54.8%) of them showed hyperdiploidy and 6 (19.35%) showed hypodiploidy. High S-phase was found in 25 (80.65%) patients while the rest of cases (19%) found to have low S-phase. Statistical analysis conducted and showed no significant correlation between aneuploidy or S-phase level with response to induction chemotherapy. *Conclusion:* As a conclusion we disprove that DNA hypodiploidy is associated with unfavorable initial response in acute leukemia and high percentage of S-phase is associated with favorable initial response in acute leukemia

P-H-43. A rare t(9; 12; 22) (q34; q23; q11) translocation in a patient with typical chronic myeloid leukemia: A case report

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Introduction: Chronic myeloid leukaemia (CML) is typically associated with reciprocal translocation between long arms of chromosome 9 and 22, t(9, 22)(q34; q11.2) and with the formation of a BCR-ABL fusion gene. In a minority of newly diagnosed CML cases, complex cytogenetic variants of the Ph chromosome can be observed with involvement of chromosomes 9, 22 and a third chromosome. *Case Presentation:* This case illustrates a CML in chronic phase with a rare complex variant translocation involving chromosomes 9, 12 and 22. The patient is a 55-year-old Malay lady known to have right breast carcinoma in which right mastectomy was performed in 1998, and now presented with progressive abdominal distension associated with low grade fever, lethargy and loss of appetite. Physical examination revealed febrile, mild pallor with massive hepatosplenomegaly. Her full blood count showed anaemia, hyperleukocytosis and mild thrombocytosis. Peripheral blood film showed leukoerythroblastic picture with all stages of granulocytic maturation seen with bipeak of segmented neutrophils and myelocytes, and 2% of blast cells. Bone marrow aspirate was consistent with CML in chronic phase. Conventional karyotyping revealed translocation between chromosome 9 and 12. FISH technique showed presence of 60% of BCR-ABL translocation with complex variant translocation between chromosome 9, 12 and 22 seen. However, DNA analysis for BCR-ABL gene was negative. She was started on cytoreductive therapy and Imatinib. She also had few cycles of leukapheresis due to retinal leukaemic infiltration and intrathecal chemotherapy was given due to possibility of CNS infiltration.

P-H-44. Diagnosis of Protein S deficiency in thrombophilia investigation: a 5-years of single center experience

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Introduction: Protein S deficiency either due to hereditary or acquired is known to be associated with venous thromboembolism. Protein S deficiency manifests as an autosomal dominant trait and thromboses are observed in both heterozygous and homozygous states. Three types of test are available for Protein S study, which constitute an important battery of tests. However interpretation difficulties and results discrepancies have been reported due to reagents variations. *Objectives:* This retrospective study was aimed to determine the prevalence of Protein S deficiency among patients with recurrent thrombotic events in this institution including analysis of their laboratory

and clinical data of potential diagnostic flaws. **Methods:** A retrospective study was conducted by retrieving laboratory data from 2007 to 2011. The data were obtained from Coagulation Laboratory, Haematology Department, Hospital Universiti Sains Malaysia, Kubang Kerian, Kelantan. Results of thrombophilia investigation for Protein S activity and free Protein S antigen were analysed in subjects with recurrent thrombosis. Clinical information was taken from the records available in the laboratory for patients investigated for thrombophilic disorder. **Results:** Total data retrieved for Protein S were 139. 10(7.2%) were found to be deficient in Protein S. Based on both free Protein S and activity levels with 4 (2.8%) had positive family history. **Conclusions:** This study suggests that Protein S deficiency is not much different from the reported finding among patients with recurrent venous thrombosis.

P-H-45. Preliminary data on incidence of heparin induced thrombocytopenia (HIT) among acute coronary syndrome patients receiving unfractionated heparin

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Introduction: Heparin is a mainstay treatment in Acute Coronary syndrome (ACS). Heparin induced thrombocytopenia (HIT) is an immune mediated, potentially life threatening, adverse effect of heparin therapy which frequently associated with venous and arterial thrombosis. HIT was defined as more than 50% reduction in platelet counts or an absolute platelet count of less $100 \times 10^9/L$ during and after heparin therapy and positive test for heparin-dependent antibodies. **Objectives:** To determine incidence of HIT in ACS patients treated with unfractionated Heparin and its outcomes of those patients in 3 months time. **Method:** ACS patients treated with unfractionated heparin were prospectively enrolled. Platelet count were measured at baseline, at day 5 and at 1 month post treatment, while anti- PF4/Heparin antibodies were measured by ELISA method (ASSERACHROM HPIA, Diagnostia Stago) at day5 and at 1 month post heparin treatment. All Patients were follow-up for 3 months for the end-point outcomes. **Results:** This is the preliminary data of twenty ACS patients whereby all have normal baseline platelet count (mean= $215 \times 10^9 /L$), none of them showed platelet count less than $100 \times 10^9 /L$ or reduction of 50% at day 5 and 1 month post heparin treatment. Anti-PF4 heparin antibodies also were not detected in all patients. Two patients noted to have platetet count drop at day5 and 1 month (29%, 24% at day 5 and 28%, 20% at 1 month respectively) with both had history of heparin exposure within 120 days, but none of them have Heparin antibodies positivity. Two (7.4%) patients have recurrent angina at 1 month follow-up. At 3 months follow-up, two(14.8%) experience new ACS, one (3.7%) recurrent angina and two (7.4%) need revascularization. **Conclusion:** Our preliminary findings suggest that unfractionated heparin quite safe to be use in Acute coronary syndrome as the heparin therapy given were short duration. However, regular monitoring of platelet count and symptoms related to thrombosis need to be exercised to ensure safety of heparin therapy.

P-H-46. Mesenchymal stromal and neural cell markers in ex vivo expanded limbal stromal cells

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Introduction: The limbus of human ocular surface which is located at the junction of the cornea and conjunctiva is known to be a unique stem cells niche for the ocular surface. While the successful use of cultivated limbal epithelial stem cells for the management of limbal stem cell deficiency (LSCD) has been reported extensively, the study of potential clinical application of limbal stromal cells is still in its infancy. Recent studies have suggested the presence of multipotent mesenchymal-like cells (MSC) in the limbal stromal. We studied the expression of MSC and neural markers in the limbal stromal cells. *Method:* Ex vivo expanded limbal stromal cells which were derived from human corneosclera rims were cultured on matrigel coated plate with defined media. When confluence, the cells were subjected to fluorescence activated cell sorting (FACS) analysis with a panel of MSC markers (CD90, CD71, CD73, CD29, CD44, CD105, CD123, CD271 and HLA-DR) and a neural stem cell marker (Nestin). The expression of neural markers including Nestin, beta-tubulin III, glial fibrillary acidic protein (GFAP), microtubule associated protein 2 (MAP2), neuron specific enolase (NSE), neuron filament (NFH) were further evaluated by immunostaining and reverse transcription polymerase chain reaction (RT-PCR). *Results:* FASC analysis revealed that the limbal stromal cells were positive for mesenchymal markers (CD90, CD73, CD29, CD44, CD105) but negative for CD71, CD123, CD271 and HLA-DR. High expression of neural marker, Nestin was also detected by FASC. Intriguingly, without any neural induction assay, expressions of neural markers such as Nestin, beta-tubulin III, MAP2, NFH and NSE were detected either by immunostaining and RT-PCR. *Conclusion:* The cultured limbal stromal stromal cells possessed mesenchymal stem cells (MSC) and neural cells properties. Given their developmental origin from the cranial neural crest, the limbal stromal cells might retain neural crest progenitor cells properties. Further studies will be needed to elucidate the neural potential of these cells.

P-H-47. Transduction of bone marrow-derived mesenchymal stem cells using non-integrating lentiviral single reprogramming cassette.

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Introduction: Transduction of a small set of transcription factors into somatic cells leads to creation of induced pluripotent stem (iPS) cells which are highly similar to embryonic stem cell (ESC). *Objective:* To transduce bone marrow-derived mesenchymal stem cells using Lentiviral single reprogramming cassette containing four transcription factors; Oct4, Sox2, Klf and c-Myc. *Methods:* HEK293FT cells were used to package the Lentiviral OSKM. Supernatant containing Lentiviral vector were harvested 48 hours after transfection and two further harvests at 12 hours interval. Lentiviral vector were titrated using abm Lentivirus-qPCR titer kit. The titer collected from supernatant of three harvests was 5 x 106 TU/ml. BM-MSC was transduced with Lentiviral OSKM at MOI20 twice and cells were cultured under human embryonic stem cells condition for 30 days. *Results:* Qualitative RT-PCR on BM-MSC transduced twice showed increased of Oct4 and Nanog gene expression as compared to non-transduced BM-MSC. *Conclusion:* This demonstrates that Lentiviral OSKM could be used to transduce somatic cells. However further optimization are needed to increase the efficiency of transduction.

P-H-48. Mesenchymal stem cells from diabetic microenvironment displayed higher adipogenic differentiation capacity

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Introduction: Mesenchymal stem cells (MSC) are promising candidate in cell-based therapies due to their multilineage differentiation capacity and their unique ability to migrate and engraft at injury or tumour sites. MSC are highly dependent on the bone marrow microenvironment for optimum growth and survival. It remains unclear whether MSC from diseased state, for example a diabetic microenvironment, displayed the same differentiation capacity. **Objective:** In this study, we investigated the effect of a diabetic microenvironment on the adipogenic differentiation capacity of MSC. **Methods:** MSC from bone marrow aspirate of normal and diabetic patients were isolated by Ficoll-Paque gradient centrifugation and expanded on DMEM supplemented with 10% fetal bovine serum. Expanded MSC were cultured in adipogenic induction medium for 10 days. MSC were fixed with 10% formalin and incubated in 60% Oil Red O solution for 30 mins at 37°C. Adipocyte conversion assay was performed by extracting the stain with isopropanol and measuring the absorbance at 490 nm. Experiments were performed in triplicate and conversion rate was expressed as percentage relative to control MSC. Total RNA was also extracted post induction and subjected to RT-PCR for detection of adipogenic genes. **Results:** MSC from normal and diabetic patients were successfully expanded and induced into adipocytes. However, MSC from diabetic microenvironment displayed greater adipocyte conversion at 1048% relative to control when compared to MSC from normal microenvironment at 235% relative to control. In addition, MSC from diabetic microenvironment exhibited slightly higher level of lipoprotein lipase (LPL) and peroxisome proliferation-activated receptor β 2 (PPAR- β 2) gene expression when compared to MSC from normal microenvironment. **Conclusion:** Current results showed that MSC from diabetic microenvironment displayed higher adipogenic differentiation capacity. Further studies on differential gene expression between adipocytes from these microenvironments could potentially shed light on the pathogenesis and potential therapeutic targets for diabetes.

P-MM-55. Parasitic infection and emergency medicine, a case report

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Introduction: Life threatening lower GI bleeding is very rare since it is usually intermittent and self-limiting. It can be caused by inflammation, neoplasm, anatomical abnormalities and infection caused by different pathogens. Trichuriasis is infection of the large intestine caused by the human whipworm *Trichuris trichiura*. This nematode was found to be the commonest soil-transmitted helminthes among Aborigine children in Malaysia. It was mostly observed in the age group of 2-7 years, where incidence of pica is the highest added to this; the hot, moist climate and poor sanitation which favor the worms survival. Infection is acquired through ingestion of parasitic ova. Patients usually present with dysentery, rectal prolapse, hypochromic anemia, abdominal pain and growth retardation. Chronic cases may also present with hypoproteinaemic oedema and cardiac failure due to severe anemia. Although the incident rate of typhoid and paratyphoid is decreasing in Malaysia, but still we have few cases admitted in our hospital in year 2008. Majority of patients in affluent countries present with the triad of persistent fever, headache and abdominal symptoms. Abdominal examination may reveal hepatomegaly, splenomegaly and diffused abdominal tenderness. In more

serious cases gaseous abdominal distention occurs and this may herald the onset of the acute abdomen that accompanies ileal perforation. Complications of typhoid infection are bleeding, perforation, circulatory collapse and relapse following treatment. We report this case as rarity and misleading diagnosis of the underlying cause of lower GI bleeding caused by helminthes infection.

P-MM-56. Biofilm formation on enteral feeding tubes by *Cronobacter sakazakii*, *Salmonella* serovars and other Enterobacteriaceae

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Introduction: This study was carried out to detect biofilm formation and the presence of *Cronobacter sakazakii*, *Salmonella* serovars and other Enterobacteriaceae, as well as to determine the safety of enteral feeding tubes used in the intensive care unit (NICU) at a Malaysian Hospital. *Method:* A total of 30 samples of enteral feeding tubes were taken and labeled as T1 to T30. The microbiological analysis involved were Total Plate Count (TPC), detection of *C. sakazakii*, *Salmonella* serovars and Enterobacteriaceae, followed by biochemical test using ID 32E kit (Biomereux). *Results:* The TPC of enteral feeding tubes were between 106 to 107 cfu/g, where the range had exceeded the FAO/WHO microbiological standard criteria for baby foods (<104 cfu/ml). No *C. sakazakii* and *Salmonella* spp. were isolated but other Enterobacteriaceae were identified, the *Klebsiella pneumoniae* spp. *pneumoniae* and *Escherichia coli* with 99% probability. Total Enterobacteriaceae calculated were below FAO/WHO safety criteria for baby foods (<103 cfu/ml). Among the Enterobacteriaceae member detected using ID 32E kit were *K. pneumoniae* spp. *pneumoniae*, *E. coli*, *Acinetobacter baumannii* and *K. oxytoca*. *K. pneumoniae* spp. *pneumoniae* was the most common bacteria detected in the enteral feeding tubes, followed by *E. coli*. Scanning Electron Microscopy (SEM) analysis showed biofilm formation on the walls of the tubes and the presence of rod-shaped bacteria, probably the Enterobacteriaceae group. *Conclusion:* Although the contamination of all the enteral feeding tubes were at a safe level for Enterobacteriaceae and no *C. sakazakii* and *Salmonella* were detected, the high total plate counts may affect the safety of the tubes in infants. Hence, the daily or routine change of enteral feeding tubes for the infants should be considered.

P-MM-57. Antibiotic resistance of *Cronobacter* spp. and its growth in infant formula milk with probiotics

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Introduction: This study was conducted to test the antibiotic resistance of *Cronobacter* spp. and its growth in infant formula milk (IFM) with probiotics. *Method:* Four strains of *Cronobacter* spp. i.e., *C. muytjensii*, *C. sakazakii*, *C. turicensis* and *C. malonaticus* were tested for their antibiotic resistance using Kirby-Bauer disk diffusion method, and *C. sakazakii* strain was used in the growth study. *Results:* All of the *Cronobacter* spp. tested for the antibiotic resistance was susceptible towards ampicillin, ceftriaxone, ceftazidime, cefuroxime, ciprofloxacin, gentamicin and norfloxacin. Only *C. muytjensii* was susceptible to streptomycin, while the other *Cronobacter* spp. reacted moderately to it. *C. muytjensi* and *C. turicensis* reacted moderately to sulphamethoxazole, while *C. sakazakii* and *C. malonaticus* were resistant towards it. All of the *Cronobacter* spp. reacted moderately towards fosfomycin. Results of growth study at 25°C have shown a higher generation time (0.45h) and lower specific growth rate (1.53/h) of *C. sakazakii* in IFM with probiotics, but no significant difference ($P>0.05$) were found when comparison was made with IFM without probiotics, with 0.39h and

1.77/h as its generation time and specific growth rate respectively. For *C. sakazakii* growth in IFM with probiotics and without probiotics at 37°C, the generation time and specific growth rates were 0.33h, 0.28h, 2.09/h and 2.45/h respectively. *Conclusion:* Significant difference ($P<0.05$) were found between growth study of *C. sakazakii* in IFM with and without probiotics at 37°C.

P-MM-58. Prevalence of *Cronobacter sakazakii* and other microorganisms in neonatal intensive care units of selected Malaysian hospitals

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Introduction: *Cronobacter sakazakii* is commonly implicated in infant formula milk but a sizeable part of *C. sakazakii* infections emerge in hospital neonatal intensive care units (NICUs). *Objective:* This study determined the prevalence of *C. sakazakii* and other microorganisms in milk preparation lines of NICUs in three Malaysian Hospitals. *Method:* 472 swabs of utensils, equipments, counter tops, milk preparation premises and infant formula samples were collected and analyzed for *C. sakazakii*, aerobic plate count (APC), *Escherichia coli* and coliforms, *Staphylococcus aureus* as well as other Enterobacteriaceae. *Result:* The highest APC (3.02 ± 0.21 log CFU/faucet) and *S. aureus* level (1.46 ± 0.11 log CFU/faucet) were detected on faucets; highest coliforms (2.69 ± 0.07 log CFU strainer) and Enterobacteriaceae levels were found in strainers. No *E. coli* were observed in any of the samples. *C. sakazakii* was suspected in 18/472 (3.8%) of the samples but using biochemical tests, only four (0.85%) were confirmed as such.

P-MM-59. Genetic determinants responsible for the extended-spectrum beta-lactamase (ESBL) phenotypes in *Klebsiella pneumoniae*

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Introduction: *Klebsiella pneumoniae* is a common hospital-acquired, as well as community-acquired pathogen. Extended-spectrum Beta-lactamases (ESBLs) producing strains are rapidly increasing. Data on ESBL genes in resistant *Klebsiella pneumoniae* isolates are limited in Malaysia. *Objectives:* We aim to explore the most common ESBL genes in Malaysian clinical isolates of *K. pneumoniae*. *Methods:* A total of 92 ESBL producing *K. pneumoniae* isolates were collected from patients admitted to University Malaya Medical Centre, Kuala Lumpur, Malaysia in 2010 and 2011. Identification of all strains as *K. pneumoniae* was done by standard biochemical methods, and confirmed by PCR. Susceptibilities to antimicrobial agents were determined by the disk-diffusion method according to the CLSI guidelines. ESBL production was confirmed by Etest (Cefepime /Cefepime + clavulanic acid) and Cefpodoxime Combination Disc Kit. The presence of blaESBL genes was examined by two multiplex PCR assays: multiplex I for TEM, SHV and OXA-1-like and multiplex II for CTX-M groups. Additional two multiplex PCR assays were used to detect the presence of carbapenemase genes (IMP, VIM and KPC) and AmpC genes (ACC, FOX, MOX, DHA, CIT and EBC). *Results:* Detected blaESBL genes were: CTX-M (92%), SHV (77%), TEM (63%) and OXA-1-like (32%). DHA gene was detected in 2% of the isolates. Carbapenemase genes were not detected in any

isolate. Combination of multiple genes was detected in 89% of the isolates with CTX-M being the most common gene in the combination. *Conclusion:* CTX-M gene is the most common blaESBL gene in this collection of *K. pneumoniae*. Further analysis by sequencing is necessary to define the most common subtype for each detected gene.

P-MM-60. Non-typhoidal Salmonellosis in a tertiary care hospital: Species distribution and antimicrobial susceptibility pattern (2009-2011)

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Introduction: Non-typhoidal Salmonella (NTS) is being recognized as an important pathogen especially if it is associated with bacteraemia. The emergence of salmonellae resistance to antimicrobial agents is a worldwide problem with multiple resistance to ampicillin, tetracycline, gentamicin and trimethoprim-sulfamethoxazole been reported from many countries during the past two decades. Certain serotypes of *Salmonella* exhibit a much higher risk of causing bacteraemia and these serotypes differ in between countries. It is noteworthy to analyze the current pattern of antibiotic resistance of salmonellae in our 600-bedded hospital which will reveal the present situation and serve as a local data to guide empirical therapy. *Method:* We analyzed the species distribution and antibiotic susceptibility data of NTS isolates that were isolated in the Microbiology Laboratory from January 2009 through December 2011. The isolates were identified by either API® or Vitek® identification and antimicrobial susceptibility was performed based on Clinical Laboratory Standard Institute (CLSI) guideline. Species identifications were determined by Institute for Medical Research which is a reference laboratory. *Results:* A total of 114 isolates of NTS were isolated. One-third of the isolates was isolated from blood cultures (31.6%). 95% of the isolates that were isolated from blood cultures were all identified as *Salmonella enteritidis*. In general, the three main species isolated were *Salmonella enteritidis* (48.2%), *Salmonella paratyphi B* (14.9%) and *Salmonella weltevreden* (13.2%). All isolates were susceptible to both ceftriaxone and ciprofloxacin. More than 90% were susceptible to ampicillin, chloramphenicol and trimethoprim-sulfamethoxazole. 54.8% resistance to tetracycline was observed. *Conclusion:* In our institution, the most common species of NTS isolated is *Salmonella enteritidis*. There is no resistance detected to ceftriaxone and ciprofloxacin and empirical treatment with either these two drugs would be the best option as empirical therapy. It would be interesting to observe the antibiotic resistance pattern of salmonellae in our institution in the future.

P-MM-61. Descriptions of third instar larvae of Boettcherisca Highlandica, Kurahashi & Tan 2009, (Diptera: Sarcophagidae): A highlander of forensic importance in Malaysia

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Introduction: A forensic entomological study was conducted in Cameron Highland, Pahang (4.49N, 101.39E) in January 2012. Three rabbit carcasses were placed at a study site which is 1,517 m above sea level. *Methods:* Visits to the study site were carried out for 22 days continuously and the mean temperature recorded was 23.4°C with mean humidity 85%. We collected adults of flesh fly (Diptera:

Sarcophagidae) on Day-3 of decomposition namely Parasarcophaga albiceps and then followed by Boettcherisca highlandica on Day-5. On Day-22, we collected three post-feeding larvae beneath a rabbit carcass which were brought to the lab for preservation or rearing purposes. *Results:* One larva developed into an adult after 17 days as a pupa while the other two larvae were preserved and processed on slides. The larval morphology of third instar Boettcherisca highlandica was studied under a light microscope and its structures, such as cephalopharyngeal, anterior spiracles, body spinations and posterior spiracles, were described for the first time. There is a mark different in the number of anterior spiracular papillae between Boettcherisca peregrina, a species common to the area, and B. highlandica, where there is 24-26 and 28-30 papillae, respectively. Species identification of sarcophagid larvae is of paramount important in forensic entomology as different species have unique life cycles and habitats. We propose that B. highlandica is a potential forensically important fly since its larvae have been recovered from rabbit carcasses in our study. *Conclusion:* Hence, there is a possibility that these larvae may be collected from human corpses found in highland which is more than 1,500 m and may play a part in providing clues in the determination of minimum postmortem interval. A key of comparison for adult and larva among Boettcherisca spp. in Malaysia is also discussed in this paper.

P-MM-62. Assessment of participating laboratories' performance in the National Quality Control Program in Bacteriology: A survey from 2009 to 2011.

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Introduction: Proficiency testing is an external quality assurance method which laboratories are sent simulated clinical specimens for testing by routine laboratory methods. *Objectives:* The goals of the present study were; i. to determine if the participating laboratories are following the NCCLS guidelines; ii. to assess diagnostic techniques used by laboratories to identify the organism; iii. to monitor the performance of the participating laboratories, for conformance of the ISO15189 guidelines ; iv. To identify the weakness in Bacteriological identification amongst Malaysian hospitals and implementation for improvements. *Method:* All participating hospitals are sent simulated bacteriological clinical specimens 3 times a year, which consist of (1) organism difficult to identify and (2) and (3), organisms with antibiotic susceptibility testing. The participants are given case report for each organism, instructions and are required to submit their findings before a given due date. *Results:* This study from 2009 to 2011, consist of nine batch of simulated specimens sent to participating hospitals throughout Malaysia. In 2009, batch 1; 83.0% of the participating hospitals had correct identifications, batch 2; 80.8% and Batch 3; 95.1%. In 2010, batch 1; 85.4% of the participating hospitals had correct identification, batch 2; 80.4% and batch 3; 83.5%. In 2011, batch 1; 86.2% of the participating hospitals had correct identification, batch 2; 87.2% and batch 3; 69.5%. The antibiotic sensitivity test method used by the laboratories is Kirby Bauer method with reference to the CLSI guidelines. In 2009, the overall performance for antibiotic sensitivity testing is 94.97%, 2010; 95.85% and in 2011; 85.13% It can be observed that the performance dropped in 2011. *Conclusions:* The performance of the participating hospitals is quite variable; it can be observed that participating hospitals perform well in identification of the simulated specimens compared to the performance for antibiotic sensitivity testing.

P-MM-63. Genetic analysis of co-trimoxazole resistance genes in *Haemophilus influenzae* isolates

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Introduction: Co-trimoxazole (SXT), a sulfonamide antibacterial combination of trimethoprim and sulfamethoxazole, has been used for the last few decades for treatment of *Haemophilus influenzae* infections. However, with the emergence of SXT-resistant strains worldwide, its usage has become less important. Several mechanisms have been reported to be associated with SXT-resistance, mainly involving variants of dihydrofolate reductase (DHFR) dfr genes responsible for trimethoprim resistance; while sulfonamide genes, sul1 and sul2 and/or mutation in the chromosomal dihydropteroate synthetase (DHPS) folP contributes to sulfonamide resistance. **Objectives:** To investigate the presence of the genes involved in SXT resistance in Malaysian *H. influenzae* strains and to compare their amino sequences with other *H. influenzae* strains isolated elsewhere. **Methods:** Antibiotic susceptibility test to SXT was carried out on 34 strains of *H. influenzae* and minimum inhibition concentrations (MIC) assay were performed. Primers targeting for five variants of dfr, folP, sul1 and sul2 were used to amplify the genes in the SXT-resistant strains. The amplicons were sequenced and multiple alignments of the assembled sequences of the test strains were compared to other *H. influenzae* strains available in the Genebank. **Results:** Of the 34 strains, seven were resistant to SXT with MIC that ranged from 8 to >32 microgram/ml. Of the five variants of dhf genes, dfrA1 was detected in three strains but all the seven strains possessed folP gene. Four had sul2 gene and none had sul1 gene. Amino acid sequence of the folP genes showed that strain H152 was genetically different from the others due to a 15-bp nucleotide insertion in the gene, which was similar to the insert in folP of *H. influenzae* strain A12, a strain isolated in United Kingdom. **Conclusion:** The SXT resistance amongst the Malaysian *H. influenzae* strains was mainly attributed to folP. Presence of sul2 in addition to folP in four strains had increased the level of resistance. The high level of resistance to sulfonamide demonstrated by a strain that lacked either sul1 or sul2 gene was possibly attributed to a 15-bp DNA insert.

P-MM-64. Kinyoun stain of acid fast bacilli (AFB): efficacy in diagnosis of tuberculosis in Faculty of Medicine, UiTM.

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Introduction: Lack of diagnostic capacity has been a crucial barrier preventing an effective response to National Tuberculosis Control Programme. A staining method for AFB, which can be practiced in developing countries and remote areas, is anticipated for successful implementation of TB Control Programme. Tuberculosis screening of AFB is the main element that serve significant role in the initial step of laboratory investigation in TB diagnosis. To date, TB screening stains includes ZN, Kinyoun and auramine-rhodamine stain. The objective of this study is to determine the effectiveness of Kinyoun stain in TB screening and diagnosis, and to ascertain the role of Kinyoun stain as an alternative to ZN stain. **Methods:** A retrospective study was conducted on respiratory and non-respiratory samples collected from Microbiology Unit, Centre for Pathology Diagnostic and Research Laboratories (CPDRL), Faculty of Medicine, Malaysia from January 2011 to March 2012. Kinyoun stain of all sample were performed immediately once the samples reached the laboratory based on the request of 'sputum for AFB'. However, if the request involved 'TB culture', the samples will be sent to Microbiology Unit, University Malaya Medical Centre (UMMC). **Results:** A total of 77 samples were collected from suspected cases of tuberculosis. Of 77 samples, 75 were respiratory

samples (73-sputum, 2-bronchioalveolar lavage) and remaining two were synovial fluid and stool. However nine respiratory samples were rejected for analysis, as eight were saliva and one was due to leaking of the container. Among 68 samples, 27 samples were processed in UMMC, whereby TB screening performed by using ZN stain. All 27 samples processed in UMMC demonstrated concordance results with Kinyoun stain regardless of AFB positive or negative. *Conclusion:* Kinyoun stain is an improvement and appropriate alternative method over the traditional ZN stain, as it makes heating step unnecessary. Furthermore, due to its simplicity and rapidity Kinyoun stain demonstrated to be equally sensitive in detecting AFB as the ZN stain method.

P-MM-65. Description of patients with methicillin resistant *Staphylococcus aureus* (MRSA) bacteremia in Hospital Sungai Buloh.

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Introduction: The frequency of methicillin-resistant *Staphylococcus aureus* (MRSA) infections continue to grow in hospital-associated settings globally. The risk factors for nosocomial MRSA bacteremia have been attributed to those who receive prior antibiotic treatment, invasive catheterization, history of intensive care unit admission (ICU) and patients with severe underlying disease. *Methods:* A retrospective study was conducted over a two and a half-year period (2009- until April 2012). The incidence of *Staphylococcus aureus* bacteremia (SAB) was determined from the data collected by the Infection Control Unit Hospital Sungai Buloh. *Results:* 65 patients had MRSA isolated from blood a single or both culture bottles. Only 59 patients were analysed and 6 were abandoned due to lack of clinical data. 22 (37.2%), 13 (22%), 12 (20%), 4 (6.7%), 3 (5%), 3 (5%), 2 (3.3%) patients had been or was admitted in ICU, medical ward, general surgical ward, neurosurgical ward, orthopaedic ward and neonatal ICU (NICU) high dependency unit (HDW) respectively during the blood culture was taken. 35 (59%) patients had intravenous catheterization through such as intravenous jugular vein, femoral vein, and central venous line, 20 (34%) patients also had a history of mechanical ventilation. 12 (20.3%) had diabetes and 10 (16.9%) had a history of multiple hospital admission. *Conclusion:* Our preliminary finding shows that patients with a history of ICU admission and intravenous catheterization were most frequently found to develop MRSA bacteremia. Further analysis is needed to ascertain the risk factors for MRSA bacteremia among patients in Hospital Sungai Buloh.

P-MM-66. Pre-operative screening for viral hepatitis infections: To do or not to do?

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Introduction: In theory, universal precautions should be able to protect healthcare workers from the risk of 'occupational exposure' to Hepatitis B and C viruses. However, strict adherence is almost impossible in practice. Although routine pre-operative screening tests may be done, universal screening is not currently recommended for asymptomatic patients. *Objectives:* This study aims to determine the frequency of Hepatitis B and Hepatitis C infections among pre-operative population of patients. *Methods:* This retrospective, hospital-based study was conducted in Clinical Training Centre, Faculty of Medicine UiTM, Malaysia. Two hundred subjects from both genders whom are scheduled for angiogram were screened. Blood specimens tested for qualitative detection of

Hepatitis B antigen (HBsAg) and Hepatitis C antibody (anti-HCV). *Results:* Between the period of May 2011 and March 2012, 200 subjects were screened for presence of HBsAg and anti-HCV. Positive (reactive) results were detected in 5.5% (n = 11) of subjects. The prevalence of Hepatitis B carrier, Hepatitis C carrier, as well as Hepatitis B and C carrier (dual infection) among the total cases studied were 3.0% (n = 6), 2.5% (n = 5) and 1.0% (n = 2) respectively. More male subjects showed positive results compared to female (n = 9 vs. n = 2). The age of subjects with positive results ranges from 42 to 61 years old. Most interestingly, out of 11 subjects with Hepatitis B or / and Hepatitis C infections, only 2 had an abnormal liver function. This were demonstrated by raised level of liver enzymes, namely alkaline phosphatase (ALP), alanine aminotransferase (ALT) and gamma-glutamyl transpeptidase (GGT). None of the 11 subjects were clinically symptomatic of hepatitis. *Conclusion:* Based on current guidelines, 9 patients in this study would not have been detected as viral hepatitis carrier, as infectious screening was neither clinically nor biochemically indicated. However, the routine pre-operative screening done for them had (1) potentially prevent transmission of infection to healthcare workers; and (2) allow these patients to manage their risk of developing viral-related chronic liver disease much earlier.

P-MM-67. Detection of *Bordetella pertussis* from suspected cases of pertussis in Malaysian hospitals by polymerase chain reaction.

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Introduction: Pertussis or whooping cough is a highly infectious respiratory disease caused by *Bordetella pertussis*. It is a vaccine preventable disease usually affecting children less than 2 years old. *B. pertussis* is fastidious and difficult to culture, thus PCR has been used to provide rapid and sensitive detection. *Method:* A total of 708 specimens were received from major hospitals in Malaysia in 2011. The specimens were cultured on Charcoal agar and subjected to PCR, which amplified the repetitive insertion sequence IS481 and pertussis toxin promoter gene. *Results:* Out of these specimens, 276 were positive, where 6 were positive by both PCR and culture, 4 by culture alone and 266 were positive by PCR alone. Among the positive cases, 41.7% were aged 0- <2 months, 35.5% aged 2- < 4 months, 5.4% aged 4 - <6 months, 6.2% aged 6- <12 months, 8.7% aged 1- <6 years, 1.8% 6-12 years and 0.7% aged >12 years. Most of the positive cases were from Kedah (15.2%), Terengganu (14.1%) and Selangor (12.7%). This study showed that most of the positive cases involved children less than 2 months old who are not in the vaccination age group, followed by children in the age group 2- <6 months, who were also at high risk of infection because their vaccination schedule have not been completed yet. *Conclusion:* This indicates that babies who were not protected by vaccination are prone to get the infection. Our study also showed that PCR technique was able to pick up more positive cases compared to culture method. Delay in transportation, improper storage and improper transportation media could be the cause of low culture positivity.

P-CP-75. Hepatoprotective activity of peel and pulp methanolic extracts of *Musa acuminata* Colla cv. Kapas against carbon tetrachloride-induced liver damage in rats

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Introduction: It is very important to discover safe and effective plant-based drugs as an alternative cure for liver disease. **Objectives:** This study was done to assess the hepatoprotective activity of the peel and pulp methanolic extracts of *Musa acuminata* Colla cv Kapas against carbon tetrachloride (CCl₄) hepatotoxicity by using biochemical parameters and histopathological studies in rats. **Methods:** A total number of 51 young adult Sprague Dawley male rats were divided into 9 groups. Group I, II and III served as normal, negative and positive control groups. Group IV, V and VI were pre-treated with 100, 200 and 400 mg/kg of peel crude extract for 7 consecutive days followed by administration of CCl₄ on the 8th day. Likewise, group VII, VIII and IX were pre-treated with the same concentrations of the pulp crude extract. Samples of rat's blood serum were collected for assessment of biochemical analysis by measuring total bilirubin (Tbil), total protein (TP) and glutathione SH (GSH) and liver samples were processed for histopathological studies. **Results:** The hepatoprotective effect of the peel and pulp methanolic extracts of *M. acuminata* were in a dose-dependent manner and the results of the biochemical analysis and histopathological studies supported each other at all doses. A marked improvement was observed in the positive control group which was pre-treated with silymarin and with groups pre-treated with 400 mg/kg dose of peel and pulp extracts as compared to the negative control group. The improvement at 400 mg/kg of both peel and pulp were comparable to silymarin as Tbil serum level was significantly reduced and TP and GSH were significantly elevated. In addition, a significant attenuation of hepatic lesions was observed by showing the normality of the liver lobule histoarchitecture. Phytochemical screening showed the presence of flavonoids, triterpenes and saponins in the extracts, which could be responsible for the observed hepatoprotection because of their anti-inflammatory and antioxidant properties. **Conclusion:** The methanolic peel and pulp extracts of *M. acuminata* have significant hepatoprotective effect at dose of 400 mg/kg in hepatotoxicity with CCl₄ which is comparable to the standard drug of silymarin, probably due to the synergistic action of flavonoids, triterpenes and saponins in the extract.

P-CP-76. Evaluation of the hepatoprotective action of peel and pulp methanolic crude extracts of *Musa acuminata* Colla cv. Kapas on induced liver damage by paracetamol in rats

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Introduction: There is an urgent need to explore possible sources of hepatoprotective drugs against liver diseases. **Objectives:** This study was carried out to evaluate the hepatoprotective effect of the unripe peel and pulp methanolic extracts of *M. acuminata* Colla cv Kapas on paracetamol-induced hepatic injury through biochemical parameters and histopathological studies. **Methods:** nine groups of 13-week old male rats (Sprague Dawley) were used with 6 rats in each group. Group I, II, III served as normal, negative and positive control groups respectively. Group IV, V and VI were pre-treated with 100, 200 and 400 mg/kg of pulp crude extract and group VII, VIII and IX received the peel extract at dose of 100, 200 and 400 mg/kg followed by administration of paracetamol.

For evaluation of liver function, total bilirubin (Tbil), total protein (TP) and glutathione SH (GSH) of the blood serum were measured and the rats liver were collected for histopathological studies. *Results:* The biochemical parameters and histopathological studies of the normal, positive and negative control groups as well as all pre-treatment groups supported each other wherein the methanolic crude extract of the peel and pulp of *M. acuminata*, showed hepatoprotective effect in a dose-dependent manner. A significant improvement was observed in the positive control group pre-treated with silymarin and in the pre-treated groups with methanolic extracts of *M. acuminata* peel and pulp at dose of 400 mg/kg while the dose of 200 mg/kg of pulp extract showed a moderate improvement. The marked improvement was due to significant reduction of Tbil, marked elevation of TP and GSH serum levels and normal histoarchitecture of the liver lobules. PCM toxicity is attributed to the oxidative action of its toxic metabolite, N-acetyl-p-benzoquinone-imine (NAPQI) which binds to DNA and proteins. Flavonoids, saponins and triterpenes present in the extracts have anti-inflammatory activities and could explain the hepatoprotective effect observed in this study. *Conclusion:* A high hepatoprotective effect, better than the commercial drug of silymarin was observed at the dose of 400 mg/kg of peel and pulp extracts and a moderate effect at the dose of 200 mg/kg of pulp extract. Further investigations should be done to explore the potential of “pisang kapas” as a possible source of liver-protecting agent.

P-CP-77. Allicin has significant hypoglycemic effect on type 1 diabetes mellitus

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Introduction: Plant drugs are frequently considered to be less toxic and more free from side effects than synthetic ones. Allicin is an organosulfur compound obtained from garlic exhibits hypoglycemic effect in type 2 diabetes mellitus but no evidence about its effect in autoimmune type 1 diabetes mellitus (T1DM). *Objectives:* The purpose of this research was to experimentally assess the anti-diabetic effect of allicin on T1DM in streptozotocin (STZ) -induced rats. *Methods:* 24 male Sprague-Dawley rats with an average weight of 150-250g and an average age of 12-16 weeks were used. The rats were divided into four groups; six rats each. Group 1 Normal control group (do not receive any treatment neither STZ nor allicin). Group 2 Diabetic control group (treatment with intra-peritoneal i.p STZ only 50 mg/kg). Group 3 Diabetic rats received i.p Allicin (8mg/kg). Group 4 Diabetic rats received i.p Allicin (16mg/kg). All rats haven't treated with insulin at any time during the experiment which lasted for 30 days. Levels of serum glucose, total cholesterol, triglycerides, and daily body weight in normal and streptozotocin-induced diabetic rats were evaluated. The results have been compared to the ones obtained from healthy and non treated diabetic rats. *Results:* Administration of Allicin significantly decreased serum glucose, total cholesterol, triglycerides levels. The antidiabetic and hypolipidaemic properties of Allicin were more effective in high doses. *Conclusions:* These experimental results indicate that Allicin must be considered as excellent candidate for future studies on diabetes mellitus and of great value in managing the effects and complications of T1DM.

P-CP-78. Paraoxonase-1 (PON1) activity in serum and various anticoagulated-plasma

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Introduction: Paraoxonase 1 (PON1) is a high density lipoprotein (HDL) associated enzyme that is known for its function to hydrolyze organophosphate (OPs) into a relatively harmless substance and inhibit oxidative modification of low density lipoprotein (LDL). It is among the commonly studied biochemical markers for cardiovascular diseases. PON 1 activity is usually measured in serum (from plain container) while some other relevant cardiovascular parameters require anti-coagulated plasma. Collection of blood in many types of container may increase the cost and require a larger amount of blood to be collected. Apart from plasma in lithium heparin, the reliability of plasma in other anti-coagulated container such as potassium-citrate, potassium- oxalate/sodium-fluoride and CPDA (from blood collection bag) on PON 1 activity have not yet been clearly ascertained. **Objective:** to compare and to study the correlation between the PON1 activity in serum and in plasma collected in various anti-coagulated containers. **Methods:** An experimental study was carried out on 50 volunteers. Blood samples were collected in plain container and containers with potassium-EDTA, lithium-heparin, potassium-citrate, potassium-oxalate/sodium-fluoride and CPDA. Serum and plasma were analyzed for PON1 activity spectrophotometrically after the hydrolysis of substrates paraoxon. **Results:** The PON1 activity in plasma from lithium-heparin container was slightly reduced but not statistically different ($p=0.062$) from that of serum ($272.95\text{U/L}\pm85.11$ vs $288.95\text{U/L}\pm91$). However, the PON1 activity was significantly reduced ($p<0.001$) to $224.13\text{U/L}\pm74.78$, $211.46\text{U/L}\pm64.18$, $184.32\text{U/L}\pm60.06$ and $48.00\text{U/L}\pm45.91$ respectively in plasma from potassium-citrate, CPDA, potassium-oxalate/sodium-fluoride and potassium-EDTA container. There were significant positive correlations ($p<0.001$) between PON1 activity in serum with PON1 activity in all anti-coagulated plasma except for plasma in potassium-EDTA container ($r=0.27$, $p=0.06$). **Conclusion:** Our finding suggested that only serum and plasma in lithium-heparin container are suitable for the analysis of PON1 activity.

P-CP-79. Evaluation on the I-CHEM velocity diagnostic iRICELL 2000 fully automated urinalysis system

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Introduction: Urinalysis is a simple routine clinical laboratory test that normally includes a number of parameters of physical and chemical characteristics in urine. Positivity of certain characteristics found in urine may followed by microscopic examination for further investigation. The information provided by the test is useful for various medical condition screening. The Core Laboratory Unit, Hospital Kuala Lumpur opts to turn to the new Iris Diagnostic iRICELL 2000 Fully Automated Urinalysis System that consists of two units of analysers; the Iris iChem Velocity which performs the biochemistry test using dipstick method and iQ 200 Urine Microscopy analyser which provides digital image captures. The objective is to achieve better TAT, efficient testing and accurate result. The evaluation test is executed to see the performance of the new system. **Method:** A total of forty fresh urine sample remainings were collected from the Urology Clinic outpatients. The urine sediments were examined by manual microscopy, Uriscan analyser and Iris Diagnostic iRICELL 2000 Fully Automated Urinalysis System. The protocols of the evaluations are comprised of a correlation study between the Iris iChem Velocity and the Uriscan Semiautomatic Urine Analyser for the urinary

biochemistry strip testing; imprecision test using quality control materials; linearity study of the iQ 200 and correlation study for RBC and WBC in urine between Iris iChem Velocity and between iQ 200 Urine Microscopy analyser. **Result:** The concordance within one grade result is determined to be the indicator for acceptance in this evaluation for correlation study in the strip test. Agreement of the semi quantitative analytes concentration within one grade is seen in all parameters. While the iQ200 shows consistent and precise microscopic readings. **Conclusion:** The automated systems demonstrated good concordance with the Uriscan Semiautomatic Urine Analyser and show a slightly better sensitivity in analysis.

P-CP-80. Inflammatory status in patients with low High Density Lipoprotein and co-morbidities-matched controls.

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Introduction: Atherosclerosis has been established to be a chronic inflammatory process. High density lipoprotein (HDL) plays a vital role in the reverse cholesterol transport pathway and has been shown to exert anti-inflammatory effects. Low HDL level is well established to be associated with increased risk of coronary artery disease. **Objectives:** To compare the inflammatory status between subjects with low HDL and normolipidaemic controls. **Methods:** Fifty one subjects with low HDL levels and 52 age, gender ethnic, hypertension, diabetes, and smoking status matched normal controls were recruited for this study. Fasting serum samples were collected to analyse for IL-6 and hsCRP levels. Enzyme-linked Immunosorbent Assay (ELISA) was performed to identify the concentration of IL-6, while the concentration of hsCRP was measured by using an automated analyser (Cobas Integra 400, Roche Systems, Germany). **Results:** There was no significant differences in IL-6 (mean \pm SEM: 2.82 ± 0.24 pg/ml vs 3.16 ± 0.25 pg/ml, $P > 0.05$) and hsCRP (mean \pm SEM: 4.82 ± 1.15 mg/l vs 2.28 ± 0.45 mg/l, $P > 0.05$) levels between patients and controls. Furthermore, there was no correlation noted between IL-6 and hsCRP. **Conclusion:** There is insignificant difference in the inflammatory status between subjects with low HDL and hypertension, diabetes and smoking status matched controls. This suggests that the co-morbidities play a role in inflammatory status.

P-CP-81. Theaflavins-rich fraction reduces lipopolysaccharide-induced expression of adhesion molecules in vascular endothelium

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Introduction: Atherosclerosis is a chronic inflammatory process during which activated vascular endothelium leads to increased expression of adhesion molecules and subsequent recruitment of monocytes to the endothelium. The effects of theaflavins, major polyphenolic compound in black tea, on the expression of adhesion molecules in activated endothelial cells in atherogenesis are still unclear. **Objective:** To investigate the cytotoxic effects of theaflavins-rich fraction (TsRF) on human umbilical vein endothelial cells (HUVECs) and TsRF effects on the expression of adhesion molecules (E-selectin, VCAM-1 and ICAM-1) in HUVECs during lipopolysaccharide (LPS)-induced inflammation. **Methods:** Cytotoxicity was assessed by methyl-thiazol-tetrazolium assay using TsRF (Organics Herbs, China) with concentrations ranging from 1.6 to 200 μ g/ml which were added to HUVECs (Cascade Biologics, USA). HUVECs were treated with 1 μ g/ml LPS (Sigma, USA) and TsRF 10,20,30,40 and 50 μ g/ml and incubated for 16 hours. Protein and RNA expression were determined using ELISA (Bender MedSystem, Austria) and qPCR (BioRad iCycler, USA)

respectively. **Results:** TsRF = 50ug/ml exhibited = 80% cell viability. TsRF 10-50ug/ml reduced E-selectin($p < 0.0001$), VCAM-1($p < 0.0001$) and ICAM-1($p < 0.05$) levels in LPS-stimulated HUVECs. Compared to controls, the lowest levels of E-selectin(1194.7 ± 0.2 vs. 1885.6 ± 0.7 pg/ml, $p < 0.0001$) and ICAM-1(1457.5 ± 1.4 vs. 1468.2 ± 1.6 , $p < 0.05$) were observed at TsRF 50ug/ml, while VCAM-1(1732.9 ± 0.4 vs. 1759.5 ± 4.7 pg/ml, $p < 0.0001$) were shown at TsRF 20ug/ml corresponding to percentage inhibition of 36.6%, 0.73%, and 2.1% respectively. TsRF 10-50ug/ml reduced gene expression of E-selectin ($p < 0.0001$), VCAM-1($p < 0.0001$), ICAM-1($p < 0.0001$). TsRF 40ug/ml demonstrated the lowest gene expression of E-selectin, VCAM-1 and ICAM-1 (0.6 ± 0.1 vs. 2.4 ± 0.3 fold, $p < 0.0001$; 0.4 ± 0.2 vs. 2.5 ± 0.1 fold, $p < 0.0001$; 0.2 ± 0.0 vs. 2.3 ± 0.4 fold, $p < 0.0001$ respectively). **Conclusion:** TsRF inhibits expression of adhesion molecules, hence attenuating endothelial activation and inflammation. This suggests the potential benefits of black tea in the preventive and treatment of atherosclerosis.

P-CP-82. Effects of long term confined isolation on inflammation and endothelial function in human

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Introduction: Inflammation and endothelial dysfunction are the key events in the pathogenesis of atherosclerosis. Space exploration journey to Mars has been suggested to enhance the status of these pro-atherosclerosis factors due to prolonged confinement. The effects of long term confinement on inflammation and endothelial function in human under 1 gravity (1g) environment are not well established. **Objective:** To investigate the effects of prolonged confinement on inflammation and endothelial function in marstronauts compared to controls. **Methods:** Six (6) males marstronauts (age + SD age = 32 ± 4.7 years) and 7 age-matched healthy free living ground controls (age + SD age = 36.6 ± 6.2 years) were recruited in the study. Fasting venous whole blood samples were serially collected at the pre, intra and post-isolation phases for the duration of 520 days. High sensitive C-reactive protein (hsCRP) levels in the serum of both Marstronauts and controls were measured on an automated analyser (Cobas Integra 400, Roche Diagnostics, Switzerland). Brachial artery endothelial-dependent flow mediated dilatation (FMD) was performed following the standard protocols set by the International Brachial Artery Reactivity Task Force by trained personnel. **Results:** There were no significant differences in the baseline characteristics of the cosmonauts and controls which include age, hsCRP, blood pressure and baseline brachial artery diameter. There were no significant differences in the hsCRP levels and mean percentage of brachial artery diameter between cosmonauts and controls throughout the 520 days of confinement ($p > 0.05$). **Conclusion:** Inflammation and endothelial function status in human are not altered by prolonged confined isolation under 1g environment.

P-CP-83. Oxidized Low-Density Lipoprotein (OX-LDL) and Lecithin-Cholesterol Acyltransferase (LCAT) are associated with low High-Density Lipoprotein (HDL)

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Introduction: It is established that high-density lipoprotein (HDL) may play an anti-atherogenic role as an antioxidant by inhibiting the oxidative modification of low-density lipoprotein (LDL). The antioxidant property of HDL has been associated with lecithin: cholesterol acyltransferase (LCAT),

a key enzyme in reverse cholesterol transport and HDL remodeling. LCAT is not only capable of esterifying free cholesterols in plasma, but can also prevent the accumulation of oxidized LDL (ox-LDL). *Objective:* To evaluate the association between ox-LDL and LCAT in subjects with low HDL-c levels. *Methods:* A total of 51 patients (20 males, 31 females, mean \pm SD age=50.6 \pm 8.5) with low HDL (HDL-c =0.6 mmol/L and = 0.7mmol/L in males and females respectively) and 52 age-, gender- and race-matched healthy normal controls (HDL-c =1 mmol/L and =1.3 mmol/L for males and females respectively) were enrolled in this study. Fasting blood samples were obtained. Lipid profile was measured by enzymatic reference method on an automated analyser (Cobas Integra 400, Roche systems, Germany). Ox-LDL and LCAT concentrations were measured using commercial double monoclonal antibody ELISA kits. *Results:* Data are expressed as median (95% CI). Patients with low HDL had a significantly higher level of ox-LDL, [25.69 (8.53 - 46.70)] U/L versus [17.05 (8.53 - 56.61)] U/L, P <0.01, compared to normal controls, and lower LCAT concentration [4.37 (1.46 - 25.41)] μ g/ml versus [6.84 (1.54 - 19.09)] μ g/ml, P < 0.05. Ox-LDL had a significantly inverse correlation with HDL levels (P<0.01) while LCAT showed positive correlation with HDL levels (P<0.01). However, no correlation was found between ox-LDL and LCAT levels (P=0.29). *Conclusions:* Decreased LCAT levels and increased oxidative stress are associated with subjects with low HDL levels, not due to secondary causes. This suggest that pro-atherogenecity of HDL is in part contributed by enhanced oxidative stress.

P-CP-84. Central obesity with and without metabolic syndrome is associated with elevated inflammatory and oxidative stress status

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Background: Metabolic syndrome (MS) is a cluster of coronary risk factors that includes abdominal obesity, atherogenic dyslipidaemia, hypertension and insulin resistance. It had been postulated that MS and central obesity are linked to enhanced inflammatory and oxidative stress, two pivotal factors in atherosclerosis. Furthermore, the association of these factors in MS with different glycaemic status is still unclear. Therefore, the objective of this study is to evaluate the inflammatory and oxidative stress in MS subjects with different glycaemic status and simple obesity compared to controls. *Methods:* A total of 260 subjects were recruited and divided into 5 groups: central obesity without MS (OBXMS), MS with diabetes (MSDM), MS with impaired fasting glucose (MSIFG), MS with normoglycaemia (MSNG) and normal control (NC). Subjects with MS were centrally obese (waist circumference =90cm for males and =80cm for females) plus = 2 out of 4 other factors. Exclusion criteria were those on antidiabetic, antioxidant and anti-inflammatory medications, current smoker, LDL-c >4.1mmol/L, chronic inflammatory disorders or severe disease that shorten life expectancy. Fasting serum sample were collected to measure high sensitivity C-Reactive Protein (hsCRP) using an automated analyzer (Cobas Integra400, Basel) while plasma oxidized low-density lipoprotein (ox-LDL) by enzyme immunoassay. Plasma malondialdehyde (MDA) was measured by method adopted from Ledwozy et al.(1986). *Results:* There was higher serum hsCRP levels in all MS compared to NC ($p<0.01$). Similarly, OBXMS had higher hsCRP compared to controls ($p<0.0001$). The plasma ox-LDL was higher in MSIFG compared to NC ($p<0.0001$) and compared to OBXMS ($p<0.0001$). MSDM group showed higher MDA levels compared to NC ($p<0.0001$) and OBXMS ($p<0.005$). *Conclusion:* There is enhanced inflammation and oxidative stress in simple obesity especially so in the presence of MS irrespective of their glycaemic status. These suggest elevated coronary risk in central obesity even in the absence of but especially so with the presence of MS.

P-CP-85. Pure tocotrienol isomers but not tocotrienol-tocopherol mixed fraction prevents hypercholesterolemia in rabbits fed with high cholesterol diet

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Introduction: Hypercholesterolaemia is an established major risk factor for the development of coronary artery diseases (CAD). Tocotrienol tocopherol mixed fraction (TTMF) contains the various tocotrienol (TCT) and tocopherol isomers. The aim of this study is to compare the effects of TTMF and TCT on the lipid profile in hypercholesterolaemic-induced rabbits. **Methods:** 17 New Zealand male white rabbits were randomized into 2 groups and given either TTMF (15mg/kg body weight) or TCT (15mg/kg body weight) throughout the study. They were fed normal diet (ND) for 8 weeks followed by 1% high cholesterol diet (HCD) for 2 weeks. Fasting serum samples were measured for total cholesterol (TC), low density lipoprotein cholesterol (LDL-c), triglycerides (TG) and high density lipoprotein cholesterol (HDL-c) at baseline, 8 weeks and 10 weeks. **Results:** TC (mean+SEM: 0.48 ± 0.66 mmol/L vs 1.36 ± 0.23 mmol/L, $p<0.005$), LDL-c (mean+SEM: 0.11 ± 0.02 mmol/L vs 0.60 ± 0.09 mmol/L, $p<0.01$) and TG (mean+SEM: 0.45 ± 0.06 mmol/L vs 1.08 ± 0.13 mmol/L, $p<0.05$) were decreased in the TCT group at 8 weeks compared to baseline. Significant percentage change in reduction was seen following 2 weeks HCD in the TCT group compared to placebo for TC and LDL-c ($p<0.05$). There were no significant differences in the lipid profile of the TTMF group at 8 or even 10 weeks compared to baseline and when compared to placebo following HCD. **Conclusion:** TCT significantly lowers cholesterol levels compared to TTMF suggesting that there is a possibility that the presence of tocopherols in TTMF may attenuate the atheroprotective properties of TCT.

P-CP-86. Effects of prolonged, confined isolation on serum levels of Intercellular Adhesion Molecule-1 and Interleukin-6

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Introduction: Long term confinement and microgravity environment have been suggested to induce inflammatory responses and modulate immune functions that may increase oxidative stress. Inflammation and endothelial dysfunction are important in the pathogenesis of atherosclerosis. The effects of long term isolation under either 1g facility, simulated microgravity environment or actual space travel on in vivo inflammation and endothelial activation are still unclear. **Objective:** To determine the effects of long term confinement under 1g on biomarkers of inflammation such as serum intercellular adhesion molecule-1 (sICAM-1) and interleukin-6 (IL-6). **Methods:** Fasting venous whole blood and urine specimens were collected serially from the 6 cosmonauts during the pre, intra (30, 90, 150, 270, 390 and 520 days) and post-isolation phases for 520 days. Age and gender-matched healthy, free living Russian and Malaysian ground controls were recruited in parallel. Serum samples were analyzed for fasting serum lipid profiles by the automated analyser Cobas Integra 400 (Roche Systems, Germany), while ICAM-1 and IL-6 levels were analyzed by Enzyme-linked Immunosorbent Assay (ELISA). **Results:** There was no significant differences in the cosmonaut, Russian and Malaysian control groups within group comparison between all the time intra and post isolation period, with their respectively baseline. Furthermore, there was no significant difference in IL-6 and sICAM-1 levels between the cosmonauts and ground controls throughout the isolation period ($P>0.05$). **Conclusion:** Prolonged confined isolation and 1g environment does not lead to increase neither inflammation nor endothelial activation.