The 2015 Annual Scientific Meeting of the College of Pathologists, Academy of Medicine Malaysia & 40th Anniversary Celebration of the Pathology Advocates was held at Berjaya Time Square Hotel, Kuala Lumpur from 13-14 June 2015. Abstracts of scientific presentations follow:

K PRATHAP MEMORIAL LECTURE

Evolving concepts in the pathological diagnosis of lung cancer

Pathmanathan Rajadurai

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The pathological diagnosis of lung cancer has transformed over the past several years from a morphological characterisation of the disease to one where molecular characterisation has become increasingly important. Molecular characterization of lung carcinoma contributes valuable information in terms of diagnosis, prognosis, and the potential for treatment with targeted therapy. Hence, the simple separation of lung cancer into small cell and non-small cell is insufficient for the modern day management of this cancer. Over the past few decades, the concept of “oncogene addiction” in malignancies has led to the documentation of a number of reproducible molecular alterations in lung cancer—the so called “driver mutations”. This has permitted the identification of specific molecular cohorts of patients who may benefit from therapy targeted at these driver mutations. Among other challenges for the pathologist of today is the demand to extract a vast body of information from increasingly small diagnostic specimens and scanty cell aspirates. The pathologist plays a pivotal role in balancing the need to preserve tissue for molecular studies, over performing other ancillary, potentially wasteful studies. With additional evidence that targeted therapy is a major improvement over conventional chemotherapy when applied to the appropriately selected patients, evaluation for epidermal growth factor receptor (EGFR) mutations, anaplastic lymphoma kinase (ALK) and ROS-1 rearrangements are now considered by many to be the standard of care in advanced-stage pulmonary adenocarcinomas. Personalized precision medicine is real, and is here, and having a firm grasp of the processes involved in molecular testing of tumour specimens should be an overriding concern for the pathologist. As more sophisticated and sensitive testing modalities evolve, it behoves the astute practicing pathologist to remain ‘future-proof’ and relevant in facing the challenges ahead.

PLENARY LECTURES

PL1. Ebola in Transfusion

Diana Teo

Senior Director at the Blood Services Group (BSG) of the Health Sciences Authority (HSA) and Chairman of the HSA Professional Board

Ebola Virus Disease (EVD) was first reported in 1976, with 25 outbreaks occurring since then. In March 2014, the World Health Organization was notified by Guinea of a rapidly evolving outbreak in West Africa. As of 8 March 2015, a total of 24,282 cases with 9,976 deaths have been reported in 9 affected countries. Although the spread of Ebola virus infection has primarily occurred within West Africa, there have been a number of importations to countries outside the affected region.

Although the extent of viraemia during asymptomatic and pre-symptomatic stages of infection is little known, the likelihood of transmission by transfusion of blood collected from healthy asymptomatic donors is assessed to be low. The impact of EVD on the blood supply is more likely if there is an outbreak in the community, which will result in significant drop in donor attendance and the potential for disruption of blood service operations. In such situations, the blood service will need to implement its contingency plans for infectious disease outbreaks.

Treatment is currently based on supportive care and hospitalization in an intensive care unit, and will include blood and blood component support for patients who develop haemorrhagic manifestations and disseminated intravascular coagulation. There is a theoretical risk that biological samples sent to the laboratory for testing may contain Ebola virus, and several countries have developed guidelines for handling samples and procedures to be followed for EVD patients requiring blood transfusion.

Several experimental therapeutic options are under study and development, which include the use of convalescent plasma and hyperimmune globulin prepared from EVD survivors. The use of convalescent whole blood and plasma is considered an empirical treatment until more definitive and targeted therapies are available, which has spurred the development of appropriate strategies and protocols for this treatment modality.
PL2. Antibiotic resistance in Enterobacteriaceae

Sally Partridge

Centre for Infectious Diseases and Microbiology at Westmead Hospital, Sydney

Enterobacteriaceae are responsible for a large proportion of serious, life-threatening infections and resistance to multiple antibiotics in these organisms is an increasing global public health problem. In many countries isolates resistant to almost all, or even all, available antibiotics are becoming increasingly common. Resistance to third-generation cephalosporins in these organisms is considered a ‘serious’ problem and resistance to carbapenems an ‘urgent’ threat.

While mutations in chromosomal genes contribute to antibiotic resistance in these organisms, the Enterobacteriaceae are adapted to sharing genetic material and much important resistance is due to ‘mobile’ resistance genes. These genes have been captured from various source organisms, often those found in the environment, where they may originally have had other functions. If transferred to plasmids, these resistance genes are then able move ‘horizontally’ between different bacterial cells, including different species, and well as being transferred ‘vertically’ during cell division.

Many different families of mobile genes conferring resistance to each class of antibiotic have been identified, complicating detection of the factors responsible for a particular resistance phenotype. Antibiotic use also may select for variants that have acquired an ‘extended-spectrum’ of resistance, which may be due to just a single amino acid change. Understanding the mechanisms of antibiotic resistance, and the means by which these mechanisms can evolve and disseminate, is important for developing ways to efficiently track the spread of resistance and to optimise treatment.

PL3. Comprehensive drug screening in the clinical laboratory by liquid chromatography mass spectrometry

Chung-Shun Ho

Department of Chemical Pathology, The Chinese University of Hong Kong

The decision of Bio-Rad to discontinue world-wide support of the REMEDI Drug Profiling System necessitated its replacement in 4 Hong Kong hospitals in 2007. In collaboration with laboratories in Denmark and the United Kingdom, a method for comprehensive urine drug screening was developed using liquid chromatography and time-of-flight mass spectrometry. Identification was achieved by comparison of retention time and spectral fragmentation data to a locally prepared library containing ~300 drugs and metabolites. Accurate mass measurement and isotope distribution pattern allowed the prediction of probable elemental composition and conferred additional confidence to the proposed drug candidates by the software. The parallel run study using 1000 routine patient samples showed this time-of-flight method was able to detect more drugs and metabolites in routine patient urine samples and demonstrated significant improvement over the REMEDI system.

Recently, a more sensitive time of flight mass spectrometry system with a commercial turn-key library of >1000 drugs and metabolites has been adopted for routine urine comprehensive drug screening at the Prince of Wales Hospital. The improved analytical performance over the previous system due to new instrument design and software will be discussed. Furthermore, the performance of tandem mass spectrometry systems for comprehensive drug screening reported in the literature will also be reviewed.

SYMPOSIA PAPERS

Symposium 1A – Anatomical Pathology

S1Aa. Recent Advances in Immunohistochemistry

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Immunohistochemistry (IHC) has been introduced to surgical pathology by Coon et al. in 1941 by using immunofluorescence to detect Pneumococcal antigen in the tissue. Since then the method was developed to use protein conjugate and enzymatic reaction to localize antigen in the paraffin embedded sections. The techniques have been improved from low sensitivity & specificity to high sensitivity & specificity. Applications of IHC include research, diagnostic and therapeutic purposes. New antibodies are expanded endlessly. Many more antibodies become commercially available. IHC has been applied to all specialties in pathology. Examples of new antibodies for diagnostic purposes are INI1 (negative expression in malignant rhabdoid tumor and other soft tissue sarcomas), JAK2V617F (in myeloproliferative neoplasms), PD1 (in angioimmunoblastic lymphoma), Sox11 (in mantle cell lymphoma), MDM2&CDK4 in osteosarcoma, bcl-3 (cytoplasmic localization in colon cancer, etc. Examples of new antibodies for therapeutic purposes are CD30 (CD30+ lymphoma), CD44v/CD44s (prognosis in pancreatic carcinoma), PD1 Ligand (lung carcinoma, lymphoma), BIRC6 (prognosis in colorectal cancer), etc.

Another area of advancement is staining technique. Automated staining is widely used and many can cover all steps from deparaffinization to coverslipping. More sensitive methods using tyramide or polymer are developed. Microwave-stimulated technique becomes generally accepted. Double stain is very useful and able to be performed in automated stainer.
Quantification of IHC signals is another area of interests. Few scoring methods such as Kline, Allred, and IRC, are introduced and often used in researches, though not completely perfect. Digital image analysis is another effort of scientists to analyze morphometry in IHC images.

Last but not least, quality assurance in IHC is a critical subject of improvement. Quality control of IHC laboratory together with external quality assessment is necessary for all IHC labs to warrant the correct interpretation, both qualitative and quantitative assessment.

S1Ab. The 2015 WHO Classification of Lung Tumors

Nor Salmah Bakar

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The 2015 WHO classification is largely driven by the molecular revolution and therapeutic breakthroughs. Major changes in this classification include the introduction of immunohistochemistry and the genetic testing (EGFR mutation and ALK rearrangement) which also applicable on small biopsy specimens. Therefore tumours previously classified as non-small-cell carcinoma, not otherwise specified based on morphology alone should be classified further by using immunohistochemical panel. A limited immunohistochemical workup is recommended to preserve tissue for molecular testing. Other changes include the terms “bronchioloalveolar carcinoma” and “mixed subtype adenocarcinoma” have been discontinued in this classification. Former mucinous bronchioloalveolar carcinomas are now called “invasive mucinous adenocarcinomas”. As for resected adenocarcinomas, new concepts of adenocarcinoma in situ and minimally invasive adenocarcinoma are added. These tumour categories, if they undergo complete resection, will have 100% disease-free survival. Invasive adenocarcinomas are now classified by predominant pattern after using comprehensive histologic subtyping with lepidic, acinar, papillary, and solid patterns; micropapilllary is added as a new histologic subtype with poor prognosis. This classification of lung tumours provides standard for pathologic diagnosis not only for patient care but also for clinical trials and TNM classification.

S1Ac. Anatomical Pathology Laboratory in the 21st century

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For more than 100 years, principle of pathology slide preparation has not been changed, starting from gross cutting, fixation, dehydrating, clearing, paraffin infiltration, embedding, microtome cutting, staining and coverslipping. Formalin fixative becomes more accepted since introduction of immunohistochemistry and molecular pathology. Researches demonstrated that the antigenicity and DNA quality are lost more in the mercury-based fixatives than in formalin fixative. Non-formalin fixative has been commercially introduced to avoid formalin toxicity but still not popular. For tissue processing, microwave stimulation is adopted in the automated machine. Xylene substitute has long been introduced but still not widely used probably due to high cost. JFC fixative solution is a special formula invented to replace multisteps in dehydration and clearing. H&E automated staining strategy has been developed to use fresh reagent in every slide. In addition, tracing system is more perfect than before. This helps the lab to do quality control easily monitored.

Nowadays the anatomical pathology laboratory needs to be quality accredited using local or international standards. Generally the standards cover management and technical aspects. Specimen identification/tracking/storage, report delivery and waste control become important issues in quality management. External quality assessment or proficiency testing turns out to be essential part of quality control in anatomical pathology laboratory. This includes staining quality and diagnostic accuracy. Pathologists have to familiarize with the quality system. Pathology residency training program should take this concern and include this topic in the education program.

Symposium 1B – Microbiology

S1Ba. Resistance gene mobility mechanisms

Sally Partridge

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Resistance to antibiotics among the Enterobacteriaceae is often due to ‘mobile’ genes captured from the chromosome of different bacterial species by mobile genetic elements (including insertion sequences, transposons and integrons). Each resistance gene is generally always seen in close association with the same mobile element, suggesting that capture events are relatively rare. Transfer to conjugative (or mobilisable) plasmids allows resistance genes to move between bacterial cells, including those of different species.

The different characteristics of these different mobile elements may influence the spread of the resistance genes that they have captured. However, as mobile elements and associated resistance genes are often found clustered together in complex
multi-resistance regions on plasmids, other mobile elements can influence subsequent spread. Multi-resistance regions may evolve by rearrangements, insertions and/or deletions and recombination between elements acting as mobile regions of homology may contribute to this. Although increasing numbers of DNA sequences relating to multi-resistance are becoming available, those in databases are often poorly annotated and analysed. Nomenclature issues, and the fact that available analysis programs generally focus on identifying open reading frames and functions of putative proteins by homology to known proteins, both contribute to this. Meaningful comparative analysis to help us understand how multi-resistance evolves and spreads requires consistent nomenclature and the correct identification of well-characterised resistance genes and of the boundaries and fragments of mobile elements.

S1Bb. Emerging Infectious Disease: MOH Malaysia Preparedness and Response
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Emerging infectious disease represent an ongoing threat to the health and livelihoods of people everywhere, including those of Malaysians. Over the last few decades, there have been several emerging infectious diseases that have taken the global community by surprise and drawn new attention to emerging infectious diseases, including SARS, Pandemic H1N1, Middle East Respiratory Syndrome Coronavirus (MERS-CoV), Avian Influenza A(H7N9) and recently Ebola. While not every newly identified infectious disease has major public health implications, a few have resulted in global pandemics.

A guiding framework for global efforts is the revised International Health Regulations (IHR 2005), a legally-binding international agreement among member-states of the World Health Organization (WHO) that requires countries to develop a minimum level of capacity to “detect, assess, notify and report” potential outbreaks and other public health emergencies and that outlines the processes for reporting, investigating, and responding to these threats at the international level. Therefore, the successful implementation of the IHR (2005) requires a strong national public health system, which is critical for response to a public health emergency of international concern (PHEIC). The Asia Pacific Strategy for Emerging Diseases (APSED) was developed in 2005 to serves as a road map to guide all countries within the WHO South East Asia and Western Pacific regions in building the IHR (2005) core capacity requirements, thus ensuring regional and global health security. Malaysia Strategy for Emerging Diseases (MySED) Workplan, 2012-2015 was formulated as part of Malaysia’s continuing commitment towards meeting the IHR (2005) core capacity requirements, thus ensuring national, regional and global health security. Development and implementation of MySED Workplan 2012-2015 involves multisectoral collaboration with the relevant stakeholders and it has been tested during the first case of Avian Influenza A (H7N9) and MERS-CoV in Malaysia in year 2014.

The Ministry of Health Malaysia has enhanced the preparedness and response from time to time to address the threat posed by emerging infectious disease including enhancing networking and collaboration with other countries. Most recently Malaysia has participated in the Global Health Security Agenda (GHSA) and was mandated as the lead country in the Action Package pertaining to Emergency Operations Centre (National Crisis Preparedness Response Centre –National CPRC). All the efforts are to accelerate the progress toward a safe and secure from infectious disease threats at national as well as at the regional and international level.

S1Bc. Molecular methods: the future in clinical practice
Ng Kee Peng

Molecular diagnostics has revolutionized the health care system. It detects specific sequences in DNA or RNA or proteins associated with a person’s risk of developing a disease or a hereditary condition; screen for diseases and the diagnosis of existing symptoms. The number of commercially available molecular assays is increasing; the current uses of molecular assay can be found in infectious diseases, oncology, pharmacogenomics, genetic disease screening, human leukocyte antigen typing and coagulation.

The clinical application of molecular diagnostics is expanding rapidly in the country. The notable impact of molecular assay on optimally managing patients through the continuum of care is the quantitative HIV, HBV, HCV and CMV viral load testing. The molecular screening of methicillin-resistant *Staphylococcus aureus*, *Clostridium difficile* or Vancomycin-resistant enterococci has made huge impact on hospital infectious disease surveillance. The Multiplex PCR system amplifies multiple targets in a single PCR reaction provides a unique new platform replacing the conventional immunofluorescence staining and culture methods currently used in many Microbiology laboratories.

The presentation will focus on the applications of molecular assay in infectious disease highlighting the cost, benefit; and the limitation of the molecular diagnostics.
Symposium 2A – Haematology

S2Aa. Patient Blood Management

Diana Teo

Senior Director at the Blood Services Group (BSG) of the Health Sciences Authority (HSA) and Chairman of the HSA Professional Board

The safety and efficacy of blood transfusion is based not only on the safety and quality of the blood or blood product (“blood safety”) transfused, but on the safety and quality of the processes used to deliver the blood to the recipient. This includes ensuring that blood transfusion is appropriate to the patient’s clinical need, that its benefits outweigh the risks, and that treatment options that are able to lower the risk or reduce the likelihood of transfusion have been considered.

Focus has therefore moved beyond blood conservation involving perioperative blood recovery (autologous) and transfusion triggers, to include issues such as identifying anaemia before elective surgery, use of point-of-care testing to guide transfusions, and use of pharmacologic agents to minimise bleeding. Thus, the practice of Patient Blood Management (PBM) is multidisciplinary, and requires treating each patient who may require a blood transfusion as an individual.

Measures used as part of PBM include: optimising pre-surgical haemoglobin; reducing phlebotomy loss; making evidence-based haemotherapy decisions; using perioperative autologous donation and red cell recovery techniques; minimising perioperative blood loss with topical haemostatics and sealants, ancillary techniques; and review of blood utilisation through auditing.

The introduction of PBM in the USA, Australia, New Zealand and many European countries has resulted in a significant reduction in transfusion needs. In Singapore, a national PBM programme was launched in 2013 following its adoption and endorsement by the healthcare leadership at the Ministry of Health and public sector hospitals. PBM concepts were introduced in hospitals through Hospital Transfusion Committees, and reinforced through educational programmes involving international and local experts. Public hospitals have now embarked on PBM initiatives, and the programme is being extended to private hospitals.

S2Ab. Molecular challenges in diagnosis of thalassaemia

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The inherited disorders of haemoglobin synthesis thalassaemia and thalassaemia-haemoglobinopathy are the most common autosomal recessive disorders in the world. High frequencies are found in areas endemic with malaria: in Southeast Asia, Southern China, India, Pakistan, Middle East, Africa and the Mediterranean. Clinically thalassaemia presents as 3 phenotypes: trait, intermedia and major. The molecular defects are targeted to globin chain synthesis: affecting specifically the globin genes of human haemoglobin including the locus controlling region (LCR) and UTR sites. Both point mutations and deletions are seen. Each ethnic group has 4-5 common mutations and some rare mutations. The mutations that cause thalassaemia in Malaysia have been elucidated over the last 30 years. Molecular diagnosis for thalassaemia is now available in number of centres. Over 17 beta globin molecular defects have been described: -29(A to G), -28(A to G), CAP site +1 (A to C), CD 8/9 (+G), CD 17 (A to T), CD 19 (A to G) or Hb Malay, CD 26 (G to A) or Hb E, IVS 1-1 (G to T), IVS 1-5 (G to C), CD 41-42 (-TCTT), CD 71-72(+A), IVS 2-654 (C to T), polyA (A to G), 100 kb 5'g3db3 and 45-kb Filipino deletion. The common mutations seen in the Malays being IVS 1-5 (G to C) and Hb E, in the Chinese CD 41-42 (-TCTT) and IVS- 654 (C to T), and in the Kadazanduzun of Sabah, the Fil deletion. In alpha thalassaemia, both deletion and non-deletion thalassaemia are see. The a0 deletion (--SEA, --FIL, --MED, -20.5 kb) and a+ deletion (-3.7 and -4.2) where in Malaysia, the a0 deletion (--SEA) and a+ deletion (-3.7 and -4.2) where in Malaysia, the a+ deletion (--SEA) and a+ deletion (−3.7) are most common. The non-deletion mutation seen are Hb Constant Spring, Hb Quong Tze, Hb Pakse and Hb Adana. The molecular methods that identify these mutations depend on whether they are point mutations or deletions. Mutation screening is by denaturing high performance liquid chromatography (D-HPLC) and restriction fragment polymorphism (RFLP). Specific molecular tests for point mutations include amplification refractory mutation system (ARMS) and reverse dot blot hybridization (RDBH) and for deletions GAP-PCR. Sequencing is used to identify unknown mutations. Errors in diagnosis may occur in compound heterozygous states. Patients with HbE and Fil deletion may erroneously be labelled as homozygous Hb E with ARMS and RDBH methods. Sequencing is not able to determine if a mutation is heterozygous or homozygous. In conclusion, the diagnosis of thalassaemia requires correlation of the clinical status and mutations identified. Patients may have more than one globin gene affected and limitation of molecular methods used need to be defined.
S2Ac. Molecular advances in haemostasis disorders

Faraizah Haji Abdul Karim

Deputy Director and the Head of the Haemophilia Centre at the National Blood Centre in Kuala Lumpur

Most bleeding disorders encountered in clinical practice will be diagnosed, at least initially, by phenotypic assays. Molecular genetic testing, for the haemophilias, in particular, is aimed to determine the carrier status, prenatal diagnosis and prediction of the likelihood of inhibitor development. For von Willebrand’s disease (VWD), significant recent advances have allowed for the establishment of genotype-phenotype correlations that have improved the understanding of the disease. Approach for molecular diagnosis includes screening the target genes for common mutations first, followed by point mutation screening by PCR and conformation sensitive gel electrophoresis and DNA sequencing strategy. Linkage analysis has also been established for some diseases such as hemophilia A, hemophilia B and Glanzmann Thrombasthenia. Molecular diagnosis for rare coagulation disorders has also been established such as Mutation analysis of Fibrinogen FI, FII (Prothrombin), Combined Factor V and VIII deficiency, Factor VII, Factor X, Factor XI, Factor XIII deficiency.

Symposium 2B – Chemical Pathology


Paul E.C. Sibley

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Learning Objectives:
- Introduction of the importance of Vitamin D in Malaysia
- Reference values recommended
- Factors which affect Vitamin D status
- Need to routinely measure Vitamin D
- Clinical areas affected by insufficient Vitamin levels
- Standardization of Vitamin D measurements

Many publications have indicated that Vitamin D insufficiency or deficiency is prevalent throughout the world. Vitamin D helps regulate calcium and phosphorus in the development and maintenance of healthy bones. There are a number of ways to obtain sufficient levels of Vitamin D in the circulation, including sunlight exposure, diet or supplementation. Recent studies have indicated a high prevalence of Vitamin D insufficiency in Malaysian children and a significant proportion of Malaysian men have Vitamin D insufficiency. In addition, Malaysian women living in urban areas had significantly lower Vitamin D status compared to those living in rural areas.

The classical areas for the impact of Vitamin D deficiency include bone health, rickets and skeletal abnormalities and there have been many reports of associations between Vitamin D levels and cardiovascular disease, cancers, diabetes, glucose intolerance, multiple sclerosis, neurological disease and autoimmune disease. It has become increasingly important to know the status of Vitamin D at all ages using methods that produce standardized results. In addition, once a patient has been assessed as being deficient or insufficient, routine testing will determine whether a correct level of Vitamin D supplementation has been prescribed.

The National Institutes of Health Office of Dietary Supplements created the Vitamin D Standardization Program (VDSP) to establish a standard for accurate and comparable results for the detection of 25(OH)D across laboratories and the Centers for Disease Controls (CDC) established a Vitamin D Standardization-Certification Program (VDSCP) utilizing the University of Ghent Vitamin D, and D, Reference Measurement Procedure (RMP). The ADVIA Centaur® Vitamin D Total assay met the criteria for VDSCP certification, which establishes an acceptable alignment to a harmonized testing standard for 25(OH) D. The ADVIA Centaur Vitamin D Total assay provides laboratories with a standardized and automated means for quickly and efficiently testing patients’ 25(OH)D levels.

S2Bb. From immunoassay to mass spectrometry: experience with immunosuppressants TDM

Chung-Shun Ho

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Before 2005, therapeutic drug monitoring (TDM) of whole blood Cyclosporin A (CsA) service in the Prince of Wales Hospital was routinely performed using Abbott TDx immunoassay, which was expensive and suffered from interference by cross-reacting metabolites. To overcome these shortcomings, measurement of CsA was transferred to a liquid chromatography tandem mass spectrometry (LCMS/MS) method. The LCMS/MS method had improved analytical performance and lower consumable cost over the TDx method. To familiarize transplant clinicians with the new CsA results, results from both methods were parallel-reported for 6 months. To establish new therapeutic ranges, pharmacokinetic study was conducted
with 158 stable renal transplant patients. New C0 and C2 therapeutic ranges for the LCMS/MS method were adopted for the routine service. Experience over the last 10 years on practical considerations in the implementation of whole blood immunosuppressants TDM service on LCMS/MS platform will be shared.

S2Bc. Pheochromocytoma and Paraganglioma: Laboratory evaluation

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Adrenal pheochromocytomas and extra-adrenal sympathetic paragangliomas (PPGLs) are rare neuroendocrine tumours, characterised by production of the catecholamines: noradrenaline, adrenaline and dopamine. Tumoural secretion of catecholamines determines their clinical presentation which is highly variable among patients, therefore, once clinical suspicion is aroused it is imperative that clinicians choose the most appropriate laboratory tests to identify the tumors.

The first step in diagnosis is the proper biochemical analysis to confirm or refute the presence of excess production of catecholamines or their metabolites. Biochemical testing is not only indicated in symptomatic patients but also in asymptomatic patients with adrenal incidentalomas or identified genetic predispositions.

Measurements of metanephrines in plasma or urine offer the best diagnostic performance and are the tests of first choice. Paying attention to sampling conditions, patient preparation and use of interfering medications is important, as these factors can largely influence test results. LC-MS/MS offers numerous advantages over other analytical methods and is the method of choice when measurements include methoxytyramine, the $O$-methylated metabolite of dopamine. The plasma test offers advantages over the urine test, although it is rarely implemented correctly, rendering the urine test preferable for mainstream use.

When initial test results are inconclusive, additional tests can be performed, such as the clonidine suppression test. Test results can also be used for estimation of tumour size or prediction of tumour location and underlying genotype. To ensure optimum diagnostic sensitivity for the plasma test, reference intervals must be established for blood samples collected after 30 min of supine rest and after an overnight fast when measurements include methoxytyramine. Similarly collected blood samples during screening, together with use of age-adjusted reference intervals, further minimize false-positive results.

Symposium 3A – Molecular

S3Aa. Molecular pathology of lymphoma

Noraidah Masir

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Lymphomas encompass a wide spectrum of disorders with varying clinical and biologic features. Based on the WHO Lymphoma classification (2008), there are more than 50 lymphoma entities, each with its own distinct behaviour. The appropriate diagnosis and classification is therefore pertinent in patient management decision and choice of treatment protocol. Current diagnostic strategies include molecular studies in addition to histomorphological assessment and immunohistochemistry. Developments in molecular pathology have enhanced our understanding of lymphomas pathogenesis. Molecular techniques are of increasing practical importance as they provide an additional level of testing. PCR-based clonality analysis and detection of chromosome aberrations by FISH support the diagnosis and classification in difficult cases. Gene expression profiling and more currently next generation sequencing methods have detected distinct molecular lymphoma subtypes. The basic molecular biology in lymphomagenesis, common molecular aberrations, and the application of molecular diagnostics as a key ancillary tool in the diagnosis and classification of lymphoma will be discussed.

S3Ab. Advance Molecular Techniques in Thalassaemia Diagnosis

Suthat Fucharoen

Institute of Molecular Biosciences, and Director of the Thalassemia Research Center, Mahidol University, Nakornpathom, Thailand

The molecular basis of both alpha and beta thalassaemia is well known. Alpha thalassemia is often caused by gene deletions of various lengths that may remove one or two linked alpha globin genes. Non-deletional defects are found with a lower frequency. The molecular defects that cause beta thalassemia are more heterogeneous. The most common are single point substitutions that produce defects in transcription, RNA splicing, or translation (via frameshifts or nonsense codons), resulting in decreased mRNA or unstable mRNA.

Molecular diagnosis can be defined as a discipline in which clinical conclusions are made from the analysis of nucleotide sequence information derived from the patient’s sample. PCR-based techniques are effectively used to identify known and unknown thalassemia mutations. DNA diagnosis provides an invaluable tool for screening of the high risk couple, genetic
counselling and prenatal diagnosis. The information also helps thalassemia patients have proper management, which is part of prevention and control program of thalassemia worldwide. Many DNA techniques have been used for point mutation detection. For the last few years there is a development of DNA chip technology and DNA MassArray has been developed to address all these issues effectively, and will therefore play a key role in future genetic profiling.

Real-time PCR with different color probes and high resolution melting (HRM) analysis has been developed for the diagnosis of alpha and beta-thalassemia. The techniques have been applied for PND in many thalassemia disorders. HRM analysis is simple, high-throughput, and is faster than the conventional PCR since it does not require the post-PCR processing steps and is more cost-effective if it could be processed as a multiplex PCR for diagnosis of several types of thalassemias. Multiplex Ligation-dependent Probe Amplification (MLPA) has also been developed to characterize large rearrangements in the alpha- and beta-globin gene cluster. Several new deletions and duplications were found by this technique.

By using the MALDI-TOF/TOF (ABI 4800) and high resolution nano flow LC/MS (LTQ Orbitrap) we have identified proteolytic peptides from α globins with stop codon mutations. The sequence of these peptides matched with the C-terminal fragments of the elongated alpha globin chain. They have been identified in all samples carrying the Hb Constant Spring (CS) or Pakse allele, including heterozygotes and CS double heterozygotes with Hb E.

The identification of different thalassemia syndromes can be revealed by the ratio of intensities between alpha/beta-globin chains and alpha/beta-mRNA ratios. However, none of these tests can accurately diagnose specific thalassemia genotype. All of these techniques have some advantage and disadvantage. We highly recommend all service labs to use the technique(s) they are most familiar with and most economic one for their daily use.

S3Ac. Molecular and Genomics-Based Diagnostics for Medical Microbiology

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Over the years, traditional clinical microbiology practice has relied on phenotypic and biochemical characterization of microbes for definitive diagnosis of an infectious agent. Nevertheless, it is estimated that between 10 and 20% of clinical isolates are novel organisms that challenge phenotype-based identification, resulting in identification errors of rarely isolated or phenotypically atypical strains. To move clinical microbiology into a dynamic age, molecular diagnostics techniques should be favoured because they do not require culture, they have rapid turnaround times, and digital genetic information can be stored in a database for epidemiological studies. Tremendous technological advances in molecular diagnostic techniques, such as polymerase chain reaction–based amplification techniques and whole-genome sequencing (WGS) as well as mass spectrometry, have propelled genomics and molecular diagnostics and revolutionized data acquisition. This talk will summarize some of the more recent technologies and applications in clinical microbiology and how genomics is changing the future of medical microbiology.

Symposium 3B – Forensic Pathology

S3Ba. The confidential enquiry into maternal deaths: Role of the pathologist

Bhupinder Singh

Department of Pathology, Penang General Hospital

The Confidential Enquiries into Maternal Deaths in Malaysia (CEMD) was established in 1991. Maternal mortality remains a critical and sensitive indicator of the state of our health delivery system and as such maternal deaths review have become an essential component of any maternity service. With our multiracial and multicultural society the need to increase the awareness and acceptance of post-mortem examination cannot be over emphasised.

Surprisingly very few pathologists are aware of the existence of the confidential enquiry into maternal deaths. Maternal autopsies are required for accurate death certification, determination of underlying causes of death, as well as maternal mortality rates. Although there is a real need for routine post-mortem examinations in all cases of maternal deaths, various legal, social and religious factors stand in the way.

Autopsy is an important investigation tool despite the introduction of new diagnostic techniques and a meticulous attitude and patience are the keys to a comprehensive examination. Since the viability and success of an enquiry will depend on a complete autopsy, histopathological and other laboratory examinations, the preferred standard for the investigation of maternal deaths is to have a post-mortem examination conducted by an experienced pathologist. It is prudent to examine every maternal death, determine the underlying factors and correct substandard practices that contribute to death.

In situations where a pathologist is not available, autopsies can be carried out by trained medical officers, who should consult the forensic pathologist or the general pathologist in the area before conducting the autopsy. Photographs should be taken during the autopsy for further review, assessment and diagnosis. Training of adequate pathologists in this field is essential if we want a high standard of autopsies to improve the health care delivery system in the future.
S3Bb. CT scan: A complimentary approach to maternal autopsy?
Mohamad Helmee bin Mohamad Noor

Forensic Radiology Unit, Hospital Kuala Lumpur

Since 1895 upon the discovery of radiography, it has been used in court to aid as complimentary or supportive evidence of premorbid condition of decedents and in assisting the evaluation of injury namely bone injury in blunt and ballistic injury and for localization of metallic fragments and foreign bodies. However, diagnostic imaging is still underused in forensics, mainly due to unawareness of its potential.

Cross sectional imaging with advanced technologies have made it possible for CT to be used as adjunct examination with forensic autopsy. It has significant contribution for forensic autopsy as it will be more effective and brings the potential to reduce the time factor and to be more anatomically focus and internal organs targeted on each forensic autopsy cases by simply allowing the radiologists and forensic pathologist to view anatomy without dissection. In United States of America, that in certain causes of death and forensic scenarios, it is possible that cross sectional imaging may serve as a triage technique to help forensic pathologists decide which decedents should have limited or complete forensic autopsy.

However, there are short falls in postmortem Ct scan imaging as it is mostly done in unenhanced examination. Furthermore, there is postmortem Ct changes causing artifacts due to decomposition which should be recognized and not be mistaken for a pathologic process or injury. This process which includes putrefactive gas, hemoconcentration from livor mortis and rigor mortis which occurs upon death is able to be visualized in post mortem Ct scan imaging. The post mortem changes and decomposition are important findings on post mortem Ct scan because they may obscure soft tissue injury or pathology, thereby limiting the Ct assessment for soft tissue for hemorrhage, laceration and wound tracks. Therefore, all post mortem Ct examinations must be followed with the forensic autopsy examinations as the gold standard.

There is a new challenging area of post mortem Ct research with regards to maternal autopsy, taking into consideration with above mentioned limitations and time the decedent body scanned. The advance technologies also made it is possible for better visualization of vessels in angiography of postmortem Ct.

Post mortem Ct imaging is reliable in forensic autopsy setting, and the combination of postmortem Ct imaging and forensic autopsy could compliment both of the fields in determining causes of maternal death.

S3Bc. A lesson learned: Investigation of maternal deaths involving private healthcare providers – A forensic perspective.

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According to the Criminal Procedure Code of Malaysia, the investigation of deaths is the duty of police officer. The system is centred to criminal investigation and conventional classified the cases into sudden and criminal deaths. Most of the maternal, surgical and hospital procedural deaths are belonged to the former category of the police investigation. The medical information provided by police officer is usually inadequate for assisting the autopsy and interpreting its findings. The forensic pathologists face a unique challenge in the investigation of maternal deaths occurring in private healthcare providers. Full clinical documentation is usually unavailable for review prior to the autopsy. Element of dissatisfaction is common among the next-of-kin ranging from sad, anger to an extent that next-of-kin lodge the police report for forensic autopsy. In few instances, the immediate next-of-kin were so shock and disbelief of the sudden dismissed of the loved ones; and unable to accept the fact. They came to us for emotional support of their helplessness.

POSTER PRESENTATIONS

P-AP1. Tuberculosis of the Prostate

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Introduction: Genitourinary system is the second most common site of tuberculosis (TB) after pulmonary system. Tuberculosis of the prostate is a rare manifestation of genitourinary tuberculosis. It is even more uncommon if occurring in an immune-competent individual. Prostate TB is usually an incidental finding in transurethral resection of prostate. Case Report: Here, we report a case of TB of the prostate in a 70-year-old man who was admitted for acute urinary retention, accompanied by a history of haematuria for three days. There was no history of fever, cough or weight loss. Ultrasound revealed an enlarged prostate that measured 6 x 6 x 6 cm. Transurethral resection of the prostate was done. TB was diagnosed incidentally post-TURP. Histopathological examination of the prostate revealed caseous granulomatous inflammation with benign prostatic hyperplasia. Ziehl Neelsen stain showed presence of acid fast bacilli, confirmed the diagnosis of tuberculosis. Discussion: TB of the prostate is almost always the result of one or repeated hematogenous seedings. Direct extension may also occur.
The predisposing factors associated with the development of TB are related to poor immune status such as long-term use of steroid, the use of immunosuppressive drugs and diseases that impaired cell-mediated immunity.

**P-AP2. The effects of intrauterine infection by *Gardnerella vaginalis* in a fetal rabbit model**

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Introduction: *Gardnerella vaginalis* (GV) is an anaerobic, non-motile, Gram variable cocco-bacillus. It is a vaginal commensal common in women of reproductive age. Most commonly recognized for its role as one of the organisms responsible for bacterial vaginosis. Effect of *Bacterial Vaginosis* in pregnancy includes chorioamnionitis, deciduitis, IUGR, premature delivery and invasive neonatal infection such as meningitis and pneumonia. The aim of this study was to develop a fetal rabbit model using GV as the cause of intra-uterine infection in order to study the outcomes of this bacterial infection, to the fetus and placenta. Methods: A total of 21 fetal rabbits were assessed. Laparotomy and inoculation of 0.5ml of 10^2 GV were performed on New Zealand white rabbits at 21 days of gestation and waited for 7 days. Subsequently, the weight of fetal rabbit was determined, meanwhile lung and placenta tissues were harvested for further analysis. Results: The average weight of GV infected fetal rabbit was lower than control group (p=0.001). The average weight of the lung and placenta of GV infected fetal rabbit was also lower than the control group (p=0.001 and p=0.027). There was a significant increase in the number of multinucleated giant cells in the placenta of GV infected fetal rabbit than the control group (p<0.001). Discussion: This is the first model of GV infected fetal rabbit using intrauterine infection method. In conclusion, mild degree of intrauterine GV infection in fetal rabbit causes chronic inflammation with giant cells that retards the growth of fetus and placenta.

**P-AP3. Effects of Recombinant soluble form of Heparin-Binding Epidermal Growth Factor-like growth factor protein (rsHB-EGFp) on necrosis and apoptosis of liver**

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Introduction: It was reported that Heparin-Binding Epidermal Growth Factor-like growth factor protein (rsHB-EGF) is rapidly increased after partial hepectomy and suggested that it is a hepatotropic factor. In the liver, Fas stimulation causes hepatocytes apoptosis and acetaminophen (APAP) induced necrosis. In this study we investigated the effects of rsHB-EGF on both apoptosis and necrosis of liver. Material and Methods: 5- to 6-week-old male C57BL/6j mice (n=8 per group) were administered 3 intraperitoneal injections of 100 µg/mouse rsHB-EGFp at 6 and 0.5hours before and 3 hours after intraperitoneal injection of 4 µg/mouse of an agonistic anti-Fas antibody (4Fas-ip) or APAP 300 mg/kg after overnight fasting for 15-16 hours. Results: Twenty-four hours after administering of anti-Fas antibody, the serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) levels were remarkably increased in the control mice (2853±814, and 1817±469 respectively) , but were drastically attenuated to normal levels in the rsHB-EGFp-treated mice (ALT, 21±5; AST, 28±4). Moreover, immunoblotting showed reduced expression of pro-apototic protein Bax. But remarkably high liver enzyme levels were seen in both treatment and control groups of APAP injection. Accordingly, all of the control mice had histopathological liver injury, including apoptosis, while none of the rsHB-EGFp treated mice had histopathological findings of liver injury in Fas-treated group. In APAP injected mice, both control and rsHB-EGFp treated groups showed massive centrilobular necrosis. Discussion: Although rsHB-EGFp attenuated Fas-induced apoptosis, it has no effect on APAP-induced necrosis in liver.

**P-AP4. Primary Intra-Osseous Squamous Cell Carcinoma Arising from Odontogenic Cyst: Case Report**

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Introduction: Primary intra-osseous squamous cell carcinoma (PIOSCC) is a rare tumour which occurs centrally within the jaws. It is thought to arise from remnants of odontogenic epithelium or from pre-existing odontogenic cysts/tumours. Case Report: We present a case of PIOSCC in a 57 year old female who had a complaint of pain and swelling over the posterior part of the right side of the mandible. A few years back she was informed that there was a cyst in the mandible but it was not removed as it was asymptomatic. On panoramic radiography, there was an ill-defined radiolucency over the right side of the mandible suggestive of an odontogenic lesion. Excision of the lesion was performed under general anaesthesia. Diagnostic histopathology revealed the presence of an invasive carcinoma arising from the walls of the odontogenic cyst. A diagnosis of PIOSCC arising in a pre-existing odontogenic cyst was made. Patient then underwent segmental mandibulectomy
and is currently under follow up. Discussion and conclusion: Long standing odontogenic cysts have the potential to undergo malignant transformation though this may not always be the case. Relying only on radiographic findings for the management of odontogenic cysts without obtaining histopathological diagnosis is extremely ill-advised.

P-AP5. Kimura’s Disease: A Case Report
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Introduction: Kimura’s disease is thought to be a chronic inflammatory condition of uncertain aetiology that is rare. However, it does have an affinity for people of Asian descent. It usually involves the head and neck region, commonly presenting either as a deep subcutaneous masses or painless lymphadenopathy. Case report: We report a case of Kimura’s disease in a 9-year-old Asian male who presented with a subcutaneous mass over the left cheek area with cervical lymphadenopathy. Results from diagnostic imaging were suggestive of an aggressive soft tissue tumour. Blood tests revealed peripheral blood eosinophilia. Histopathological examination of an incisional biopsy was suggestive of Kimura’s disease. Oral corticosteroids were given for a two-week duration and the lesion was seen to reduce in size. The patient is currently under follow up with the pediatric haematology unit. Discussion & Conclusion: Diagnosis can at times be difficult as there can be some confusion with another condition, angiolymphoid hyperplasia with eosinophilis (ALHE), which has some overlapping features with Kimura’s disease. The two are however distinctly different pathological processes that can be distinguished on the basis of histopathological as well as clinical characteristics.

P-AP6. Silent coeliac disease among first degree relatives. Histological and HLA typing study
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Introduction: Coeliac disease (CD) is a common diagnosis among children and adults in Iraq; however to the best of our knowledge, no documented data is available about its familial prevalence yet. This study was carried out to determine the prevalence of potential coeliac disease in a group of first degree relatives of coeliac patients. Materials & Methods: We studied 106 first degree relatives of coeliac patients attending Gastrointestinal Hospital at Medical City in Baghdad, Iraq. Their sera underwent serological screening for CD using the IgA anti-endomysium antibody test (EMA), in addition to human leukocyte antigen HLA class II typing. Duodenal biopsies were performed in all subjects positive to EMA. Coeliac disease diagnosis was established according to modified Marsh classification. All family members were on a gluten-containing diet when serological tests and HLA typing were performed. Results: Fifteen (14.1%) patients were positive for EMA among 106 relatives and thirteen (12.2%) were found as new cases of coeliac disease depending on histology results (Marsh III). However, the DQ2 antigens ratio was 39.6%, DQ8 antigens ratio was 35.8%, meanwhile, DR3 ratio was 16% and the DR5/7 ratio was 8.5%. Discussion: Silent CD cases were more than expected in Iraq, therefore, serological testing is recommended for all first-degree relatives of CD patients. Moreover, they should undergo HLA typing to detect those whose HLA phenotype is consistent with CD.

P-AP7. Cinnamon has significant histological repairing ability on damaged hepatic tissue induced by acetaminophen
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Introduction: The analgesic acetaminophen causes a potentially fatal, hepatic centrilobular necrosis when taken in overdose. Cinnamon is commonly used as a spice and has also been widely used in traditional medicine. The study was carried out to investigate the potential effect of cinnamon administration in repairing the damaged hepatic tissue in acetaminophen induced liver injury. Material & methods: Fifty-four (54) adult male Sprague-Dawley rats comprising of nine normal and 45 acetaminophen hepatotoxic rats were used for this study. Hepatotoxicity was induced by single administration of acetaminophen at 750 mg/kg ip on the first day of the experiment and the 45 rats were received or not different doses of cinnamon. For evaluation of liver function, total bilirubin, total protein and glutathione SH of the blood serum were measured. After completion the experimental protocols (12 weeks), liver tissues were collected and dissected using routine histological preparation. Results: The normal control group showed normal hepatic cells and tissue, while hepatocyte necrosis, fatty change, hyaline degeneration, and infiltration
of inflammatory cells were prominent in the acetaminophen control group with no treatment. Meanwhile, the treatment by low dose (0.01 g/kg) and medium dose (0.05 g/kg) of cinnamon protected the majority of histoarchitecture, however the high dose (0.1 g/kg) of cinnamon treatment showed a picture looks like normal control group so that resulted in significant elevation of biochemical markers level (p<0.005). Discussion: These experimental results indicate that cinnamon treatment has a therapeutic protective effect against liver histological damage that induced by acetaminophen.

P-AP8. Histological and immunomodulatory effects of Nigella sativa oil in treatment of extraintestinal features of refractory coeliac disease

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Introduction: Coeliac disease (CD) is an autoimmune inflammatory disorder of the small intestine triggered by wheat, rye and barley in the diet. Diagnosis is made by a small intestinal biopsy and using “Marsh classification” that explains characteristic inflammatory changes and flattening of intestinal villi which reverts back to normal on a gluten-free diet (GFD). Refractory CD is considered in patients who do not get better on GFD. Case report: A 34-year-old man presented with CD gastrointestinal complaints in addition to three extraintestinal features; chronic urticaria, stomatitis and iron deficiency anemia. Diagnosis of CD was established by intestinal biopsy and positive coeliac serological antibodies including antigliadin, anti-endomyseal and anti-tissue transglutaminase antibodies. He had started gluten-free diet for one year. After follow-up period, his extra-intestinal features didn’t resolve with persisting partial villous atrophy (Marsh IIIa) despite strict GFD and he was considered to have refractory CD. Treatment by Nigella sativa oil (NGO) was suggested to him for 3 months in addition to continuation of GFD. After these 3 months his extraintestinal features were completely resolved and his gastrointestinal symptoms were disappeared with complete histological remission and disappearance of serum antibodies. Discussion & conclusion: Administration of NSO with GFD in treatment of extraintestinal features of CD can lead to complete histological recovery and complete absence of CD antibodies. Ultimately, this may help to provide a scientific basis for the immunotherapeutic application of NSO in clinical management of refractory CD patients.

P-AP9. Infectious Bowel Disease; Case Reports of Unusual Complication

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Introduction: Intestinal perforation due to infectious cause is still prevalent in many developing countries. Despite the advances in the management, the outcome in these patients is still very poor. We present herein 2 unusual cases of intestinal perforation complicated by acute peritonitis caused by Typhoid with concurrent Hookworm infestation and Amoebiasis with concomitant Strongyloides infestation. Case report: Two male patients, illegal immigrants from Myanmar; 20 years old male, presented with the initial symptoms of mild diarrhea, fever with chills rigors for 6 days and severe generalized abdominal pain for 2 days prior to admission. Clinical examination showed features of acute peritonitis. Emergency laparotomy with small bowel resection was performed and intraoperative finding showed pus contamination in the peritoneal cavity and small perforation of the ileum. Typhoid rapid antibody test was positive. Blood culture result was also positive for Salmonella typhi. Histopathological examination of the bowel showed areas of mucosal and transmural necrosis with prominent Peyer’s patches. Post operatively, he developed lower gastrointestinal bleeding. Colonoscopy revealed multiple hookworms throughout the colon. The second patient, 34 years old male, presented with abdominal pain associated with septic shock symptoms. Emergency laparotomy was performed. Right hemicolectomy with double barrel ileostomy and colostomy was done due to large perforation of the caecum extending to the ascending colon. Histopathological examination shows numerous protozoa of Entamoeba spp. and few larvae belong to Strongyloides spp. in the bowel lumen. Conclusion: Both cases illustrate the diagnostic challenge of caring for young immunocompetent persons with bowel perforation due to treatable infectious agents.

P-AP10. Expression of Anaplastic Lymphoma Kinase in Neuroblastoma

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Introduction: Anaplastic Lymphoma Kinase has been described as causative oncogenic driver in neuroblastoma. ALK genetic alteration in neuroblastoma is thought to be one of important factors in management of neuroblastoma. Objectives: The
P-AP11. Expression of Class II Beta Tubulin and Class III Beta Tubulin Protein in Neoplastic and Non-Neoplastic Lymphoid Tissue

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Introduction: Lymphoma presents a challenging field in medical investigations where new concepts and techniques are constantly tested. Here we investigated the expression of microtubule system proteins, namely Class II and Class III Beta Tubulins in neoplastic and non-neoplastic lymphoid tissues. Because of their involvement in cell proliferation and migration, microtubule system protein expression may signify tumour aggressiveness and it has been a target for anticancer therapy. Materials and Methods: We performed immunohistochemistry on tissue microarrays of 304 lymphoma cases and 20 non-neoplastic lymphoid tissues diagnosed over seven years at PPUKM. Results & Discussion: Our results showed that Class II Beta Tubulin expression is ubiquitous i.e., it is seen in the germinatal center, mantle and paracortex of normal lymphoid tissues. In contrast, Class III Beta Tubulin expression is restricted to the germinatal center. Among tumours, Class II Beta Tubulin is expressed in 15/15 (100%) precursor neoplasms, 229/231 (99%) mature B cell neoplasms, 22/22 (100%) mature T-NK-cell neoplasms and 36/36(100%) classical Hodgkin lymphomas. Class III Beta Tubulin is expressed in 2/15 (10%) precursor neoplasms, 84/231 (36%) mature B cell neoplasms, 9/22 (41%) mature T-NK-cell neoplasms and in 34/36(94%) of Classical Hodgkin lymphoma. Conclusion: Class III Beta Tubulin is germinatal centre restricted and almost consistently expressed in neoplastic cells of Classical Hodgkin lymphoma. In addition its frequent expression in a proportion of diffuse large B cell lymphoma, Burkitts and mantle cell lymphomas is of interest as this may be related to their aggressiveness. Class II Beta Tubulin expression is non-selective rendering it an unsuitable marker for differentiating lymphoma subtypes.

P-AP12. Cross Lineage Differentiation of Induced Pluripotent Stem Cells Derived from Oral Squamous Cell Carcinoma (IPSC-OSCC)

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Introduction: Available models for OSCC were derived from primary tumours and have limitations for in-vitro studies due to lack of cell number and continued mutations on propagation. Recent advancement in induced pluripotent stem cells (iPSCs) technology provides a new platform to study tumour characteristics via reprogrammed cancer cells. iPSCs provides a pathway for generating previously inaccessible cells and hold differentiation capacity which permits development of patient-specific disease model that could be used for research and therapy. Materials & Methods: IPSC-OSCC were subjected to Embryoid bodies (EB) formation and directed differentiation into adipocytes and osteocytes. Presence of mesoderm layers in EB was assessed by immunofluorescence staining. Differentiation into mesodermal lineage cell was demonstrated with Alizarin Red S solution and Oil O red. Results: Mesoderm specific markers were detected in EB using immunofluorescence staining. OSCC-iPSCs were shown to differentiate into adipocytes and osteocytes confirmed by respective specific staining protocol. Conclusion: IPSC-OSCC cells were shown to possess progenitor cells of mesodermal layer and be able to differentiate into mesodermal lineage target cells. IPSC-OSCC though derived from both ectodermal and endodermal lineage cells are capable of cross lineage differentiation into mesodermal lineage cells.
**P-AP13. Rhabdomyosarcoma presenting as an aggressive neck swelling with neurological complications in an infant: A case report**

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**Introduction:** Rhabdomyosarcoma (RMS) is the most common soft tissue malignancy in children and adolescents. The rarity of its occurrence in infant posed a great difficulty in terms of diagnosis and management. Here, we report an aggressive case of alveolar rhabdomyosarcoma in an infant which presented as neck swelling with neurological complications. The MRI revealed a soft tissue swelling of the neck with intraspinal extension and spinal cord compression, raising the possibility of a neurogenic or malignant nerve sheath tumour. Histopathological examination revealed a primitive, small round cell tumour with no rhabdoid differentiation. The clinical presentation, neurological symptoms, tumor location and the histopathologic features were highly suggestive of neuroblastoma. However, the tumour cells were positive for desmin, and exhibited weak nuclear immunoreactivity with antibodies to myoD1 and myogenin, features in favour of rhabdomyosarcoma. Fluorescent in situ hybridization (FISH) confirmed the presence of a translocation t(2;13)(q35;q14), supporting the diagnosis of alveolar rhabdomyosarcoma. Despite chemotherapy, patient succumbed to death after 2 months due to disseminated disease. **Discussion:** Rhabdomyosarcoma is highly aggressive mesenchymal neoplasm which may present with diagnostic difficulty. This case highlights the importance of molecular studies in making an accurate diagnosis so that appropriate chemotherapy may be instituted.

**P-AP14. Atherosclerosis in Chronic Organic Arsenic (Monosodium Methylarsonate) Exposure**

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**Introduction:** Human worldwide is exposed to arsenic mainly through drinking of arsenic-contaminated ground water. Arsenic is one of the environmental toxins reported to be associated with atherosclerosis with more attention given to inorganic arsenic as it was thought to be more toxic. Since organic arsenic particularly monosodium methylarsonate are still popularly being used and produced for agricultural activities, this study aimed to investigate the effects of chronic organic-arsenic exposure on the development of atherosclerosis in a rat model based on real human exposure. **Materials & Methods:** Fifty five male Sprague-Dawley rats were divided into 5 groups including a control group. Four treatment groups received oral intubation of monosodium-methylarsonate (MSMA) at 42.13, 63.30, 126.4 and 210.67 mg/kg body weight respectively every day for 16 weeks. Aorta were harvested and stained for H&E and Verhoef Van Gieson as well as for immunohistochemistry exposure in the development of atherosclerosis.

**Results:** Rats treated with 126.4 and 210.67 mg/Kg BW of MSMA was noted to have high mortality due to severe diarrhea and drastic weight reduction and therefore was discontinued from our study. Rats treated with 42.13 and 63.3 mg/Kg BW MSMA showed positive early atherosclerosis changes microscopically with positive VCAM-1 and ICAM-1 expression. **Discussion:** This study highlighted that chronic organic arsenic exposure with MSMA also leads to the development of atherosclerosis. This indicates that chronic organic arsenic exposure is as equally toxic as inorganic arsenic exposure in the development of atherosclerosis.

**P-AP15. Detection of EML4-ALK in lung adenocarcinoma with immunohistochemistry and Fluorescent-in-situ hybridization technique**

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**Introduction:** The echinoderm microtubule-associated protein-like 4 anaplastic lymphoma kinase (EML4-ALK) is now considered as an important driver oncogene in lung cancer. ALK +ve lung adenocarcinomas exhibit a significant therapeutic response to crizotinib, a TKI inhibitor. This study is aimed to determine the frequency of ALK mutations in adenocarcinoma of lung using both immunohistochemistry (IHC, anti-ALK D5F3 rabbit monoclonal antibody, Ventana, USA) as well as fluorescent-in-situ hybridization (FISH, Vysis LSI ALK Break Apart Rearrangement Probe). Clinical features such as age, ethnicity, gender and smoking history were analysed. **Materials and Methods:** In this retrospective study, a total of 50 biopsies confirmed, primary lung adenocarcinomas at the University of Malaya Medical Centre were analysed. All cases were negative for Epidermal Growth Factor Receptor (EGFR) mutation. Those cases which were ALK protein positive by IHC were further assessed by FISH. **Results:** Six cases were positive for ALK protein (12%) by IHC, of which four cases were also FISH positive. In one case, tissue was insufficient for analysis by FISH. Statistical analysis showed that
ALK mutation was significantly higher among the females (p=0.003) and non-smokers (p=0.002). Conclusions: IHC is a reliable, sensitive and specific method to detect ALK protein. ALK positive adenocarcinomas occur with higher frequency in females and non-smokers.

P-AP16. Case Report: Mixed Adenoneuroendocrine Carcinoma (MANEC) of Anorectum

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Introduction: Mixed adenoneuroendocrine carcinoma (MANEC) is a rare tumor entity of gastroenteropancreatic (GEP) system. Diagnosis is often challenging to the pathologist as both components i.e. adenocarcinoma and neuroendocrine carcinoma must be evaluated on routine H&E stains and immunohistochemistry study. We would like to share a case of MANEC presented to Hospital Selayang. Case report: A 72-year-old Sarawakian lady presented with 5 months history of progressive anal pain, occasional constipation and anemia. Initial presentation to local university hospital showed multiple rectal polyps, which were biopsied and reported as adenocarcinoma. The tumor has metastasized to right inguinal lymph node and liver as captured radiologically. The CEA tumor marker was not raised. Due to logistic cause, she came to Selayang Hospital for follow up and underwent an uneventful laparoscopic APR. The specimen showed an anorectal tumor involving the dentate line with microscopic features of poorly differentiated MANEC (G3), strongly stained for CD56, focal synaptophysin, weak to absence Chromogranin A and high Ki 67 index.

Discussion and Conclusion: MANEC is a high-grade tumor and behaves aggressively, often metastasized at diagnosis and has a poorer prognosis. Patient usually presented late, with symptoms and signs similar to other common carcinomas. However, without systematic histological examination, this rare tumor can be misdiagnosed. On histology, MANEC is defined with presence of at least 30% of each tumor components. Staging and grading helps physicians to plan treatment and prognosticate. In current practice, MANEC is removed surgically. Chemotherapy and radiotherapy are used concurrently in few cases.

P-AP17. Maternal Floor Infarction: An old entity yet a finding not to be missed

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Introduction: Maternal Floor Infarction (MFI) is an uncommon pathological entity characterised by the presence of massive and diffuse perivillous fibrinoid material in the maternal surface of the placenta. It was first described in the 1960s. MFI has clear associations with intrauterine fetal demise, IUGR and recurrent pregnancy losses. We would like to share two cases of MFI, which had similar clinical presentations. Case reports: Case 1: A 31-year-old lady who is in her sixth pregnancy and at 32 weeks of gestation. She has a history of three intrauterine fetal deaths and two live births. Her recent pregnancy was complicated with Chromogranin A and high Ki 67 index. Case 2: A 34-year-old lady who is in her third pregnancy and at 35 weeks of gestation. Similarly she had IUGR and oligohydramnios. In both cases, the babies were delivered alive by caesarean sections. Tissue examination of both placentas showed extensive fibrin deposition on the decidual floor, extending up into the intervillous spaces with encasement of the villi amounting to MFI.

Discussion and Conclusion: Although MFI is uncommon, it has significant perinatal morbidity and mortality with the risk of recurrence. In general, practicing pathologists are unfamiliar with MFI which is macroscopically and microscopically different from ischaemic infarcts. The etiology of MFI is unknown, but evidence support an alloimmune or autoimmune mechanism. Awareness of this old entity and recognizing them on placenta tissue sections would aid in management of future pregnancies and in the adequate follow up of the surviving infants.

P-AP18. Placental teratoma: Unexpected mass following a spontaneous vaginal delivery

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Introduction: Placental teratoma is an extremely rare benign non trophoblastic tumor of the placenta. It consists of various mature tissues derived from germ cell layers. We report a case of placental teratoma which was an unexpected finding during a normal per vagina delivery. Case report: The patient was a 35-year-old lady at full term gestation presented to the hospital with signs of labour. She had spontaneous vaginal delivery and her baby was well. However, following the delivery
of the placenta she passed out an oval mass measuring approximately 7.5 cm in largest diameter with skin-like appearance of the outer surface. The cut surface of the mass appeared heterogenous with solid and cystic areas. On histology the cystic areas were lined by skin with an underlying adnexal tissue. The components of the solid areas were bony, cartilagenous and adipose tissues as well as brain, blood vessels and glands. No immature component was noted. The placenta tissue was unremarkable.

Discussion and conclusion: The differential diagnosis is fetus acardius amorphus which is due to blighted fetus or failed twin associated with multiple pregnancy. On the contrary, placental teratoma is almost always associated with a normal pregnancy outcome and fetal development. Whether it is a true neoplasm or an extreme form of fetus acardius, the issue of origin remains unsolved. Obstetricians and pathologists should be made aware of this entity. In understanding the molecular insights of their histogenesis, further studies are required.

P-AP19. Actinomycosis: Unexpected finding of acute appendicitis in a child
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Introduction: Actinomycosis is a chronic progressive suppurative disease commonly caused by Actinomyces israelii. Abdominopelvic actinomycosis is rare and the appendix is the most common organ involved. Here, we would like to share a case of actinomycosis presented as acute appendicitis in a child. Case report: A 13-year-old boy presented with 5 days history of colicky periumbilical and right iliac fossa pain which was associated with fever and vomiting. He was febrile on examination. There was rebound tenderness on right iliac fossa. His blood count showed leucocytosis with neutrophils predominant. Acute appendicitis was suspected clinically and surgery was performed. Intraoperative findings showed an appendicular mass with localized abscess collections. The appendix was enlarged with macerated mesoappendix. Histology of the appendicular tissue showed acute appendicitis with dense suppurative inflammatory exudates seen in the outer appendicular wall, serosa and mesoappendix with abscess formation. Actinomyces colonies with typical sulphur granules and inflammatory exudate are seen in the lumen. Discussion and conclusion: Abdominopelvic actinomycosis may have resemblance to other disease such as acute appendicitis, diverticulitis or Crohn’s disease. However, histological examination would be able to distinguish them. A combination of long term antibiotic therapy and adequate surgery is necessary to ensure complete eradication. Actinomycosis should be included in the differential diagnosis, especially in patients with abdominal pain, fever, and leukocytosis.

P-AP20. Combination of Metformin and severe hyperthermia induces DNA damage and apoptosis in osteosarcoma cells in vitro
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Introduction: Osteosarcoma is a rare malignancy strongly associated with the development of chemotherapy resistance and late detection leading to a reduced 5 year survival. A new strategy for cancer treatment especially breast cancer involves a combination of hyperthermia with chemotherapeutic drugs. However this hasn’t been tested on bone cancer cells. Our previous studies have shown that the osteosarcoma cell line MG-63 is sensitive to severe hyperthermia. Moreover Metformin as an anti-glycaemic drug has been shown to have anti-carcinogenic properties. Targeting the Warburg effect, we applied a combination of Metformin and hyperthermia and measured the induced DNA damage and apoptotic cell death. Materials and Methods: MG-63 cells were treated with Metformin IC50 = 30M for 48h followed by exposure to moderate (39°C) and severe (45°C) hyperthermia for 30 mins, 1h and 2h meanwhile 37°C served as control. After each time point, damage to DNA was accessed by comet assay stained with SYBR® Green and type of cell death was determined via Annexin V-FITC and PI staining. Result: MG-63 cells in combination with Metformin and hyperthermia conditioning resulted in increased DNA damage compared to Metformin alone except after 39°C for 30 mins and 1h. Exposure to 45°C after 30 mins onwards led to grade 4 DNA damage. Meanwhile the rate of apoptosis was significantly increased according to temperature severity. Discussion: Damage to DNA and rate of apoptosis was hyperthermia severity and exposure longevity-dependent. The adjuvant effect of Metformin with severe hyperthermia increases DNA damage and apoptosis, suggesting a possible anticancer activity of metformin.
P-AP21. Effect of short term hyper- and hypothermia on bone cancer cells

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Introduction: Hyperthermia, the raising of body temperature locally or systemically has been experimented with the treatment of cancers. Local treatment of tumor cells with severe hyperthermia is often combined with other therapies i.e. radiation to enhance cancer cell death. At the moment hyperthermia is mainly used to target tumors near the skin surface like breast cancer. Little is known about the effect of temperature treatment on bone cancers for example osteosarcomas. Materials and Methods: In the present study the effect of short term hyperthermia (27°C) and hyperthermia (45°C) was examined on human osteosarcoma cell line MG-63 for one hour and compared with control (37°C). MTS assay, PCR array, Annexin V/PI and Phallolidin Conjugates with Anti-tubulin staining assay were used to determine cell viability, gene expression, apoptosis and cytoskeleton changes. Results: cells viability was reduced (P< 0.01) after short term hypo- and hyperthermia treatment. Hyperthermia induced apoptosis of osteosarcoma cells (p < 0.001) at 1 hour of hyperthermia and after 72 hours of recovery at 37 °C compared to control. However hypothermia did not induce apoptosis. Furthermore, cells cytoskeleton showed some changes under hyperthermia such cell shrunken and rounded cells, p nueclear tubulin condensation under hyperthermia. mRNA of Caspase 3, 8 and 9 showed no significant difference compared to untreated control. Discussion: Hyperthermia caused cell death and reduction of cell viability at 1 hour and after recovery at 37 °C. Hypothermia attenuates cell viability but doesn’t induce cell death. In conclusion hyperthermia induce apoptosis in osteosarcoma cell thus it can be applied for future anticancer therapy.

P-CP1. MethyLight TaqMan® Assay for quantification of DNA methylation of Reelin (RELN) gene in schizophrenia: Preliminary data

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Introduction: Epigenetic changes, particularly DNA methylation are an important mechanism in the regulation of gene expression. Since DNA methylation occurs without genetic sequence alteration, it offers a new insight on the pathogenesis of various complex diseases like cancer and psychiatric disorder such as schizophrenia. Reelin (RELN) gene encodes an extracellular matrix protein that is responsible for neuronal migration during brain development. The methylation study of RELN is needed to clarify its association in schizophrenia. Materials and Methods: DNA samples were extracted from blood of patients diagnosed with schizophrenia in Hospital Tengku Ampuan Afzan, Pahang. The DNA samples were subjected to bisulfite conversion to enhance the methylation areas. Primers and probe were designed to cover CpG rich sites of RELN gene whilst ALU sequences were used as reference target. Sensitivity and specificity of the MethyLight TaqMan® assay were determined by running the assay in serial dilution for the distinction of methylated and unmethylated DNA. With the optimised assay, the DNA methylation of 10 schizophrenia patient samples was measured. Results: This study showed a decreasing detection of methylated alleles in each subsequent dilution using universal human methylated DNA with the Cq value for RELN gene increased from 16.89 to 29.68. We detected the percentage methylation ratio, which valued above zero for the RELN gene in all ten patients ranging from 0.91 to 1.17. Discussion: Our preliminary data suggests the presence of DNA methylation of RELN gene in schizophrenia patients.

P-CP2. Pre-transplantation serum ferritin level in allogenic stem cell transplant patients in Hospital Ampang

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Introduction: The principle cause of iron overload in patients with haematological malignancies is recurrent red cell transfusions for anaemia. Serum ferritin reflects iron burden in the body in the absence of inflammation or liver disease. Materials and Methods: A cross-sectional study using retrospective data between 2008-2011 of 106 post-transplant patients (HLA-matched sibling) with haematological malignancies was carried out at Hospital Ampang to investigate the relationship between pre-transplant serum ferritin level with post-transplant outcome (alive or dead) and survival time. Patients were divided into two groups according to iron status: serum ferritin ≥ 1000 mg/L (iron overload) and < 1000 mg/L. Results: The median age for patients was 30.5 (18-58) years old. Prevalence of pre-transplantation iron overload in these patients was 87.5%. Median pre-transplantation serum ferritin level was 2423 (408.2-7664) μg/L. There was no significant association
between iron status and sociodemographic factors, type of haematological malignancies and post-transplant complications. Although insignificant, patients with iron overload had a shorter survival time (36 months) compared to non-iron overload patients (40 months). There was also no significant association between iron status and post-transplant outcome. 

**Discussion:** Serum ferritin is an acute phase reactant and levels increase in the presence of tissue necrosis and inflammation. Both these events are occurrences in haematological malignancies. Although serum ferritin is a non-invasive, relatively cost effective, widely available and practical indicator of iron status, it is not specific to iron overload. Therefore, true association between serum ferritin and iron burden is problematic in patients with haematological malignancies.

**P-CP3. Adrenal incidentaloma with pulmonary tuberculosis**

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**Introduction:** Adrenal incidentalomas are adrenal tumours detected through imaging procedure performed for reasons unrelated to adrenal dysfunction. It is increasingly being detected with more frequent use of various imaging techniques. Although most such tumours are benign and hormonally inactive, a minority has hormone hyperfunction and malignancy risk, requiring further investigation. 

**Case report:** A 28-year-old lady with hypertension and diabetes mellitus presented with two days history of fever. She also complained of intermittent shortness of breath for the past two weeks. She had a history of consuming traditional sliming pills for four years. On examination she was febrile, hypertensive, in respiratory distress and had truncal obesity. Chest x-ray revealed collapse consolidation over right upper lobe with cavitation and pneumothorax. She was subsequently intubated for respiratory failure. A CT-scan of the thorax incidentally revealed a hypodense lesion at left adrenal gland (3.6cm x 2.7cm x 2.8cm). The sputum was negative for acid-fast bacilli; however she was treated as smear-negative pulmonary tuberculosis (PTB). A markedly increased morning serum cortisol level and 24-hour urinary free cortisol excretion with a low plasma adrenocorticotrophic hormone suggested hypercortisolaemia due to cortisol-secreting adrenal mass. 

**Discussion and Conclusion:** This patient who presented initially with severe respiratory symptoms secondary to complications of PTB was incidentally noted to have an adrenal mass on CT-thorax. The discovery of an adrenal mass requires further evaluation for diagnosis and management in order to reduce morbidity and mortality. Currently there is no universal guideline on the laboratory investigations for workup of adrenal incidentaloma.

**P-CP4. Diagnostic utility of maternal serum cystatin C and pregnancy outcome in pre-eclampsia**

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**Introduction:** Pre-eclampsia typically manifests as renal impairment, monitoring of which is traditionally done by serum creatinine and uric acid. Cystatin C is potentially a better marker of renal function in pre-eclampsia. 

**Materials and Methods:** A cross-sectional observational study was conducted at the Universiti Kebangsaan Malaysia Medical Centre from August 2009 until November 2010. Fifty-three (53) patients with pre-eclampsia and fifty (50) normotensive women were recruited. Patients were followed up and foeto-maternal outcomes were recorded. 5ml of blood were drawn and serum was analysed for creatinine, uric acid and cystatin C. 

**Results:** Twenty (39%) and thirty-three (61%) had mild and severe pre-eclampsia, respectively. Severe pre-eclampsia showed significantly higher cystatin C (1.33mg/L) compared to mild pre-eclampsia (1.16mg/L) and normal control (0.89mg/L). Uric acid was only significantly elevated in severe pre-eclampsia; whilst creatinine was within the normal range in both categories. Receiver Operator Curve (ROC) analysis showed that at a cut-off of 1.00mg/L, cystatin C had 85% sensitivity and 82% specificity, with the largest area under curve of 0.91 (95% Confidence Interval (CI) 0.86-0.96), compared to that of serum uric acid and creatinine of 0.77 (95% CI 0.66-0.86) and 0.65 (95% CI 0.55-0.76), respectively. Foetal and maternal complications occurred in 17 and 6 patients with severe pre-eclampsia, respectively. Urine protein was the only significant predictor of foetal outcome with an odds ratio of 3.53 (95% CI 1.3-4.9). 

**Discussion:** Cystatin C was better in differentiating pre-eclampsia from normotensive pregnancy than uric acid and creatinine. However, cystatin C was a poor predictor of foeto-maternal outcome in pre-eclampsia.
P-CP5. Effect of haemolysis on potassium analysis

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Introduction: Haemolysis represents the number one reason for specimen rejection in clinical chemistry, occurring five times more frequently than the second most cited reason i.e. insufficient specimen quantity to perform the test. It is also one of the common causes of pre-analytical factors affecting potassium level. This study aimed to investigate haemolysis interference on potassium analysis and to study the visual effect (colour changes) of serum at different degree of haemolysis. Materials and Methods: Haemolysate was prepared by modified osmotic shock method. Stock solutions of haemolysate in saline were prepared to give haemoglobin (Hb) concentration range of 0-100g/L in increments of 10g Hb/L. Two pooled plasma potassium (mmol/L) free of any visible haemolysis were prepared, of 4.2 and 6.7 respectively. Different Hb concentrations of haemolysate were added to the two pooled plasma samples. Control was prepared by adding 100uL of saline to 900uL pooled plasma. For each 900uL of pooled plasma, 100uL of haemolysate with different Hb levels were added. Haemolysis index (HI) and potassium were measured in triplicate. Percentage change from baseline value was calculated. A change of >5% from baseline value was considered significant. Change of serum colour was captured and recorded. Results: Haemolysis interference was seen when the HI was >100. The potassium level was linearly dependent on HI. Visible haemolysis was seen when the HI was ≥30. Discussion: It is inappropriate to reject a haemolysed specimen for potassium analysis based on visible red colour changes without measurement of HI. Thus, all laboratories should perform HI measurement to confirm significant haemolysis interference which was shown to occur when the HI ≥100.

P-FP1. Pheochromocytoma: Accidental finding during autopsy

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Introduction: Pheochromocytoma is a rare but clinically important disease. The most common clinical sign of pheochromocytoma is sustained or paroxysmal hypertension, and the most common symptoms are headache, excessive sweating and palpitation. In some cases, the clinical symptoms are not clear. Surgical removal of tumour is the treatment of choice in most circumstances. Case report: A 38 years old lady, known case of Neurofibromatosis, presented with complete miscarriage and twisted right endometriotic cyst. She was discharged well post operation day 8. She presented again at the Emergency Department five days after discharge with complaint of restless, nausea, vomiting, profound sweating, and cough with whitish sputum. She developed Type II respiratory failure, intubated and resuscitated. Unfortunately, she collapsed and was pronounced dead 30 minutes post active resuscitation. Autopsy revealed pulmonary edema and adrenal mass, with no other significant findings. Pathological examination confirmed that adrenal mass was pheochromocytoma. Discussion and conclusion: The lack of specificity of the clinical symptoms lead to undiagnosed Pheochromocytoma. It is potentially curable if surgically removed.

P-H1. B-Acute lymphoblastic leukaemia with “double hit” - a diagnostic and treatment challange

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Introduction: B-Acute lymphoblastic leukaemia (B-ALL) in adults carry a poorer prognosis than in children. Double-hit lymphomas are high-grade B-cell lymphomas characterized by dual chromosomal rearrangements. Treatment remains difficult and it has a bad prognosis. To our knowledge, ALL with ‘double hit’ has not been reported. Case report: A 39-year-old man, presented with lethargy and bilateral neck swelling for two weeks duration. Examination showed cervical lymphadenopathy and hepatosplenomegaly. Peripheral blood and bone marrow aspirate showed increase in blasts, morphologically that of L3-FAB. Flowcytometry confirmed B-ALL, with positive HLA-DR, CD19, CD10 with aberrant CD117 and CD38. However trephine biopsy and immunohistochemistry revealed positivity for PAX5, CD 10, BCL-2 and BCL-6 and a diagnosis of diffuse large B cell lymphoma (DLBCL) was made. He also had abnormal male karyotype with t(1;3)(q31;q27) and trisomy12. C-MYC and BCL6 rearrangement with IGH/MYC gene translocation were seen by FISH cytogenetics. He was treated with HYPER-CVAD (B-ALL protocol regime) and showed no response, following which CODOX chemotherapy regime (Burkitts lymphoma) was given. However there is still no remission achieved. Discussion and conclusion: This case (leukaemia/lymphoma) illustrated difficulties in attaining the diagnosis. With the presence of the double hit rearrangements and complex cytogenetics, the management and treatment options, remains difficult and challenging.
P-H2. Potentially fatal acquired Haemophagocytic Lymphohistiocytosis (HLH) due to dengue infection

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Introduction: Haemophagocytic lymphohistiocytosis (HLH) is a potentially fatal clinical syndrome that can be familial or acquired. Acquired HLH commonly occurs after strong immunologic activation following variety of exogenous agents such as infectious organisms or malignancy. Dengue is a serious viral infection that is potentially life threatening, usually presents with acute febrile illness caused by infection with mosquito-transmitted dengue viruses (Aedes species). Case report: We report a case of 32-year-old gentleman who presented with severe dengue infection, and his condition deteriorated despite supportive treatment and empirical antibiotics. He was treated as dengue shock syndrome with hepatitis and acute kidney injury with metabolic acidosis. Despite treatment, he continued to deteriorate requiring intensive care. At this juncture, he had bicytopenia (anaemia and thrombocytopenia), elevated LDH, raise triglyceride, high serum ferritin but normal fibrinogen level. Bone marrow demonstrated presence of haemophagocytic activity. He was diagnosed to have dengue fever with virus-associated haemophagocytic syndrome based on the criteria of HLH 2004 protocol of the Histiocyte Society. The patient recovered with corticosteroid therapy and intravenous immunoglobulins and was discharged well. Discussion and conclusion: There have been only a few reported cases of acquired HLH in dengue patient. This condition is potentially fatal. Therefore, acquired HLH should be excluded in patient with severe dengue infection who does not respond to treatment.

P-H3. A Rare Case of Biclonal Gammopathy in Plasma Cell Myeloma

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Introduction: Biclonal gammopathies in plasma cell myeloma is a rare group of disorders with only 2% of cases reported worldwide. Case report: Here we report a rare case of plasma cell myeloma with biclonal gammopathy (IgG lambda and IgA lambda type) in a 76-year-old man who presented with smouldering plasma cell myeloma. He presented with unexplained persistent anaemia (7.7g/dL) with reverse albumin to globulin ratio for the past 3 months. The blood film exhibited hypochromic microcytic anemia with presence of rouleaux formation. Bone marrow biopsy done revealed a cellular marrow with M:E ratio of 4:1 with presence of 50% abnormal plasma cells. Serum protein electrophoresis showed a distinct monoclonal band (M band) in the gamma region with presence of Bence Jones protein in the urine. The corresponding serum immunofixation electrophoresis (Sebia) revealed biclonal gammopathy with the presence of two distinctly separate bands in the IgG lambda and IgA lambda region whilst the urine immunofixation exhibited band only in the IgG lambda region. The patient was treated with cyclophosphamide, thalidomide and dexamethasone. Unfortunately, he died after 1 cycle of chemotherapy due to neutropenic sepsis. Discussion and conclusion: This is the first case of such reported in our institution. Further extensive study on molecular events responsible for this exceptional combination may be helpful in determining their pathogenesis and clonal origin of whether they belong to a truly biclonal population or rather a single neoplastic clone.

P-H4. Filipino β°-deletion: a unique mutation causing -thalassaemia major in Sabah and Northern Sarawak

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Introduction: Beta thalassaemia major (β-TM) is commonly caused by point mutations and rarely as deletions. In Malaysia, the total number of transfusion-dependent thalassaemia patients reported was 4768 in May 2010, where over 1000 cases were from East Malaysia (Sabah and Sarawak). Materials & methods: We studied mutations in β-TM patients from Sabah and Northern Sarawak. Gap-PCR was performed for the detection of Filipino β°-deletion (NG_000007.3:g.66258_1847 34del118477) among 252 and 26 β-TM patients from Sabah and Northern Sarawak, respectively. Results: Homozygous Filipino β°-deletion was found in 219/252 (86.9%) β-TM patients from Sabah where 137/219 (62.5%) were Kadazandusun. Homozygous Filipino β°-deletion was found in 16/26 (61.5%) β-TM patients from Northern Sarawak in the indigenous population, consisting of Kedayan (7/17) and Bisayak (4/17). Discussion: A clear genetic difference was found between Sabah, Northern Sarawak and West Malaysia where point mutations are commonly reported in β-TM patients. Filipino β°-deletion is a unique mutation found in Sabah and Northern Sarawak especially in the indigenous population. This deletion is 118 kb with the 5’ breakpoint deletion at position -4279 relative to the mRNA capsite of the β-globin gene and the 3’ breakpoint extending to the downstream of β-globin gene, six olfactory reception (OR) genes (four functional OR genes and two OR pseudogenes) including one γ-globin enhancer. The homoyzogous state of the Filipino β°-deletion results in transfusion dependent β-TM. Our study suggests that the indigenous population of Sabah and Northern Sarawak may belong to the same origin historically most likely from early migration from the Philippines.

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Introduction: Chronic myeloid leukemia (CML) is typically characterized by the presence of Philadelphia chromosome, the product of reciprocal translocation between 9q34 and 22q11, resulting in generation of BCR/ABL fusion protein. In rare cases, another translocation involving 9q34 and 12p13, encoding ETV6/ABL fusion protein has been reported. Case Report: Here we describe a 44-year-old male who presented with Philadelphia-negative CML but positive for the ETV6/ABL fusion gene. He was referred for hyperleucocytosis with total white cell count of 450.0 x 10^9/l. He complained of vomiting, diarrhoea and loss of appetite for one week duration. Physical examination revealed lymphadenopathies and hepatosplenomegaly. The bone marrow examination was consistent with CML in chronic phase. Bone marrow cytogenetic displayed normal male chromosome. Multiplex reverse transcriptase-polymerase chain reaction analysis capable of detecting 28 mutations, had only identified ETV6-ABL gene, or translocation (9;12)(q34:p13). Cytoreductive and imatinib therapy were initiated. Unfortunately, he had poor response to this treatment; hence he was advised for allogeneic haematopoietic stem cell transplantation. Discussion & conclusion: ETV6-ABL fusion is a rare cryptic chromosomal rearrangement with oncogenic properties, reported so far in 29 patients with haematological malignancies. Like BCR/ABL fusion gene, it leads to increased tyrosine kinase activity, activate similar signal transduction pathways and show similar transforming activity in CML. However, the inhibitory effect of imatinib was short lived and unable to induce a complete remission in ETV6/ABL-positive CML. The present case highlights the importance of molecular studies in identifying the cryptic chromosomal translocation and the therapy resistance of ETV6/ABL-positive CML to imatinib.

P-H6. Case report of anti-G, anti-C and anti-D in a pregnant woman

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Introduction: The G-antigen of the Rh blood group system is present on almost all D-positive or C-positive red cells but absent in red cells that lack both D and C-antigens. The differentiation of anti-D and anti-C from anti-G for routine transfusion is not necessary as these patients will receive Rh-D-negative and C-negative blood that would be G-negative. However, during pregnancy it is important to correctly identify the specificity because anti-G can masquerade as the anti-D and anti-C antibodies with initial testing. In pregnant women, presence of anti-D without passive immunization excludes the need for prophylactic anti-D administration. Moreover, patients with anti-D or anti-G are at risk of HDFN and need close monitoring. Thus, proper identification allows the clinicians to take necessary steps in patient management. Case report: A 32-year-old, gravida-4, para-3 woman at 28-weeks of pregnancy, grouped as A-RhD-negative and on initial antibody testing showed presence of anti-D and anti-C. She has previous history of baby with neonatal jaundice but denied any blood transfusion. In view of the presence of combined anti-D and anti-C, presence of anti-G needs to be excluded. Double adsorption and elution using R2R2 and r’r cells confirmed the presence of combined anti-D, -C and -G. No anti-D immunoglobulin was administered and the patient was monitored closely. Discussion & conclusion: This report highlights the importance to differentiate the anti-D and anti-C from anti-G to determine the management plan for the pregnant mother. For transfusion, D and C-antigen negative red cells that are also G-antigen negative should be used for this patient.

P-H7. Influence of multiple, regular platelethpheresis donations on immature platelet fraction and platelet count

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Introduction: Platelethpheresis procedures are generally safe without significant major adverse reaction to the platelethpheresis donors. However, donor safety on recurrent long term platelethpheresis with short interdonation intervals should be emphasized and informed to publics to encourage more people to become platelethpheresis donors thus helping blood transfusion services met the increasing demand for platelethpheresis product. Methods: A cross-sectional study was performed in the National Blood Center, Kuala Lumpur during a period of 9 months from January to September 2014 and retrospective platelethpheresis records were reviewed. A total of 184 platelethpheresis and 60 whole blood donors aged between 17 to 62 years old were enrolled in this study. The blood samples were collected and all the blood samples were measured for full blood count and
immature platelet fraction using automated haematology analyser (Sysmex XE-5000) in the Pathology Department, Hospital Kuala Lumpur. We compared the baseline and recent predonation platelet count in the plateletpheresis donors and analysed the influence of frequency, interval and duration of plateletpheresis donation on the platelet count and immature platelet fraction. **Results:** There was statistically significant in reduction of predonation platelet count (273 x 10^9/L, 172-443) observed in the plateletpheresis donors when compared with the baseline predonation platelet count (291 x 10^9/L, 164-478). However, no clinically important decrease in predonation platelet count was seen in donors donating plateletpheresis components up to 24 times per year, regardless of interdonation interval and plateletpheresis duration. Immature platelet fraction for the plateletpheresis donors (1.5%, 0.5-7.5) were also comparable with the healthy whole blood donors (1.5%, 0.6-4.8; p=0.848). **Conclusion:** Frequent plateletpheresis donation with at least 14 days interdonation intervals and 24 times a year does not cause thrombocytopenia or increase in the immature platelet fraction.

**P-H8. Reprogramming Of Induced Pluripotent Stem Cells Using mRNA Transcription Factors**

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**Introduction:** Induced Pluripotent Stem Cells (iPSC) can be reprogrammed using synthetic modified mRNA encoding transcription factors from somatic fibroblasts. The objectives of this study are (1) to perform mRNA reprogramming encoded by transcription factors (Stemgent®, Cambridge, MA, USA) on primary cells and cell lines, and (2) demonstrate iPSC properties. **Material & Methods:** We isolated primary cell lines fibroblast using enzyme dissociation techniques from human foreskin (HDF UiTM). We also derived iPSC from other primary cell lines i.e. Primary Dermal Fibroblasts (ATCC® PCS-201-012™) and two cell lines which are Human Normal Foreskin (BJ ATCC® CRL-2522) and Human Skin Fibroblasts, (WS1 ATCC® CRL-1502™). BJ ATCC® CRL-2522 acted as control. Then we transfected these cells using mRNA reprogramming technique without feeder cells. mRNA reprogramming cocktail were transfected daily with a molar stoichiometry 3:1:1:1:1 for Oct4, Sox2, Klf4, c-Myc, and Lin28 mRNAs, respectively. Characterisation methods were used and results were recorded. **Results:** Morphology characteristics were observed to be similar to iPSC. Expression of pluripotency were confirmed by using live-staining antibodies StainAlive™ DyLight™ 588 Mouse anti Human TRA-1-60 (Stemgent®) and a high level of alkaline phosphatase (AP) expression (Stemgent®). **Discussion:** The TRA-1-60 antibody reacted with a pluripotent, stem cell specific antigen expressed on undifferentiated human embryonic stem (ES) cells. iPSC were stained using AP Staining Kit, undifferentiated cells appear red or purple and differentiated cells appear colorless. We conclude that mRNA reprogramming method is a viable alternative to reprogramming primary cells and cell lines to iPSC.

**P-H9. Concomitant HbH-Paksé with JAK2V617F mutation: Case Report**

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**Introduction:** Haemoglobin (Hb) Paksé is a low incidence alpha (α) thalassaemia caused by a mutation at the termination codon (codon 142) of the α2 gene (TAA → TAT or Leu → Pro). Myeloproliferative neoplasms (MPN) are a clonal haematopoietic stem cell disorder which results in proliferation in one or more of the myeloid lineages. We described a lady with a rare co-existence of HbH-Paksé disease and Myeloproliferative Neoplasm (MPN). **Case report:** A 20-year-old lady was diagnosed to have HbH disease since the age of 2 years old and was subsequently on 2-3 monthly blood transfusion. No molecular services were available at that time to prove her genotype. Recently, Hb analysis was repeated using capillary electrophoresis (CE) showed presence of HbH (14.7%) with a low Hb A2 level (0.4%). There was also presence of a Hb variant at zone 2 (1.2%) suspected to represent Hb Constant Spring (HBCS). H inclusion was positive. The findings were suggestive of non-deletional Hb H disease. However, DNA analysis for 6 types of alpha globin gene deletions and 7 types of non-gene deletions using multiplex PCR yielded only a (−SEA) deletion. Additional ARMS PCR for non - deletion was performed and finding was consistent with Hb H Paksé disease. Her platelet counts were noted to be increasing in trend for the past few years. The latest platelet count was 2600 x 10^9/L. Jak2V617F mutation analysis was performed and yielded a positive result. A diagnosis of essential thrombocytopenia (ET) was made and she was started on oral hydroxyurea. **Conclusions and discussions:** This case may be of interest not only due to the rare form of thalassaemia and its coexistence with a possible MPN, but also because of the undetermined clinical significance of JAK2 mutation in this subset of patients.
P-H10. Trisomy 8 and Double Philadelphia chromosomes in a CML patient on imatinib mesylate therapy

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Introduction: From the time when secondary chromosomal changes were first identified in patients with chronic myeloid leukemia (CML), questions as to whether these cytogenetic evolutions are due to the type of therapy given during chronic phase (CP) have been raised and imatinib mesylate has been reported to cause this.

Case report: Here we report a 30-year-old man who was initially diagnosed with Philadelphia (Ph) positive CML in chronic phase of the disease in 2008. He was started on imatinib therapy in which unfortunately he was non-compliant to. Two years later, fluorescence in situ hybridization (FISH) with the Vysis LSI BCR/ABL Dual Colour fusion probe revealed 36% and 12% of the peripheral blood cells with one and two BCR/ABL fusion signals detected respectively. Five years after starting the therapy, leukocytosis recurred (89 x 10^9/L) and bone marrow aspirate showed granulocytic hyperplasia with 2% of blast detected. Cytogenetic analyses revealed presence of trisomy 8 as well as double Ph clones in seven metaphase spreads. The finding was further confirmed by FISH analysis. He had undergone allogenic peripheral blood stem cell transplant (PBSCT) a few months later. Nevertheless, he had succumbed to the disease. Discussion and conclusion: These findings suggest that the emergence of trisomy 8 and double Ph chromosome may be due to therapy related CML.

P-H11. Screening of α-thalassaemia in newborns by capillary electrophoresis system on fresh and dried cord blood samples

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Introduction: Hemoglobin Bart’s may also occur in α-thalassaemia carriers (1-15% of total haemoglobin). This study explored the ability of capillary electrophoresis (CE) method for screening of α-thalassaemia in newborns by means of quantifying Hb Bart’s level on fresh and dried cord blood samples. Method: Newborn cord blood samples were subjected to capillary electrophoresis method by means of two different kits (Neonat kit, using dried blood spot and the Cord blood kit, using fresh cord blood). α-thalassaemia genotypes were confirmed by molecular analysis. Results: Six hundred samples were analysed, 32 and 33 showed Hb Bart’s peak by Neonat and Cord blood kits respectively. Molecular analysis confirmed the presence of α-gene deletions in all the positive samples. Of the 32 positive samples by Neonat kit, 23 were αααα, four –αααα, two αααα-αααα, and three ααααα. Cord blood kit was able to identify a very low level of Hb Bart in one additional sample with αααα-αααα. Molecular analysis on 50 randomly chosen samples with absence of Hb Bart peak revealed 4/50 and 3/50 false negative results by Neonat and Cord blood kits respectively, all of which carried a single α-gene deletion. The sensitivity and specificity of CE in α-thalassaemia carrier states detection were 88.89% and 100% respectively. The range of Hb Bart’s using Neonate Kit and CB Kit were 0.5 – 4.1% and 0.5-7.1% respectively. Conclusion: Capillary electrophoresis using fresh or dried spot cord blood samples were able to detect newborn α-thalassaemia carrier states with a high sensitivity and specificity, especially for two α-gene deletions.

P-H12. A systematic review on the involvement of apoptosis in the pathogenesis of myelodysplastic syndrome

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Introduction: Myelodysplastic syndrome (MDS) is a clonal haematopoietic stem cell disorder characterized by peripheral pancytopenia, despite the normo- or hypercellularity appearance of bone marrow. Accelerated apoptosis has been postulated to be involved in the pathogenesis of MDS, leading to ineffective hematopoiesis. The aim of this systematic review was to study the role of apoptosis in the pathogenesis of MDS. Methodology: We searched Proquest and Ebscohost databases up to March 2015. The search yielded 966 articles using keywords: Myelodysplastic Syndrome or MDS or Myelodysplasia and apopto* or cell death. Results/Discussion: A total of 18 experimental papers have been found to meet the inclusion criteria. Apoptosis has been found to occur in CD34 positive, mononuclear cells as well as stromal cells of the bone marrow microenvironment with high expressions of pro-apoptotic mediators such as Fas/Fas L, TNF-α, caspase family proteins and Granzyme-B. Bcl-2, p53 mediators and mc11 and bfl1 genes are highly expressed to compensate the apoptosis process while allowing the accumulation of blast cells within the bone marrow. Mitochondrial membrane potential, phosphatidylserine expression and DNA fragmentation act as a marker to quantify the level of apoptosis. Clonogenic assay with appropriate apoptosis inhibitors resulted in significant growth of progenitor cells. In conclusion, apoptosis is involved in various stages of MDS development. Apoptosis is up-regulated at the early stage of MDS and is diminished with disease progression.
P-H13. Thrombotic thrombocytopenic purpura masqueraded by severe dengue

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Introduction: Thrombotic thrombocytopenic purpura (TTP) is characterized by severe thrombocytopenia and microangiopathic hemolytic anemia. Although some patients also have a combination of fever and neurologic and/or renal manifestations, these are not essentially required for diagnosis. Ultra-large von Willebrand factor (vWF) multimers found in the patient’s plasma are the basis for the platelet thrombi. Recent evidence has linked the abnormal fragments of vWF with deficiency of a plasma enzyme named vWF-cleaving protease or ADAMTS-13. Our aim is to raise awareness that clinical suspicion is paramount in clinching the diagnosis and highlight the importance of early detection and prompt management to improve patient’s chance of survival. Case Report: We present a case of a 41-year-old Malay lady initially diagnosed with Severe Dengue, who subsequently demonstrated clinical and laboratory findings of TTP. When it was thought that she was recovering from the Dengue infection, she developed marked direct hyperbilirubinemia, which was quickly complicated by encephalopathy. Further laboratory investigations strongly pointed towards ongoing haemolysis with evidence of microangiopathic haemolytic anaemia. Despite, a within-range ADAMTS-13 level, three cycles of plasma exchange with fresh frozen plasma were promptly constituted to the patient of which she made a remarkable recovery. Discussion and Conclusion: The absence of severe ADAMTS-13 deficiency, as measured by current static assays, should not be used to argue against the use of plasma exchange, which has been highly effective even several years before the discovery of ADAMTS-13. Thus the determination of ADAMTS-13 activity has only a limited role in the diagnosis of TTP.

P-H14. A comparison between two sampling methods for extended red cell genotyping using TaqMan single nucleotide polymorphisms

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Introduction: Blood has always been the preferred sample in determining the different blood group types. However, in repeatedly transfused patients, accurate red cell antigen typing by serology is a constant problem because the exposure to donor’s blood in patient’s circulation renders it unreliable. The main aim of this study was to study the genotype of red cells for RH (C,c, E, e), KEL (Kell, Celano), Kidd (JKA, JKB) and Duffy (FYA, FYB) using buccal swab and peripheral whole blood amongst transfusion dependent thalassaemia patients. Method: Repeatedly transfused thalassaemia patients from the Thalassaemia Clinic of Ampang Hospital and Universiti Kebangsaan Malaysia Medical Centre participated in this study. Paired samples consisting of peripheral whole blood and buccal swab samples were collected prior to the scheduled blood transfusion and on day 7 after the transfusion. Serological phenotyping by tube method and DNA genotyping was performed using TaqMan Single Nucleotide Polymorphism (SNP) RT-PCR assays. Results: Discrepancies were found between the phenotype and genotype results for all blood groups tested in both pre- and post-transfusion samples. Full concordances of red cell genotype between pre- and post-transfusion blood samples were observed. The genotyping results between different sampling methods, blood and buccal swab samples showed concordant results. Discussion: Accurate red blood cell antigen profiling is important for patients requiring multiple transfusions. The SNP RT-PCR platform is a reliable alternative to the conventional method. Buccal samples offer a simple and inexpensive alternative collection method that may be used for accurate blood group genotyping when blood samples are unavailable.

P-H15. Neonatal jaundice in G6PD-deficient females and the association with commonly occurring genetic mutations using single-nucleotide polymorphism detection via real-time PCR

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Introduction: G6PD deficiency is an important cause of jaundice in neonates with more than 180 mutations reported. We studied the relationship between different G6PD variants and the severity of neonatal jaundice using single-nucleotide polymorphism (SNP) assay. We also observed the rate of discrepancy of G6PD deficiency diagnosis between fluorescence spot test (FST) and enzyme assay (EA) method. Method: All female neonates diagnosed as G6PD-deficient by FST and EA were consecutively studied. Molecular mutations of the G6PD gene and clinical manifestations of neonatal jaundice in 19 females with G6PD deficiency were analyzed using SNP method. Real-time PCR were performed and analyzed for

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Introduction: β-thalassaemia causes expression defects mainly by point mutations on chromosome 11. The aim of this study was to characterize β-globin gene mutations by using multiplex ARMS polymerase chain reaction (MARMS-PCR) as compared to flow-through hybridization (FTH) kit. Method: 56 β-thalassaemia cases were selected based on hypochromic microcytic red cell indices and raised HbA2 level. Mutations analysis was performed using nine primers of common β-globin gene mutations for MARMS-PCR and correlated with 25 mutations on designed FTH kit. Results: MARMS-PCR successfully detected β-globin gene mutations in 49/56 (87.5%) of samples. 24/49 (49.0%) were due to the common IVS 1-5 and Cd 41/42 mutations. The rest were IVS 1-1, Cd 17, Cd 26, IVS 2-654, Cd 8/9 and -28. There was one patient with compound heterozygous for Cd 41/42 and Cd 26 mutation. Seven samples are negative for the mutations tested. FTH results were in agreement with MARMS-PCR in 47 cases, except for the two cases where Cd 8/9 mutation was not part of FTH panel. FTH detected additional mutations (51/56 cases (91.1%)) as the panel incorporated more mutational primers in. Some of these comprised del45kb and poly-A mutation. Some of these mutations were seen to be compounded with inherent with other more common β-globin gene mutations hence more severe phenotypes of the affected patients. Discussion and conclusion: Both MARMS-PCR and FTH techniques are reliable and laborious, but FTH is able to detect 25 mutations in the single test for one patient. However, hybridization is costly for large sample sizes. Thus, MARMS-PCR is more cost effective to characterize the spectrum of β-thalassaemia mutations.

P-H17. Assessment of Iron deficiency in the Paediatric Age Group: The clinical utility of Reticulocyte Haemoglobin Equivalent (Retic-He)

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Introduction: Early identification of iron deficiency anaemia (IDA) in children is essential to prevent cognitive deficits affecting memory, attention and disruptive irritable behavior. The aim of this study was to evaluate the Ret-He in detecting IDA in children. Transferrin saturation is a known sensitive marker in detecting IDA but is biologically variable and not cost effective as a screening tool. Method: A prospective study was performed in Hospital Sultanah Aminah Johore Baharu from November 2013 to September 2014, involving 120 children with hypochromic microcytic anaemia (Hb <12g/dL, MCV <83fl, MCH <27pg). Ret-He and conventional parameters: serum iron, TIBC, ferritin and transferrin saturation, were measured. Results: 81 out of 120 subjects (67.5%) were found to be iron deficient using 20% as cut-off point of transferrin saturation. The Ret-He optimal cut-off value using the Receiver Operating Characteristics (ROC) curve analysis, was 22.65pg , based on the diagnosis of IDA using transferrin saturation of <20% and/or serum ferritin of < 12μg/L. The sensitivity and specificity for the Ret-He test was good with a value of 77.8% and 66.7% respectively. As compared with Ret-He, the sensitivity of serum ferritin was only 18.9%. Discussion: The study has shown that Ret-He could potentially be an alternative screening tool due to its good sensitivity, cost effectiveness, and minimal blood sample requirement. We suggest Ret-He at a cut-off point of 22.65pg as a sensitive marker for iron deficiency anaemia and may be utilized as a screening tool for the diagnosis of IDA in the paediatric population.
P-H18. Reducing high rejection of blood samples sent for group screen and hold (GSH)/ group cross match (GXM) due to haemolysis in Blood Bank, Hospital Kajang.

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Introduction: Haemolysis is the highest cause of rejection of blood samples sent for GSH / GXM in Blood Bank, Hospital Kajang. High rejection of blood samples sent for GSH/GXM can lead to serious consequence that include delay in blood supply to patients causing delay in treatment and management of patients especially in cases of polytrauma and massive haemorrhages. In addition, it also causes unnecessary distress to patient due to repeated blood taking. Our aim is to reduce rejection of blood samples sent for GSH/GXM due to haemolysis and also total rejection of GSH/GXM samples in Blood Bank, Hospital Kajang. The target is to reduce the number of rejection to \( \leq 0.5 \% \) for haemolysis and to \( \leq 1\% \) for total rejections.

Material and Method: Study was conducted from Jan to March 2013, July to September 2013, October to December 2013 and May to July, 2014. Data were collected through ‘Rejection Slip’, ‘Laporan Penolakan Sampel Ujian GSH/GXM’ and GXM forms. Initial data was presented during Hospital Transfusion Committee. Every 2 weekly, letters were distributed to all clinical HODs about rejection statistics that contains causes for rejection with location and names of the doctors responsible. Other intervention activities included CMEs with hands-on on phlebotomy technique and adjunct conducted at various clinical locations, houseman refresher and transfusion course. Results: Analysis from Cycle 1 revealed 1% rejection due to haemolysis with 3% total rejection. Cycle 2 & 3 showed 0.8 % and 1% rejection due to haemolysis with 2.8% & 4 % total rejection. The rejection for haemolysis decreased to 0.5% with only 1% total rejection during Cycle 4. Discussion: There was significant drop in rejection of haemolysed samples sent for GSH/GXM from 1% to 0.5% and also decrease in total rejection from 3% to 1% during the study period. This signifies that various activities that have been conducted help in reducing the haemolysed samples and overall rejection of GSH/GXM samples, therefore helps in contributes to more efficient patient care.

P-H19. Small Interfering RNA Silencing of Interleukin-6 in Mesenchymal Stromal Cells Inhibits Multiple Myeloma Cell Growth

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Introduction: Bone marrow mesenchymal stromal cells (MSC) were shown to produce high concentration of interleukin-6 (IL-6) that promotes multiple myeloma growth. Current IL-6 monoclonal antibody-based therapy failed to demonstrate significant clinical responses and RNA interference (RNAi) provides a new approach to target IL-6 overexpression in MSC. Previously, we successfully suppressed IL-6 expression in MSC transfected with synthetic IL-6 siRNA. In this study, we evaluated the in vitro and in vivo antitumour efficacy of the transfected MSC on U266 multiple myeloma cells. Materials & Methods: Conditioned medium from transfected MSC were added into wells containing U266 at the ratio of 2:1. Viability and IL-6 production post co-culture were determined on fixed intervals using MTS and ELISA assays respectively. The antitumour efficacy of transfected MSC was subsequently evaluated in a murine subcutaneous model of human multiple myeloma followed by histological assessment of the harvested tumours. Results: Significant inhibition of cell growth and IL-6 production was observed in U266 co-cultured with transfected MSC compared to U266 co-cultured with control MSC. In U266 tumours co-injected with transfected MSC, tumour volume and mitotic index were significantly reduced compared to tumours of mice co-injected with control MSC. In addition, there were slight increase in apoptotic index along with marked increase of necrosis and lymphocytic infiltrates in the U266 tumours with transfected MSC. Discussion: MSC with suppressed IL-6 displayed in vitro and in vivo antitumor efficacy against multiple myeloma cells suggesting the feasibility of using RNAi as an alternative approach for targeting IL-6 in multiple myeloma therapy.
P-H20. Induced pluripotent stem cells derived from G-292 osteosarcoma cells formed teratoma in nude mice

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Introduction: Osteosarcoma (OS) is a malignant cancer with unsatisfactory treatment outcome, new intervention is needed to improve the overall survival. New intervention can only progress with a better understanding of the pathogenesis of OS. Reprogramming of OS cells to induced pluripotent stem cells (iPSCs) is an innovative way for disease modelling and understanding of pathogenesis of OS. Materials & methods: To establish an iPSC line for disease modelling, we reprogrammed G-292, an OS cell line using Yamanaka’s approach. Reprogrammed cells were transferred to inactivated mouse embryonic fibroblasts (iMEF) on Day 3 post transduction. Colonies were manually picked after Day 15 and transferred to new iMEF. Reprogrammed osteosarcomas were characterised by observation on morphology, alkaline phosphatase and pluripotency markers expression and teratoma formation. Results: We were able to reprogram G-292 cell line to the pluripotent state. Embryonic stem cell (ESC)-like clusters started to appear between 15 to 20 days post transduction. Morphology of the colonies resembles ESC colonies with defined border and tightly packed cells. Reprogrammed G-292 expressed alkaline phosphatase and pluripotency markers, such as, OCT4, SSEA4, TRA-1-60 and TRA-1-81, similar to ESC. In our in vivo study, reprogrammed G-292 colonies formed teratoma, showing tissues derived from 3 lineages, but no tissue of OS. Discussion: These results show that OS cells can be reprogrammed. iPSC line from G-292 has not been reported before. Reprogramming changed the property of the OS cell line, G-292, to a teratoma-forming cell line rather than a primary tumour-forming cell line. This iPSC line can be propagated indefinitely and allows further studies to be carried out on the reprogrammed cells.

P-H21. A case report of in vitro reactions due to antibody that reacts with chemicals present in the commercial red blood cells suspension media

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Introduction: In vitro red red blood cells (RBCs) reactions due to reagent related antibodies occasionally occurred in the routine pretransfusion testing. This type of antibodies can give rise to erroneous result of ABO blood grouping, false positive reaction with antibody screening and identification or incompatible cross-matches. Case report: A 43-year-old lady was admitted for elective surgery. A blood sample was sent for pretransfusion testing. She did not have any history of blood transfusion. The blood grouping showed group B Rh D positive and antibody screening (Diamed) was positive. Direct Coombs test was negative. Antibody identification (Diamed) revealed pan-agglutination with same reaction strength and auto control was negative. The current findings were suggestive of antibody towards high frequency antigen. RBCs phenotyping for high frequency antigen showed kk, Kp(a-b+) and Lu(a-b+) and cross-match result was compatible. The RBCs phenotyping and compatible cross-match results did not support the diagnosis of antibody towards high frequency antigen. Repeated antibody screening and antibody identification (Phenocell) showed no reaction. Further antibody screening performed on washed RBCs (Diamed) was negative. These findings confirmed that patient had antibody that reacts with chemicals that was present in the RBCs suspension media of Diamed. Discussion and conclusion: It is important to differentiate between antibody towards high frequency antigen and antibody that reacts with chemicals present in the RBCs suspension media. This is because the former is clinically significant while the latter is clinically insignificant but may delay the provision of blood to patient due to additional time and efforts required for investigations.

P-H22. Haemoglobin Profiles and Haematologic Features of Newborn with Alpha Thalassemia and Haemoglobin E in Malaysia

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Introduction: Thalassemia and haemoglobinopathies are major public health problems worldwide. Newborn screening for thalassemia and haemoglobinopathies has been recommended as a tool for morbidity prevention. We use cord blood samples which were less invasive and more readily accepted by parents for screening purposes. In this study, we look at the haemoglobin and hematologic characteristics of normal and common thalassemia and haemoglobinopathy among newborns in Malaysia and to assess the effectiveness of MCV, MCH and Hb Bart’s as screening methods for α-thalassemia in newborns. Material and methods: A cross sectional study was done by collecting 300 cord blood samples. Consent was obtained from the mothers before delivery. The samples were tested for red cell indices (Hb, RBC, MCV and MCH). Haemoglobin analyses were performed using CE (Sebia CAPILLARYS2). DNA analyses were performed for --SEA, --α3.7, and -α4.2 deletion.
Results: From 300 cord blood samples examined, α-thalassemia was detected in 29 (9.6%) newborns, Hb E was detected in 33 (11%), 2 of the newborns were having compound heterozygous for Hb E and α-thalassemia 2 (βEβ/-3.7αααα) and 1 (0.3%) was having Hb S. The Hb, MCV and MCH were significantly lower in newborns with α-thalassemia 2 compared to normal newborns. The cut off value for MCH is 33.65pg and the cut off value for MCV is 101.65 fL for prediction of α-thalassemia 2 with sensitivity of 88.7% and 85.5% respectively.

Discussion: From this study, we observed the MCV and MCH in those cases with Hb Bart’s were significantly lower than that of newborns without Hb Bart’s. The haemoglobin, MCV and MCH of newborns with α-thalassemia 2 were observed to be significantly lower than those newborns with αα/αα genotype. However, we observed that Hb Bart’s in cord blood was not a useful marker for identification of α-thalassemia 2.

P-H23. Frequency of anaemia among UiTM medical undergraduates

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Introduction: Anaemia is a global public health problem in both developing and developed countries. World Health Organisation (WHO) reported the prevalence of anaemia in women of childbearing age is 30.2%, of which 35.7% is found in South-East Asia. A local data reported the presence of hypochromic microcytic red cell indices in 14.5% medical students. Methods: This was a cross-sectional study aiming to estimate the number of medical students with anaemia. A total of 100 medical students, aged 19 to 23 years old, enrolled. Blood samples collected in EDTA tubes were analyzed for haemoglobin (Hb) level, mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH). A structured questionnaire in relation to anaemia, was also given to the subjects. Results: There were more female (n=87) than male (n=13) participants. 16 students were found to have anaemia, of which all were females. None of the male students have anaemia. The mean Hb was 13g/dl (min 9.6g/dl, max 15.9g/dl), of which 9% have hypochromic microcytic red cells. The mean MCV is 87.36fl (min 66.2fl, max 95.3fl) and mean MCH is 28.18pg (min 18.3pg, max 32.4pg). Of 16 students with anaemia, only two complained of tiredness and one gave a history of menorrhagia. 2 of 29 students who claimed they exercise regularly had anaemia. None of the participants gave a history of chronic disease. Conclusions: This study highlighted the presence of anaemia amongst medical students. Unfortunately, due to financial constraint, no further investigation could be carried out within the scope of this study.


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Introduction: Haemoglobin Constant Spring (Hb CS) results from mutation (T-C transition) of the termination codon 142 of the α2-globin gene and produce an elongated α chain. The mRNA is very unstable and the rate of α chain synthesis is reduced to about 1% of normal. Hb CS is the most common non-deletional α thalassemia and is important cause of HbH-like disease in Southeast Asia. The Hb CS heterozygote is clinically and haematologically normal, however the homozygote shows a clinical picture of thalassemia intermedia. Case report: 11-year-old girl presented with fever and non-immune haemolytic crisis with abnormal Hb analysis. From the high performance liquid chromatography (HPLC), small peak of abnormal Hb (1.7%) in the C-window at retention time of 5.01 min was detected and capillary electrophoresis (CE) showed probable of Hb CS in zone 2 (4%). Further DNA analysis using multiplex ARMS-PCR confirmed presence of termination codon (TAA à CAA) mutation (Hb CS). Parental screening showed father had heterozygous α-thalassemia 3.7 deletion and Hb CS mutation, whereas mother had HbCS mutation only. Both parents are asymptomatic. Discussion and conclusion: α−chain may have deterious effects on cellular and membrane properties of Hb CS-containing RBCs and these changes in turn could account for increased hemolysis. These could explain the pathology of the hemolysis in the patient and thus had clinical picture resemble thalassemia intermedia. For this case, the zigosity of the Hb CS is not known yet as the test is not preceded yet.
P-H25. Gene expression in obstetric antiphospholipid syndrome: A systematic review
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Introduction: Antiphospholipid syndrome (APS) is a multisystem disease that may present as venous or arterial thrombosis and pregnancy complications. Previous studies indicate that genes are differentially expressed between normal and in the disease state. Hence, we undertook a systematic review to systematically search the literature on human gene expression that was differentially expressed either on placental tissue or blood in Obstetric Antiphospholipid Syndrome. Methodology: We performed an electronic search till March 2015 through PUBMED and EMBBASE databases; using keywords related to gene, antiphospholipid, obstetric, pregnancy. From 502 studies retrieved from the search, only original publications that had performed gene expression analyses of human placental tissue or blood that reported on differentially expressed gene in pregnancies that had Obstetric APS were included. Two reviewers independently scrutinized titles and abstracts before examining the eligible of studies that met the inclusion criteria. For each study, patient data, diagnostic criteria for APS, method for analysis and the gene signature were extracted independently by two reviewers. Results: Four eligible gene expression studies involving the obstetric APS comprising the datasets on gene expression were identified. Three studies showed the reduction of transcript expression on PRL, STAT5, TF, DAF, ABCA1 and HB-EGF in Obstetric APS. Only one study discussed the polymorphism in STAT4 and BLK that have strong association with Obstetric APS. Discussion: ABCA1, STAT4, STAT5, BLK, PRL, TF, DAF, and HB-EGF are differentially expressed in Obstetric APS. Conclusion: These can be targeted as candidate genes for further analysis to elucidate the pathogenesis of this condition.

P-H26. Regulation of microRNA in antiphospholipid syndrome
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Introduction: Antiphospholipid antibodies are autoantibodies that attack phospholipid through anti-b2-Glycoprotein-1. The actions by these antibodies are associated with various sites of thrombosis and pregnancy morbidity which is also known as the antiphospholipid syndrome (APS). The pathogenesis of APS is still not elucidated. Recently, microRNA expressions in many types of diseased tissues have been identified and linked to the involvement in both pathology and progression of diseases. Therefore, a systematic review was performed to search the literature for research papers focusing on microRNA expression profile in APS. Methodology: An electronic search was performed till March 2015. Three search engines; EBCOHST, PROQUEST AND OVID were used to identify literature related to expression of specific microRNA in APS. Two reviewers independently scrutinized titles and abstracts before examining the eligible of studies that met the inclusion criteria. A total of 350 papers were found and screened. Results: Only one study fulfilled the inclusion criteria. The microRNAs found to be associated with APS regulation were miR-19b and miR-20a. No data was found on specific microRNA expressed in obstetric antiphospholipid syndrome. Discussion: Limited data on expressions of microRNA in APS suggests that further research in this field is required. Conclusion: Characterization of microRNA profile in blood and also in placenta tissue of patients with APS could be a useful model to explain the regulation of certain genes involved.

P-H27. Systematic review on maturation pattern sensitivity in the diagnosis of myelodysplastic syndrome (MDS)
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Introduction: Clinical history, morphological appearance and cytogenetic data are required in identifying cases of Myelodysplastic Syndrome (MDS). However, this clonal stem cell disorder is still widely heterogeneous. The current approaches in diagnosis are inherently subjective and lack sensitivity. Altered maturation patterns using flow cytometry analysis have been reported to be useful for identification of MDS. This systematic review aims to assess the sensitivity of maturation pattern in obtaining MDS diagnosis. Methodology: Electronic databases (MEDLINE, PROQUEST, OVID, Scopus, Web of Science) were searched till April 2015 which yielded 677 articles. Snowballing and hand-search was also employed. Two reviewers assessed each article independently using the following inclusion criteria: all types of MDS; WHO or FAB classification; diagnostic; immunophenotyping. Results: Twenty-one papers that met our inclusion criteria were analysed. Four-colour strategy was mostly used in the analysis. Samples were bone marrow aspiration and prepared using either whole blood lysis or Ficoll-density gradient centrifugation. The most studied lineage in diagnosing MDS using maturation pattern was myeloid, mainly look at CD34 positive, CD11b/CD16, CD13/CD16 and CD235a/CD71 expression patterns. Five studies showed sensitivity between 70 to 98 percent. The variation of parameters used was justified to increase the sensitivity of maturation pattern in diagnosing MDS. Discussion: Highly reproducible parameters increased the sensitivity of maturation pattern. Strategies
employed varied in assay design and instrument set-up. Standardization on flow data analysis and reporting is also lacking. Conclusion: Maturation pattern has shown significantly high sensitivity and may be used as an ancillary technique for the diagnosis of MDS.

P-H28. The relation of adiposity and haematological malignancy

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Introduction: Excess bodyweight or adiposity is an epidemic health problem that increases the risk of various types of cancer. Adipose tissue produces hormones known as adipocytokines, which participate in carcinogenesis in many solid tumours. Leptin was shown to have mitogenic effects in cancer cell lines thus promoting the malignant behavior of cancer. Adiponectin has a significant anti-inflammatory effect and showed an inverse relation with solid tumours risk. It was hypothesized that adiponectin provides protection against carcinogenesis. However, the knowledge on the relation of adiposity and adipocytokines with haematological malignancies is limited and the findings were not consistent. Materials and Methods: We studied this feature in newly diagnosed haematological malignancy cases in Malaysia. Diagnosis was made according to World Health Organization (WHO) guidelines or the French-American-Britain (FAB) classification. The body mass index (BMI), waist hip ratio, adipocytokines levels (leptin and adiponectin) were measured in subjects (n=29) and healthy control (n=18).

Results: There was no significant difference in the mean BMI of control and subjects. However, the mean waist hip ratio in subjects were significantly higher (0.91) compared to control (0.82) with p=0.04. The mean level of leptin was markedly raised in subjects compared to control (1.80 vs 17.41) with p=0.00. The mean adiponectin level was significantly suppressed in subjects (6.54 vs 0.15) with p=0.00. Discussion: This study supports the evidence that adiposity and adipocytokines are related to haematological malignancy similar to that of solid tumours. We also concluded that waist hip ratio is a better index of adiposity compared to BMI.

P-H29. Beneficial effect of date palm (Phoenix dactylifera) in iron deficiency anaemia: A systematic review

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Introduction: Iron deficiency anaemia (IDA) is a global health problem. It is considered as an important contributing factor to global burden of disease. It is an indicator of poor nutrition and poor health. Based on the Al-Quran and prophetic Sunnah, dates are mentioned as the superfood for preservation of health. Therefore, we are evaluating the potential of dates as a treatment for IDA from published reports. Methods: The search was conducted on four electronic indexed databases namely Medline, Ovid, Scopus, and PubMed. The search yielded 2156 articles. Bibliographies of screened reports and relevant reviews and manuscripts were also searched using Google search engine. Data reporting involved systematic reviews and report of the study according to PRISMA guidelines. Results: There were only three articles on the effects of dates in IDA that meets the inclusion criteria. Significant increase in haematological parameters (haemoglobin level, red blood cell count, packed cell volume and platelet count) was reported in all three studies (p<0.05). There was no change on the total white blood cell count, differential white blood cell and bone marrow (p>0.05). Conclusion: This review suggested that Phoenix dactylifera gives beneficial effect stimulates in iron deficiency anaemia. However, the reports on this matter are very limited. There are several gaps in the available evidence. Further studies are needed to provide a comprehensive understanding of this matter.

P-H30. An unusual case of post-partum haemorrhage

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Introduction: Acquired haemophilia A is a rare bleeding disorder due to production of autoantibodies that inhibit factor VIII (FVIII) activity. Reported incidence is 1.3 to 1.5 cases per million populations per year. Case report: We reported a case of a 34-year-old Para 1 woman with underlying systemic lupus erythematosus who experienced profuse bleeding from a left inferior epigastric artery and subcutaneous haemorrhage after delivery a healthy baby by cesarean section. Her activated thromboplastin time (aPTT) was prolonged at 90.1 seconds. Immediate mixing study was corrected but 2-hour incubation mixing study was not corrected and a time dependent inhibitor was suspected. Further testing confirmed the presence of a high titer [26 Bethesda Unit(BU)] factor VIII (FVIII) inhibitor and a very low (1%) FVIII level. The patient was started on prednisolone, cyclosporin, and rituximab in successive courses. Despite this, the patient’s aPTT remained elevated and rebled from the inferior epigastric artery. A follow up FVIII inhibitor titer increased to 80 BU. Recombinant activated clotting factor VII (rFVIIa) was given to achieve haemostasis. As well, plasma exchange followed by plasmapheresis was initiated and
she responded well. At discharge, she was asymptomatic of bleeding with 2BU FVIII inhibitor titer and 10% FVIII level.

Discussion and conclusion: Acquired haemophilia A should be suspected in unusual bleeding associated with a prolonged aPTT. Risk factors include underlying malignancy, post-partum state and autoimmune diseases. Most cases are related to first pregnancies. Prompt recognition is essential in order to establish diagnosis and initiate early treatment. Fortunately, almost 100% patients with pregnancy associated factor VIII inhibitors survive.

P-H31. A case of therapy related myeloid neoplasm with complex aberrant karyotypes

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Introduction: Individuals who are exposed to cytotoxic agents are at risk of developing therapy-related myeloid neoplasms (t-MN). t-MN is a heterogeneous disease comprising of acute myeloid leukemia (t-AML), myelodysplastic syndrome (t-MDS) and myelodysplastic syndrome/myeloproliferative neoplasms (t-MDS/MPN). Case report: A 59 year-old man was diagnosed with acute promyelocytic leukaemia (APML) in 1999. He underwent all-transretinoic acid (ATRA) based chemotherapy and achieved complete remission. In 2003, the disease relapsed and he was given idarubicin, mitoxantrone and ATRA followed by maintenance chemotherapy (ATRA, mercaptopurine and methotrexate). He was in remission until 2014 when full blood picture showed leukocytosis (21.4x10^9/L), anaemia (Hb 8.4g/dL) and leukoerythroblastic picture. Bone marrow examination showed hypercellular marrow with trilineage dysplasia and 3% blasts. Fluorescence in situ hybridization study of PML/RARA was negative. Karyotyping revealed complex abnormality (49,XY,-4,del(7)(q31),-9,-12,t(19;?)(q13.4;?),+21,+5mar). A diagnosis of t-MDS/MPN with unfavorable karyotypes was made. The disease progressed rapidly and transformed into t-AML in less than four months complicated with severe pneumonia. Despite aggressive treatment with antibiotics and chemotherapy (daunorubicin and cytarabine), patient succumbed to the illness 2 weeks after the diagnosis. Discussion and conclusion: Diagnosis of t-MN should be suspected in patients who have a history of exposure to cytotoxic agents. The prognosis of t-MN is poor and risk stratification by karyotype is a strong predictor of survival. Complex aberrant karyotypes have placed this patient into unfavorable group with poor outcome. There is limited data on the treatment of t-MN and clinical practice differs vastly. More research is required to address the optimal management for this population of patient.

P-H32. The need of routine pre-operative coagulation screening tests for patients undergoing cardiac surgeries in Clinical Training Centre (CTC) UiTM

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Introduction: For the past two years, CTC UiTM has been performing a number of cardiothoracic surgeries and pre-operative coagulation screening tests are done routinely. The aim of the study is to review whether there is positive correlation between prolonged coagulation tests and usage of blood/blood product during/after the operation. Materials and Methods: 52 patients who underwent various cardiac surgeries from May 2014 to January 2015 were divided into 2 categories; Group A (15 patients) characterized by abnormalities in either one or both prothrombin time/activated partial thromboplastin time (PT/ aPTT) and Group B (37 patients) characterized by normal PT/aPTT tests. The baseline pre-operative haemoglobin (hb) and platelet were compared with intra-operative hb and platelet. The blood/blood product usage during/after operation were reviewed. Results: The mean of pre-operative hb was 13.3g/dL and platelet 261x10^9/L while the mean of intra-operative hb was 10.3g/dL and platelet 181x10^9/L. 14 patients (93.3%) from Group A and 33 patients (89.2%) from Group B required blood/blood product transfusion. 6 from 14 patients (40%) of Group A and 20 from 33 patients (54.1%) of Group B required single transfusion only. One patient (6.7%) from Group A and 4 patients (10.8%) from Group B did not require blood/blood product transfusion. Discussion: Generally, pre-operative coagulation screening tests do not appear to be useful in predicting usage of blood/blood product during/after operation (p=0.646). Hence, these tests are not indicated routinely unless other medical conditions associated with bleeding tendencies are suspected.

P-H33. Penicillium Marneffei: A case report from Hospital Ampang

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Introduction: Penicilliosis is a disseminated and progressive fungal infection caused by a facultative intracellular pathogen and the only thermally dimorphic fungus of this genus, Penicillium Marneffei. It is an opportunistic pathogen which has emerged to become an AIDS-defining illness among HIV-positive patients in endemic areas such as South East Asia. Case report: We report a case of a 29-year-old HIV infected Thai female who presented with pancytopenia, fever, weight loss, skin lesions and hepatomegaly. Full blood picture confirmed the pancytopenia; MGG stained marrow aspirate showed numerous macrophages filled with fungal spores. These fungi show the presence of intracellular fission and confirmed as a
fungal organism by GMS and PAS staining of trephine. Hematopoietic elements were markedly reduced in both aspirate and trephine consistent with involvement by fungaemia. Discussion and conclusion: The identity of this organism was established by marrow culture, though uncommon. Histologically, the cells of Penicillium marneffei are resemble those of Histoplasma capsulatum. This may account for early cases being misdiagnosed as histoplasmosis. However, detection of non budding yeast cells with characteristic central transverse septum would give a presumptive diagnosis of Penicillium marneffei and this should be confirmed by culture. P.Marnefferi should be considered as one of the differential diagnosis for infective causes in the work up of a young immunocompromised patient presented with fever and pancytopenia. Prompt diagnosis followed by effective antifungal therapy may lead to improve clinical outcome for the patient.

P-M1. Can the usual risk factors associated with candiduria also predict for non-albicans candiduria?
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Introduction: Candiduria is the presence of Candida sp. in urine. Many centres do not routinely identify yeasts in urine samples, so it is important to know if the risk factors for candiduria in general may also predict for non-albicans candiduria due to the higher occurrence of fluconazole resistance in non-albicans Candida. Materials and methods: A total of 64 urine samples with yeasts were collected from 64 different patients over a 1-year period. The yeasts were speciated through culture on CHROMagar and carbohydrate assimilation testing using ID 32 C. Demographic data were collected for all patients with candiduria. Results: Candida albicans accounted for 38 (59.4%) and non-albicans Candida accounted for 26 (40.6%). Of the 13 patients admitted in intensive care, 8 had albicans candiduria and 5 had non-albicans candiduria (p=1.000). Out of 37 female patients, 24 had albicans candiduria and 13 had non-albicans candiduria (p=0.316). Of the 49 catheterized patients, 28 had albicans candiduria and 21 had non-albicans candiduria (p=0.563). Of the 56 patients who received antibiotics, 33 had albicans candiduria and 23 had non-albicans candiduria (p=1.000). Out of 9 patients who received antifungals, 5 had albicans candiduria and 4 had non-albicans candiduria (p=1.000). Of the 42 patients with diabetes mellitus, 28 had albicans candiduria and 14 had non-albicans candiduria (p=0.116). Discussion: Risk factors for candiduria cannot be relied upon to predict for non-albicans candiduria. Thus, laboratory identification should still be done to ascertain if a patient has non-albicans candiduria, especially if specific antifungal therapy is warranted.

P-M2. Molecular typing study of carbapenemase-producing Klebsiella pneumoniae isolates in Malaysia for genetic relationship determination
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Introduction: The emergence and spread of carbapenem-hydrolysing β-lactamases amongst Enterobacteriaceae represent a serious issue in hospitals as these organisms have been associated with major outbreaks and are becoming important nosocomial pathogens. DNA-based strain typing methods are increasingly used for epidemiological purposes. We report a study on Pulse Field Gel Electrophoresis (PFGE) and Multilocus Sequence Typing (MLST) of carbapenemase-producing Klebsiella pneumoniae isolates obtained from various hospitals in Malaysia. Materials and method: A total of 26 isolates from various states hospitals throughout Malaysia were selected and subjected to MLST typing. Primer pairs that amplify seven housekeeping genes for Klebsiella pneumoniae were used. Results were blast in the database at Institute Pasteur MLST website to determine distinct sequence typing (ST). Nineteen isolates were also analyzed using PFGE and results were interpreted by fingerprinting software (Bio-Rad Laboratories). Result: Malaysian isolates are clonally diverse. Based on MLST, most common was ST147. PFGE identified 15 unique patterns that form into 2 major groups of organisms (defined as 87% band identity). Discussion: Carbapenemase-producing Klebsiella pneumoniae in our hospital settings are genetically diverse. PFGE and MLST are useful tools for epidemiological study. However, based on the unique STs or patterns determined, PFGE is more discriminatory for discerning outbreak isolates.

P-M3. Melioidosis in Eastern Coast of Peninsular Malaysia
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Introduction: Melioidosis is a fatal disease caused by gram negative bacteria, Burkholderia pseudomallei. The disease is endemic in Southeast Asia and northern Australia. Following recent massive flood in Kelantan, Terengganu and Pahang, the incidence of melioidosis becomes a public concern. Previous reports from Australia and Thailand have shown higher incidence of melioidosis during monsoonal wet season. A study done in Singapore has also shown strong association of melioidosis with total rainfall. In Malaysia, there is little information on the occurrence of melioidosis during northeast
monsoonal season. The eastern coast areas (Kelantan, Terengganu and Pahang) are the main areas affected by the monsoonal wet season. **Material and methods:** Institute for Medical Research Kuala Lumpur is the main centre for the serological diagnosis of melioidosis in Malaysia. All positive results for melioidosis from Kelantan, Terengganu and Pahang from November 2010 to February 2015, totalling up to 2,188 cases were evaluated. The positive cases were divided into three groups: pre-monsoon (July – October), monsoon (November - February) and post-monsoon (March – June). **Results:** There was a higher incidence of melioidosis cases in monsoon group with average of 188.6 cases, followed by pre-monsoon group, 181.25 and post-monsoon group, 146.25. However, there is statistically no significant difference between the three groups (Mann-Whitney test, p<0.05). Patients aged between 41-50, males and Malays had higher incidence compared to other groups. **Discussion:** This study shows that there is no strong linkage between monsoon events and melioidosis infection. There are other factors to be considered for the occurrence of melioidosis.

**P-M4. Analysis of Non-typhoidal Salmonella in Northern Malaysia Tertiary Hospital in Perlis**

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**Introduction:** This retrospective study was conducted at Hospital Tuanku Fauziah, Kangar, Perlis describing prevalence of non-typhoidal *Salmonella* (NTS), serogroups and serovars distribution, bacteraemia potential with clinical association and trends of antibiotic resistance patterns. **Materials and methods:** Demographic, clinical, laboratory and antimicrobial sensitivity data from 2010 to 2014 were collected and analyzed. **Results:** Mean patients age was 22.4 years old, predominantly Malay (87%, 145/169), 41 serovars identified with highest being *Salmonella* serogroup E (31%, 54/169). Paediatric group was highest in NTS gastroenteritis and the elderly was highest in extraintestinal salmonellosis (P<0.05). The commonest serovars in gastroenterointestinal site was *Salmonella* Weltevreden (34.5%, 50/145). *Salmonella* Enteritidis was highest for extraintestinal sites (81.3%, 15/24), reflecting true blood invasiveness ratio (BIR) of 36.6%. AIDS was the main underlying disease (5 cases, 27.8%) and 14 patients (78%) had at least one underlying disease. Hepatitis C was higher in immunosuppressed patients (P<0.05). Mortality rate (16.7%, 3/18) and other underlying factors were not associated (P>0.05) with an immunosuppressed state, indicating possible good patient management with prolonged hospitalization. Different antibiotic resistance levels were noted for ampicillin, AMP (12.8%), ciprofloxacin, CIP (3.6%), chloramphenicol, C (5.9%) and trimethoprim/sulfamethoxazole, SXT (0.8%). Multi-resistant case was low (1%) with AMP-CIP-SXT resistance in *Salmonella* Typhimurium and AMP-CIP-SXT-C resistance in *Salmonella* Billa. **Discussion:** The geographical location of Perlis state may be a factor in the diversity of *Salmonella* serovars resulting in slight prevalence and resistance pattern differences compared to other Malaysian states. Ongoing surveillance for NTS infections and antibiotic resistance is needed to control this pathogen.

**P-M5. The Prevalence and Demographic Study of Positive Antinuclear Antibodies (ANA) in Northern Region Hospitals of Malaysia**

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**Introduction:** This is a prevalence study of antinuclear antibodies (ANA) conducted in Hospital Tuanku Fauziah, Kangar, Perlis. **Materials and methods:** This is a convenience sampling over a 6 month period of year 2014. Samples collected from Northern Region Hospitals in Malaysia (Perlis, Kedah and Pulau Pinang). **Results:** ANA test was performed for patients with suspected connective tissue diseases (CTD) and autoimmune disorders. Mean patients age was 22.4 years old, predominantly Malay (87%, 145/169), 41 serovars identified with highest being *Salmonella* serogroup E (31%, 54/169). Paediatric group was highest in NTS gastroenteritis and the elderly was highest in extraintestinal salmonellosis (P<0.05). Patients aged between 41-50, males and Malays had higher incidence compared to other groups. **Discussion:** There is a higher incidence of melioidosis cases in monsoon group with average of 188.6 cases, followed by pre-monsoon group, 181.25 and post-monsoon group, 146.25. However, there is statistically no significant difference between the three groups (Mann-Whitney test, p<0.05). Patients aged between 41-50, males and Malays had higher incidence compared to other groups. **Discussion:** The sex hormones estrogen, androgen and prolactin have all been proposed as having a role in autoimmune diseases. These prevalence data will serve as a useful baseline for future investigations of changes in ANA prevalence over time.
P-M6. Syphilis and HIV co-infection in UKM Medical Centre

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Introduction: Syphilis is a sexually transmitted disease caused by Treponema pallidum subspecies pallidum. Recent epidemiological studies show an increasing number of cases in relation to HIV infection which predominantly occur among men who have sex with men (MSM). This study is done to describe syphilis and HIV co-infection. Materials and Methods: This retrospective study was conducted on patients who were serologically confirmed to have syphilis from year 2010 to 2012. Those without HIV serology results were excluded from further analysis. Patients’ epidemiological and clinical data were obtained from patients’ files at the Department of Health Information and recorded in a data collection form. Results: A total of 53 syphilis-confirmed patients were included in the study (58.5% males, 41.5% females). The age of the patients ranged from 13-84 years, with a mean age of 46.43 ± 16.55 years. The majority of the patients were aged between 20-39 years (41.5%). Most patients were diagnosed at the latent stage (60.4%). HIV was diagnosed in 14 patients (26.4%). Syphilis and HIV co-infection occurred predominantly in males (p=0.0001), are frequently symptomatic (p=0.0001), are diagnosed at an early stage of syphilis (p=0.0001) and has a rapid plasma reagin (RPR) titer of ≥1:32 at diagnosis (p=0.017). Discussion: Syphilis in a patient with HIV is likely to be diagnosed at an early stage because of the symptomatic nature of the disease. The RPR titre at presentation in this group of patients is also high.

P-M7. Prevalence and type distribution of human papillomavirus (HPV) in Malaysian cervical cancer patients

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Introduction: Assessment of the prevalence and type distribution of human papillomavirus (HPV) is important for designing effective cervical cancer prevention programs. In this study, we evaluated the prevalence and type distribution of HPV among women with cervical cancer in Malaysia. Materials and Methods: Total DNA was isolated from the cervical smear specimens of 120 histopathologically-confirmed cervical cancer patients. Viral-specific DNA was amplified with biotinylated primers and hybridized to HPV type-specific probes via a flow-through process for determination of the presence and type of HPV. Results: Overall, HPV infection was detected in 106 specimens (88.3%). Among the HPV-positive samples, the most prevalent HPV types were HPV-16 (38.0%), HPV-18 (27.1%) and HPV-58 (6.2%). A similar distribution was observed in Malaysia when the data was stratified by ethnicity, with the prevalence of the three HPV types being 38.3%, 32.1% and 8.6%. In non-Malays, however, the three most prevalent HPV types were HPV-16 (47.1%), HPV-18 (26.5%) and HPV-81 (5.9%). In Malays when the data was stratified by ethnicity, with the prevalence of the three HPV types being 38.0%, 32.1% and 8.6%. Among the 53 specimens for which data on cancer histopathological subtype is available, the most prevalent HPV types in squamous cell carcinomas were HPV-16 (43.8%), HPV-18 (18.8%) and HPV-33 (12.5%). In adenocarcinoma, HPV-16 and HPV-18 remained the most prevalent HPV types (41.2% and 35.3% respectively), while HPV-31, HPV-45, HPV-58 and HPV-81 each made up 5.9% of the total infections. On the other hand, cervical carcinomas of other histopathological types contained HPV-18 exclusively (100.0%). Discussion: Slight difference exists on the distribution of HPV types in Malaysian cervical cancer cases of different ethnicity and histopathological subtypes.

P-M8. Hepatitis B immune status among non-physician healthcare workers born pre and post vaccination era: The UiTM experience

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Introduction: Hepatitis B immunity in healthcare worker (HCW) is mandatory to prevent occupational-related infection. Those born in 1990 onwards are expected to be immunised, following routine immunization in 1989. Those born prior to that are immunised if vaccine were taken as part of their occupational requirement. We investigated the Hepatitis B immune status in non-physician healthcare workers, among those born in pre and post-vaccination era. Materials and Methods: We retrospectively studied the immune status of relevant HCWs which include nurses, ‘pembantu perawatan kesihatan’ (PPK), science officer (SO) and Medical Laboratory Technician (MLT) in Faculty of Medicine, Universiti Teknologi MARA. Sera were tested for presence of Hepatitis B antibody (anti-Hbs) using Elecsys 2010 immunoassay. Staffs with titre of more or equal to 10mIU/L were considered immunised.
Result: A total of 216 personnel were screened in 2014 which consist of 158 nurses, 37 PPKs, 3 SOs and 18 MLTs. The respective numbers and percentages of immunised HCWs by categories are as follows, nurses 128 (81%), PPK 4 (11%), SO 1 (33%) and MLT 8 (44%). Discussion: Almost all nurses, with the exception of one, born post-vaccination era were immunised. As expected, the majority of non-immunised nurses were those born before 1990. Interestingly, all four immunised PPKs were those born pre-vaccination era but the six PPKs born in post-vaccination era were not immunised. All SO and MLTs tested were born in pre-vaccination era. This study highlighted the fact that Hepatitis B immune status screen is mandatory for all HCW, regardless of whether they were born pre, or post vaccination era.


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Introduction: The prevalence of antimicrobial resistance of both encapsulated and non-typeable Haemophilus influenzae has been increasing in recent years. This phenomenon engenders upsurge of beta-lactamase-negative ampicillin-resistant (BLNAR) strain too. However, the impact of these important changes has received little attention. This study was undertaken to describe the resistance profiles and BLNAR occurrence in clinical H. influenzae isolates. Materials and methods: Fifteen H. influenzae isolates were isolated from sputum between August 2010 and April 2015, from Microbiology laboratory, Faculty of Medicine, UiTM. These isolates were tested for susceptibility testing towards ampicillin, amoxicillin-clavulanic acid, co-trimoxazole, azithromycin, chloramphenicol, cefuroxime and ceftriaxone by disc diffusion method. Detection of BLNAR was performed by using nitrocefin disc on ampicillin resistant isolates based on recommendation from Clinical Laboratory Standards Institute. Results: H. influenzae isolates demonstrated 53%, 33%, 20% and 7% of resistance rate towards ampicillin, amoxicillin-clavulanic acid, co-trimoxazole, and chloramphenicol respectively. However, all isolates were susceptible to azithromycin, cefuroxime and ceftriaxone. There was 47% of BLNAR detected from 15 H. influenzae isolates. Discussion: The resistant rates of H. influenzae isolates from our centre are comparable worldwide. This includes prominent ampicillin resistant rate, as well as significant BLNAR circumstance. The consequence of BLNAR includes reduce activity of ceftriaxone, while usage of oral amoxicillin-clavulanic acid and cephalosporin has contributed to its occurrence. Moreover, BLNAR involves mutation of the ftsI gene. Hence, determination of minimum inhibitory concentration of antibiotics and molecular studies need to be performed, to assist surveillance to effectively guide empirical antibiotic therapy.

P-M10. Therapeutic effect of Ipomoea Batatas extracts on induced psoriasis in mice

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Introduction: Psoriasis is a non-curable, T-cell mediated autoimmune chronic skin disease with a prevalence of 5% in Malaysia (cf. world average of 2%). Treatment options are essentially symptomatic dominated by oral or topical steroids and in severe cases require anti-cancer drug such as methotrexate or anti T-cells drug likes cyclosporine. Sweet potato (Ipomoea batatas) leaves (SPL), a common edible plant in Malaysia has been anecdotally reported by a number of psoriatic patients to be effective in inducing remission. Materials and methods: Psoriasiform lesions were induced in 15 male BALB/c mice using imiquimoid for 21 days and divided into 3 equal groups. One group was designated as control and the other two groups were subjected to topical application of aqueous SPL extract and lipophilic SPL extract respectively. Modified psoriatic Area and Severity Index (PASI) score, histological changes and intracellular cytokines of TNF- and IFN- were compared between the groups for pre-induction, post induction and post treatment. Result: Both groups subjected to topical SPL extracts showed significant improvement compared to control group with slight therapeutic superiority shown by the lipophilic extract in all parameters. No toxicity effect evidenced by histological examination noted. Discussion: Synergistic effect of bioactive compounds within the SPL extracts especially by the lipophilic components showed promising potential to be a steroid-sparing anti-psoriatic medication. Further confirmation of its potential using psoriatic patients should be undertaken to assess its true potential.
P-M11. Antimicrobial effect of green tea leaves “Camellia sinensis” on the skin normal flora

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Introduction: Medicinal plants have been a major source of therapeutic agents for alleviation and cure diseases. Camellia sinensis (green tea) is known for its therapeutic properties (anti-inflammatory, anti-oxidative and anti-ageing). Although, anti-microbial properties of green tea have been studied, its role against skin commensal bacterial is not well known. The aim of this study was to determine in vitro inhibitory activity of green tea extract on some odorous skin commensal flora. Materials and methods: Tea leaves were collected from MARDI Agro technology Park, Cameron Highland. A standardized protocol was used to extract green tea. Aqueous green tea extracts were tested for antibacterial activity by well diffusion method. Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) assays were performed by broth microdilution assays using green tea extract concentrations from 16 to 0.0313 mg/ml. Results: Camellia sinensis extract showed higher antibacterial activity against skin commensal bacteria. The high antimicrobial effect was achieved against Micrococcus luteus with MIC and MBC (0.125 and 0.25 mg/ml) followed by Staphylococcus epidermidis (0.25 and 0.25 mg/ml), Bacillus subtilis (0.5 and 0.5 mg/ml), and Corynebacterium xerosis (0.5 and 1.0 mg/ml). Discussion: This study confirms in vitro anti-microbial activity of green tea extract against skin commensal bacteria. The antibacterial effects of green tea against odors skin bacteria with its anti-oxidant and anti-aging properties will help in keeping skin healthy, fresh and reducing unpleasant odors.

P-M12. Hepatitis B prevalence among Malaysian indigenous population

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Introduction: In Malaysia, Hepatitis B prevalence is about 5%, a data reflective of major population groups, the Malay, Chinese and Indian. Studies in other countries demonstrated that prevalence among minor, indigenous groups of population, are higher compared to general population within the same area. In Malaysia, little is known about its corresponding status in Malaysian indigenous population, the Orang Asli. Materials and methods: This study estimated Hepatitis B prevalence among the Orang Asli. One hundred and forty-four samples were collected from Orang Asli of three subtribes; the Bateq and Mendriq (from Negrito tribe) as well as Temiar (Senoi tribe). Sera were tested on the HBsAg assay on Elecsys 2010 analyser. Reactive samples were retested in duplicate. Full blood count and liver function tests done to assess clinical status. Results: Twelve positive samples gave a prevalence rate of 8.3%. Male to female ratio was of 2:1 and age ranges between 20 to 46 years old. Additional parameters for corresponding individuals fell within normal range. Demographic data classified them as hunter-gatherers; and primary school as highest level of formal education. None were injecting drug user but half of them (n=6) admitted to heterosexual promiscuity. Discussion: This pilot study suggests that Hepatitis B prevalence among Orang Asli is higher than that of non-indigenous population, in keeping with those demonstrated from worldwide studies. Not surprisingly, sexual transmission appeared to be the main route of transmission. More comprehensive study is required to support this preliminary data, in effort to curb this disease among Orang Asli specifically and Malaysians as a whole.

P-M13. Elevated level of Serum Amyloid A and reduced level of apoA-I expression in severe human leptospirosis

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Introduction: Leptospirosis is a zoonotic infectious disease affecting many people in tropical, subtropical and temperate countries. The mortality caused by severe leptospirosis is very high. Material and Methods: Nine paired samples were obtained from subject admitted at Hospital Sultan Haji Ahmad Shah with written consent. The subjects had a history and clinical manifestations suggestive of leptospiriosis. Patients were classified as mild leptospirosis (ML) or severe leptospirosis (SL) using the classification outlined in Leptospirosis Clinical Practice. In this study, two-dimensional electrophoresis analyses in combination with MALDI-TOF spectrometry results were compared among mild and severe leptospirosis-infected patients. The expression level of protein was verified by ELISA/ western blot and significant difference of spots in two groups were analysed statistically. Results: The biomarker, serum amyloid A (SAA) and ApoA1 were found consistently upregulated and
downregulated respectively in severe case. The results obtained from ELISA/ western blot test also strongly supported the finding. Discussion: The increasing level of SAA repressed the expression of apoA-I. The apoA-1 protein was decreased four fold in severe compared to mild leptospirosis cases and it was significant different between two groups ($p < 0.05$). This might be due to SAA displacing apoA-1 and associates rapidly with high density lipoprotein (HDL). SAA is mainly deposit in spleen, kidney and liver. It may suppress lymphocytic response to antigens and inhibit platelet activation and aggregation. In conclusion, our results suggest SAA protein level is elevated while apoA-1 protein level is reduced in severe human leptospirosis infection.