

**The 9<sup>th</sup> Annual Scientific Meeting of the College of Pathologists, Academy of Medicine Malaysia was held at the Faculty of Medicine & Health Sciences, Universiti Putra Malaysia, Serdang, Selangor from 26-27 June 2010. Abstracts of scientific presentations follow:**

**SYMPOSIA PAPERS**

**SP1. Virtual autopsy**

Mohd Shah Mahmood

*National Institute of Forensic Medicine, Hospital Kuala Lumpur*

Virtual autopsy is the utilisation of high-resolution imaging technology such as multi-sliced computed tomography (MSCT) or magnetic resonance imaging (MRI) on a dead body and has been proposed to complement or even replace the well established conventional autopsy. There are advantages and limitations to this new non-invasive postmortem examination technique. The advantages are (i) non-invasive 'scaple-less' technique (ii) digital imaging takes only a few minutes (iii) locating retained foreign bodies / bullets and three dimensional reconstruction of bullet tract (iv) better detection of bony injuries especially spine fracture (v) detection of air embolism and pneumothorax (vi) three dimensional digital images can be stored permanently without loss of imaging detail which will allow for later re-examinations and second opinions. There are several limitations to virtual autopsy; (i) histological and metabolic informations not available to conclusively determine the cause of death (ii) not able to detect causes of death for all natural diseases (iii) not able to demonstrate active contrast extravasations to visualise the circulatory activity (iv) cost-effectiveness as compared to conventional autopsy.

HKL has the first Forensic Postmortem CT Research Facility in Malaysia and has officially started scanning all deaths investigated by the Police since 1<sup>st</sup> June 2010. This research facility will provide the Forensic Pathologists, Radiologists and Odontologists the avenue to conduct and promote research activities in postmortem CT technology. Research priority will be on the future possibility of performing postmortem CT ONLY on road traffic accidents fatalities in determining the cause of death.

Many studies worldwide has shown that imaging autopsies still could not totally replace the conventional autopsies and postmortem CT is a very good complementary modern tool for conventional autopsy.

**SP2. Human papilloma virus vaccine: are our ladies protected?**

Ilina Isahak

*Universiti Kebangsaan Malaysia Medical Centre*

Infection with human papillomaviruses (HPV) is universal. Development of technologies for cloning and sequencing genes showed that HPV DNA could be found within many cervical cancers and precancer lesions. The low grade cervical dysplasias identified in Pap screening tests are simply productive HPV infections. In contrast, high grade dysplasias and cancers do not produce virions because viral gene expression is limited to two viral oncogenes, E6 and E7. HPV is the most common sexually –transmitted virus and many strains of HPV progress into cancers of the cervix, mouth, anus, vagina and penis.

The World Health Organization (WHO) estimates worldwide annual incidence of HPV infection

is 660 million, with low- and high dysplasia being 30 million and 10 million cases, respectively. WHO also estimates 30 million cases of genital warts occur every year. In Malaysia, cervical cancer rank second in the 10 most frequent cancers in females 2003-2005 after breast cancer. Cervical cancer is a major cause of life-years lost in women in the developing world. Many women in developing countries contribute more than men to the food and health needs of the family. The incidence of vulva intraepithelial neoplasia (VIN) is increasing worldwide. HPV 16 appears to be the predominant HPV type associated with high-grade VIN. 42-65% of VIN1 cases are associated with HPV types 6 and 11. For cervical cancer, the predominant type in all regions, ranging from 52% in Asia to 58.1% in Europe, was HPV 16. HPV 18 was the second most common type worldwide with prevalence ranging from 12.7% in South/Central America to 22.2% in North America. Vaccination against HPV types 16 and 18 has the potential to prevent more than two thirds of invasive cervical cancer worldwide.

Primary prevention through vaccination is the foundation of a comprehensive approach to cervical cancer prevention. HPV is a sexually transmitted infection, usually acquired within the first few years after sexual debut. Therefore, the ideal age for HPV vaccination is prior to sexual debut before any exposure can occur. Higher neutralizing antibody responses were observed in female adolescents, compared with young adult women. Although screening programmes have obvious benefits, the burden of inadequate Pap smears and the moderate number of cervical abnormalities is still evident. The primary target population for vaccination should be based on age of sexual initiation and feasibility of delivery infrastructure to reach young adolescent girls (i.e. through schools, healthcare facilities, community-based methods). For most countries, this population is likely to be girls 9 or 10 through 13 years of age. It is commendable that Malaysia's Ministry of Health will embark on HPV vaccination programme for the ladies this year.

### **SP3. Influenza A (H1N1) – is the second wave real?**

Yasmin A Malik

*Department of Medical Microbiology, Faculty of Medicine and Health, International Medical University*

On 24 April 2009, the World Health Organisation (WHO) put out its first disease outbreak mention of the 'swine flu'. Within 3 days, the pandemic alert was elevated from phase 3 to phase 4, and it quickly moved to phase 5 two days later. On 11 June 2009, with 80 countries confirming their first cases, the WHO raised the pandemic alert level to phase 6. The virus had spread throughout the world in 6 weeks.

An epidemic exists when new cases exceed the prevalence of a disease, in excess of the usual level of expectancy. The seriousness and severity of the disease also influences the definition of an epidemic. If the disease is life threatening only a few cases needs to occur to constitute an epidemic. In light of the significantly high case fatality rate for 2009 influenza A (H1N1) in young adults, this may be the reason why the WHO made such an early announcement alert.

Second pandemic waves do not occur in situations when there is a specific vaccine available to induce herd immunity, for it will modify the distribution of the strains. Herd immunity is based on the notion that if a population is mostly protected from a disease by immunization, then the chance of a major epidemic occurring is highly limited. The second wave of the 2009 Influenza A (H1N1) that occurred in Europe and North America was probably due to the delay in producing enough of the specific monovalent vaccine in time to initiate adequate herd immunity. In the United States, the first wave occurred from April to July 2009. During the first wave, the rate of symptomatic cases was 1.4% (1/70) and the death rate was 0.05% (1/2000). While it appeared that the death toll was comparable to most influenza seasons, there have been many more deaths in young adults. At the end of August, the influenza activity began to increase again, peaking in October. This was inevitable since adequate vaccines was only made available at the time when the second wave had already begun. By the end of November, 50 million (15% of the US population) people have been infected with 10,000 deaths and the second wave is finally resolving. Even then, 97.7% of the viral

isolates were still the 2009 Influenza A (H1N1). Meanwhile, the seasonal flu had not started yet. By 15 May 2010, only Hawaii reports local influenza A (H1N1) activity. Canada also experienced the second wave since the initiation of mass vaccination only began in late October, which was the same time when peak influenza activity was occurring. As expected a substantially greater number of cases were recorded. By January 2010, rates of ILI dropped below the historical seasonal baseline in the United Kingdom, the first wave occurred also at around the same time from April to July 2009. By mid-October, the second wave of the pandemic had begun with 27,000 confirmed cases in England alone and 106 deaths. In light of the limited number of vaccine at the that time, vaccination was only given to those at risk. By the end of December 2009, enough vaccines was available to be given to the general public.

Fortunately, Australia did not experience a second wave because the pandemic occurred at around the same time as the seasonal flu. The first wave of influenza hit mid-May 2009 and lasted 18 weeks. The pandemic strain of influenza A (H1N1) actually accounted for 90% of influenza isolates by week 8. The influenza A (H1N1) had pushed out seasonal flu and was the dominant flu during their winter season. By late August 2009, WHO reported that most countries in southern hemisphere had passed their peak influenza activity and returned to baseline activity. Australia initiated their mass vaccination in late September 2009.

As of 30 May 2010, more than 214 countries and overseas territories or communities had reported laboratory confirmed cases of the pandemic influenza A (H1N1) 2009, with over 18,138 deaths. Considering that seasonal influenza kills 250,000 to 300,000 people worldwide, this pandemic H1N1 is mild by comparison.

#### **SP4. Apo E: the culprit?**

Baizurah Mohd. Hussain

*Department of Pathology, Hospital Ampang, Selangor, Malaysia*

Apolipoprotein E (ApoE) plays a major role in transport of cholesterol. Apo E is the activator of lecithin-cholesterol acyltransferase (LCAT), which forms cholesteryl esters in HDL. Apo E exists in different polymorphic genetic isoforms and coded by three alleles  $\epsilon 2$ ,  $\epsilon 3$  and  $\epsilon 4$  at a single gene locus. There are variations of Apo E depending on the different genotype. Apo E, part of very low density and high-density lipoproteins, mediate the binding of lipoprotein particles to LDL and chylomicron receptors, thereby mediating plasma response to dietary cholesterol. Apo E acts as a ligand between triglyceride rich lipoprotein particles, hepatic low-density lipoproteins and chylomicron remnant receptors.

ApoE is the new culprit that has been associated with diseases like Alzheimer's Disease and Coronary Artery Disease. ApoE also influences dietary fat that determines the size of LDL. It also increases the risk of carotid atherosclerosis and hence, increases susceptibility to strokes. It has been known to modulate accelerating actions of alcohol and obesity in atherosclerosis. It has also been associated with other diseases including colon cancer, rectal cancers, lipoprotein nephropathy, severe Ig A nephropathy, gallstones, development of clinical macroalbuminuria in Non-Insulin Dependant Diabetes Mellitus. The role of ApoE has been implicated in many other diseases but needs further research.

**SP5. Cytogenetics in the age of DNA**

Roziana Ariffin

*Genetics Department, Hospital Kuala Lumpur*

**Background:** Comparative genomic hybridization, otherwise known as CGH, analyzes chromosome at the DNA level. The advancement of array CGH enables the identification of chromosomal abnormalities through digital karyotyping. **Methods:** Since 2008, 175 patients were referred to Kuala Lumpur Hospital (HKL) with dysmorphisms and various congenital anomalies albeit a normal karyotype. Array comparative genomic hybridization (CGH) was then carried out in these patients using BlueGnome 1Mb Enhanced Cytochip. **Results:** aCGH discovered 57.7% positive cases including 75 deletions and 36 duplications in the patients. The copy number variants ranged from approximately less than 1Mb to 25Mb with an average 9-10 CNV in each individual. Eleven familial studies had been done so far. Eight cases were identified as *de novo* while three were familial inheritance. The study in the remaining families is on going. In cases of CNV which had been reported as polymorphic in less than 10% of the population refer (Redon et. al, 2006) will be followed up with parental FISH validation study if there is a clinical suspicion of pathogenicity of the CNV. **Conclusion:** Identification of chromosomal imbalances has significantly contributed to the detection of genes that play a pathogenic role and the elucidation of molecular mechanisms responsible for defined phenotypes in malignant or congenital diseases.

**SP6. Updates in the management of haematological malignancies – the role of the laboratory**

K M Chang

Imatinib has revolutionized the management of chronic myeloid leukaemia. From a median survival of 3-5 years whereby majority of patients would need to undergo a stem cell transplant, imatinib is now the first line therapy for almost all CML patients. It inhibits the kinase activity of the BCR-ABL1 oncoprotein and the IRIS study (International Randomised Study of Interferon and STI571) showed an actuarial overall survival of 86% at 7 years. However only 57% of the patients are still in complete cytogenetic response at the end of 7 years. With this success and the cost of treatment RM8000-16000 per month, it is imperative that patient responses are monitored carefully and any loss of response or resistance would warrant a change in dose or change in therapy. The European Leukaemia Net has published consensus guidelines (revised in 2009) with definitions of suboptimal response and failure of response taking into consideration the availability of newer more potent TKIs and need for stem cell transplant as alternative. Amongst the tests recommended are cytogenetic responses based on karyotyping or FISH, molecular responses based on an International Scale and the detection of mutations.

The advent of novel therapies and targeted agents has change the paradigm of treatment in the management of haematological malignancies. Classifications are now based on biologic entities, therapies are tailored based on these biological markers and better prognostic markers based on biologic features are developed to determine the need for more intensive treatments e.g. monoclonal antibodies and stem cell transplantation. This has created a tremendous stress on the routine haematology lab to develop newer tests to meet these demands.

In the Ministry of Health, an additional fund of more than 50 million per year has been specially apportioned to making these expensive treatments available. In order that these treatments are optimally used and be made cost-effective, we need to make laboratory tests available for diagnosis, monitoring and prognostication. We need to seriously sit down and strategize on how to develop and centralize these tests.

**SP7. Science and serendipity in the era of molecular pathology**

John E.J. Rasko

*Cell and Molecular Therapies, Royal Prince Alfred Hospital; Gene & Stem Cell Therapy, Centenary Institute; Sydney Medical School, University of Sydney, Australia*

In this talk I wish to highlight the importance of molecular pathology and ‘accidental’ discoveries that arise from clinical observations. My aim is to encourage increased activity in all aspects of biomedical exploration – especially emphasising the discipline of Pathology. I will describe our research involving inborn errors of human renal metabolism, as it has provided surprising insights into human metabolic pathways and genetics. Amino acids are crucial components of most essential biological processes. Following absorption in the intestine and delivery to peripheral tissues they can serve a plethora of functions including protein synthesis, regulation of cellular metabolism, production of metabolic energy, cell growth and neuronal signalling. Our particular interest was originally focused on autosomal recessive disorders of neutral amino acid transport. In Hartnup disorder (OMIM 234500), neutral amino acid absorption is severely impaired in both kidney and intestine, resulting in excessive amounts of neutral amino acids in the urine. In familial iminoglycinuria (OMIM 242600) excessive proline, hydroxyproline and glycine are excreted; and in Dicarboxylic aminoaciduria (OMIM 222730) there is defective renal and perhaps intestinal transport of two acidic amino acids, glutamic and aspartic acid. The hallmark massive urinary loss of neutral amino acids in Hartnup disorder can be compensated by a protein-rich diet if available; whereas a poor diet may lead to severe symptoms such as a photosensitive pellagra-like skin rash, cerebellar ataxia and other neurological symptoms. We identified the SLC6A19 gene that encodes a sodium-dependent neurotransmitter transporter (B<sup>0</sup>AT1). Our Consortium and a group from NIH reported that mutations in SLC6A19 represent the underlying defect leading to wastage of neutral amino acids in Hartnup disorder (1). More recently we have discovered the complex genetics of human iminoglycinuria and hyperglycinuria and modeled the development of transport systems in mice (2). This knowledge has provided further insight into the Hartnup and iminoglycinuria phenotypes, whilst furthering our understanding of the complex renal and gastrointestinal amino acid transport systems.

1. Seow, H, Bröer S, Bröer A, Bailey, CG, Potter, SJ, Cavanaugh, JA, Rasko JEJ Hartnup disorder is caused by mutations in the neutral amino acid transporter, SLC6A19 *Nature Genetics* 2004; 36:1003-1007
2. Bröer, S, Bailey CB, Kowalczyk, S, Ng, C, Vanslambrouck, JM, Rodgers, H, Auray-Blais, C, Cavanaugh, JS, Bröer, A and Rasko JEJ, Iminoglycinuria and hyperglycinuria are discrete phenotypes explained by complex mutations in proline and glycine transporters, *J. Clin. Investigation*, 2008;118(12):3881-92

**SP8. Pathology training in Malaysia**

Lai-Meng Looi

*Department of Pathology, University of Malaya*

In post-independence Malaysia, most pathologists acquired their training and qualifications through the Diploma in Clinical Pathology Courses (UK) or the Royal Colleges of Pathologists of Australasia or UK. Recognising that overseas training would not be able to meet National needs, local training programmes for medical specialists were mandated in the early 1970s and vested in the Universities. The University of Malaya (UM) pioneered the Master of Pathology (MPath) Programme in 1973. It started as a 2-year course modeled after the MRCPPath Part I, and was upgraded in 1987 to a 4-year programme modeled towards the MRCPPath Part II (Final) examinations. Similar programmes were established by UKM in 1988, USM in 1992 and UPM in 2009. The MPath programmes were to address 3 major National needs: (1) diagnostic pathologists for patient care services, (2) academic

pathologists for Universities & training institutions, and (3) medical researchers in pathology. Over the years, the programmes have evolved in complexity and sophistication, finally adopting a common curriculum and examination system designed through an Inter-University Postgraduate Pathology Committee (Pathology Conjoint Board). Since 1995, the MPath programme has taken a two phase structure: Phase 1 (year 1) comprising equal rotational training in the 4 main disciplines of pathology followed by a vetting multidisciplinary Part I examination, and Phase 2 (years 2, 3 & 4) comprising advanced training in a monodiscipline, with research exposure, ending with a probing exit examination. The acceptance of an “open” system alternative, whereby trainees can be trained in centres outside the Universities (such as Ministry of Health Pathology Departments) was a landmark development. While much has been achieved, many challenges remain. Among them are issues of quality versus quantity, accreditation of training centres, research supervision, progress monitoring and examination hosting. The time has also come to consider our standing in the international arena, which has implications on Malaysia’s competitiveness in the global scene.

### **SP9. Clinical pathologist: a new requirement?**

Norain Karim

#### *Ministry of Health Malaysia*

The number of pathologists in the Ministry of Health of Malaysia has increased steadily over the years. Currently, there are 184 Pathologists (78 Anatomical pathologists, 48 Haematopathologists, 28 Microbiopathologists, 13 Chemical pathologists, and 21 Forensic pathologists). In the 1980’s, Anatomic pathology was the main discipline of interest. Over the last 10 years, other disciplines including transfusion and genetics have become more developed. Overall, the developments in the field of Pathology have been substantial, both in terms of technological advances and greater impact on patient outcomes. Along with higher patient expectations, subspecialties have sprung up in all disciplines. With this development, it is apparent that no one person can master all the new knowledge and integrate it into diagnostic decision-making especially in the larger and more specialized hospitals (major state hospitals and hospitals with specialization in centers of excellence). We have now reached a tipping point. There is need and demand for pathologists with highly specialized expertise.

While most of the clinical disciplines have been expanded in state hospitals and the centres of excellence, district hospitals with specialists have also developed clinical specialties which require the full complement of pathology services. It may be ideal to have pathologists in all sub-specialties at all these hospitals, but attainment of this ideal may not be possible in the foreseeable future. Although Anatomical Pathology (AP) service is required by clinicians in all hospitals, their placement as resident pathologists in smaller settings generates some concerns. The low workload and less variety of cases will impact the skillset of these pathologists. Several other issues also arise. Should the Anatomical pathologists cover the haematology service especially in morphologic diagnosis? Should they also provide consultations and supervise the other clinical disciplines? What is the role of non-AP pathologists in other disciplines particularly haematopathologists as their numbers are increasing and they are being posted to the district hospitals. Should their role be extended to areas such as microbiology and chemistry too? Should the clinical pathology service be left to technologists and scientists? How much professional oversight of these services can be provided remotely in a safe fashion? Don’t we need professional pathology oversight in the non-AP pathology services in these hospitals? While the answers to these questions are far from easy they merit serious consideration for the provision of a 21<sup>st</sup> century diagnostic service.

While the profession may seek solace in the fact that this problem is not unique to pathology, but also faced by the other clinical disciplines like medicine and surgery. A proposal for some rationalization will be discussed in detail.

**SP10. Challenges of private practice in Pathology**

Pathmanathan R

*Sime Darby Medical Centre Subang Jaya*

The challenges of a private pathology practice are varied, and depend very much on the type of practice one is in. In Malaysia, the 2 main types of private pathology practices are institutional/hospital based practices (such as in government based academic departments of pathology or private hospitals) and corporate providers of anatomical pathology services (in short, private laboratory services). Group practices either in general pathology or as multispecialty groups are not here yet, but may represent a next step in the development of private pathology services. The challenges faced by a pathologist in private practice are many. Many of these impact on issues of professional competency, career development, financial and ethical issues, the ever present threat of litigation, and the challenge to continue to remain current and relevant in an environment that emphasizes, quality, rapid turn-around time and patient safety.

**SP11. Emerging roles of the College of Pathologists, AMM, in Malaysia**

Soon-Keng Cheong

*President, College of Pathologists, Academy of Medicine Malaysia*

The College of Pathologists, Academy of Medicine of Malaysia (CPathAMM) was formed on 22<sup>nd</sup> June 1999 upon merger of the Malaysian Society of Pathologists of Malaysia with the Chapter of Pathologists, Academy of Medicine of Malaysia. With its formation, CPathAMM becomes the only body representing the pathologist profession in Malaysia. The First Annual General Meeting was held on 27<sup>th</sup> May 2000 and this year is our 10<sup>th</sup> Annual General Meeting. It is timely to examine the roles CPathAMM in the face of new challenges in the immediate future. All these issues have been raised in the strategic planning of CPathAMM in February 2009 and are in various stages of implementation by the Council of CPathAMM. The first issue was the formation of a not-for-profit company to establish external quality assurance programmes (EQAP) for different disciplines of pathology. There is an urgent need for CPathAMM to play this coordinating role as many of the pathology laboratories in Malaysia could not afford the high cost to subscribe to the overseas EQAP and local EQAP are limited and uncoordinated. The second issue is postgraduate training in pathology specialties. Every year, there are many potential candidates who are unable to enter the local Master of Pathology Programmes due to a variety of reasons. Perhaps we should develop an alternate pathway to serve these candidates with the help of our colleagues in private practice (the lost talents) and overseas sister colleges. This is especially true in pathology subspecialty training such as genetics, neuropathology, metabolic medicine, haemostasis medicine, and so on. The third issue is the promotion of continuous professional development (CPD) for our members and fellows and also the pathology community at large. Undergraduate medical education has gone from the traditional face to face teaching to adopting the blended learning model. The computer professionals have evolved from an institution-based computing environment to a cross-boundary cloud computing new order. Are we to be left behind? CPathAMM should play an active role to embrace the new web generation and offer web-based CPD programmes for our colleagues, especially gaining CPD points would soon be mandatory for specialty practices. The fourth issue is perhaps the hardest – to appeal to our fellow colleagues to get ready for the new challenges ahead. Pathology services in Malaysia like in other places of the World have entered an era where the number of specimens coming to the laboratory would progressively dwindle, albeit slowly. This is due to advances in science and technology where diagnoses could be achieved without tissue or fluid specimens such as the advent of virtual colonoscopy, virtual histology, microendoscopy, tissue-specific *in-vivo* biomarker studies, advanced point-of-care testing devices, and so on. Resistance among pathologists to train in new technology and adopt new roles further compounds these challenges. If we recognise that pathology is medicine, we must now strengthen our position as practising clinicians. Pathologists

should emerge from the perception by our other clinical colleagues that we are “back room persons” and elevate the departments under their charge from a supporting service to a clinical department. The return to physician role has been adopted by some pathology practices quite successfully such as the fine-needle aspiration, clinical blood transfusion, clinical haematology, metabolic medicine, and infection control services provided by the respective pathology specialties. There is an urgent need for us to broaden these scopes so that pathologists will remain relevant to the new era of medical care.

## POSTER PRESENTATIONS

### P1. DNA isolation from whole blood using in-house method versus commercialized techniques: Quality and cost consideration

Norlelawati AT<sup>1</sup>, Salina M<sup>1</sup>, Nor Zamzila A<sup>1</sup>, Mustafa Fadzil FW<sup>2</sup>

<sup>1</sup>Kulliyah of Medicine, International Islamic University Malaysia; <sup>2</sup>School of Distance Education, Universiti Sains Malaysia.

**Background:** Isolation of deoxyribonucleic acid (DNA) from whole blood is among the crucial steps prior to molecular diagnostic or research in human genetics. As the isolation techniques have progressively evolved, the manual techniques for DNA purification have been gradually replaced by automation and purification kits. Though expensive, these techniques were preferred due to their superiority in extracting DNA from small volume of sample and their safer and rapid procedure. **Objective:** The purpose of this study is to compare the cost and quality of DNA purification between manual technique and the commercialized techniques. **Method:** Manual salting out procedure technique was adopted and optimized to purify DNA from small volume of blood. The quality and quantity of DNA were compared to two commercialized techniques, QIAmp DNA extraction kit (Qiagen) and fully automated technique (Magstration 12C, Precision). The costs per extraction were calculated based on the actual cost incurred for the procurement of all reagents, chemicals and kits used in this study. **Result:** Apart from being a relatively safe and cheap technique, we found that the optimized salting-out procedure was able to extract comparable quantity and quality of DNA from small volume of blood. **Conclusion:** The main advantage of in house DNA purification method was its minimum costs. The method is also suitable in laboratory with good human resource but limited financial support.

### P2. Oxidized-low density lipoprotein level among organophosphates (OPs) pesticide exposed workers

Nor Zamzila Abdullah<sup>1</sup>, Ishaka Aminu<sup>1</sup>, Niza Samsuddin<sup>2</sup>, Razman Mohd Rus<sup>2</sup> and Abdul Hadi Mohamed<sup>3</sup>

<sup>1</sup>Department of Basic Medical Sciences,<sup>2</sup>Department of Community Health and Family Medicine, <sup>3</sup>Department of Anesthesiology and Intensive Care, International Islamic University Malaysia, Bandar Indera Mahkota, 25200 Kuantan, Pahang.

**Background:** Organophosphate (OP) is one of the widely used pesticides worldwide. It has been shown to induce oxidative stress in both animal and human. Oxidative stress may stimulate the oxidation of lipoprotein particularly the low density-lipoprotein (LDL) which is known to promote atherogenesis. PON1 is a high density-lipoprotein (HDL) related enzyme which is recognized for its function to hydrolyze OP into a relatively less toxic substance and prevent atherosclerosis by hydrolyzing oxidized-LDL (ox-LDL). Low PON1 activities were reported in OP exposed individuals, while in different studies it was associated with a higher risk of coronary artery disease (CAD).

However, a link between chronic OP exposure, PON1 activity and ox-LDL which is known for its contribution in the development of atherosclerosis has not yet been reported. The aim of this report was to compare the level of PON 1 activities and ox-LDL between workers who are exposed to OP and the comparative non-exposed group. **Methods:** A cross sectional study was carried out and 51 pesticides sprayers were selected from four farms in Kuantan who fulfilled the criteria while 48 control subjects were selected based on matching process of age, ethnicity and income bracket. Serum samples were analyzed for ox-LDL by ELISA method while PON1 activities were determined spectrophotometrically after the hydrolysis of its substrates paraoxon, phenylacetate and diazoxon. **Results:** A significantly lower diazoxonase activity and higher ox-LDL level ( $p < 0.001$ ) was observed among the OP exposed group. The PON1 to ox-LDL ratio which probably reflect the ability of PON1 to hydrolyze ox-LDL were also significantly lower ( $p < 0.05$ ) among the OPs exposed group. **Conclusion:** Our data demonstrated that the increased ox-LDL may result from the reduced serum antioxidant capacity of PON1 in OPs exposed individuals. This may contribute to the development of atherosclerosis. A larger scale study is required to confirm our observation.

### P3. Evaluation of Anti-TPO assay on Roche Cobas e411 Immunoassay System

Intan NS<sup>1</sup>, Baizurah MH<sup>2</sup>, Subashini CT<sup>1</sup>, Hannah P<sup>2</sup>, NorBaizurah B<sup>2</sup>

<sup>1</sup>Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Selangor; <sup>2</sup>Hospital Ampang, Ministry of Health

**Background:** Anti-TPO antibody is the most commonly measured autoantibody for the investigation of thyroid autoimmune disorders. Anti-TPO antibody is present in 90% of cases of Hashimoto's thyroiditis and in 60-80% of Graves' disease. **Aim:** To assess the analytical performance of Roche Cobas e411 for the analysis of anti-TPO antibody. **Methods:** Imprecision and accuracy studies were performed using 2 quality control (QC) levels. For within-run imprecision, both QC were assayed 30 times on the same day, whereas for between run imprecision, analyses of each levels of QC were performed daily on 30 working days. Interference study involved the analysis of haemolytic, lipaemic and icteric samples on anti-TPO assay. Stability of anti-TPO on storage were evaluated by comparing fresh anti-TPO samples and samples stored at 3 different storage temperatures (4°C, -20°C and -80°C) on day 3 and day 12 of storage. **Results:** The within-run coefficient variations (CV) for low and high QC were 7.7% and 2.5%, whilst the between-day coefficient variations (CV) for low and high QC were 6.5% and 11.3%, respectively. The percentage deviation from the true value was -4.9% and -3.6% for low and high QC, respectively. Anti-TPO showed variable recovery from 92.0% to 119.0% on day 3 and day 12 of storage. **Conclusion:** Roche Cobas e411 immunoassay has demonstrated acceptable within-run precision for anti-TPO assay. The inaccuracies are acceptable as their deviations from the target value are within  $\pm 10\%$  and within  $\pm 2SD$ . It is recommended that samples for anti-TPO are analyzed immediately as variable recovery was obtained on storage.

### P4. Evaluation of Anti-TG assay on Roche Cobas e411 Immunoassay System

Subashini CT<sup>1</sup>, Baizurah MH<sup>2</sup>, Intan NS<sup>1</sup>, Hannah P<sup>2</sup>, NorBaizurah B<sup>2</sup>

<sup>1</sup>Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Selangor; <sup>2</sup>Hospital Ampang, Ministry of Health

**Background:** Thyroglobulin, produced in the thyroid gland, has an essential function in the iodination of L-tyrosine and in the formation of the thyroid hormones T4 and T3. The frequency of thyroglobulin autoantibodies (anti-TG) is approximately 70-80% in subjects with autoimmune thyroiditis, including Hashimoto's disease, and 30% in individuals with Graves' disease. Anti-TG assay is important for use in monitoring the cause of Hashimoto's thyroiditis and for differential diagnosis of (cases of suspected autoimmune thyroiditis of unknown origin with negative anti-TPO

test result, Graves' disease without lymphocytic infiltration and to rule out interference by anti-TG in TG test). *Aim:* To assess the analytical performance of Roche Cobas e411 for the analysis of anti-TG antibody. *Methods:* Imprecision and accuracy studies were performed using 2 quality control (QC) levels. For within-run imprecision, both QC were assayed 30 times on the same day, whereas for between run imprecision, analyses of each levels of QC were performed daily on 30 working days. Interference study involved the analysis of haemolytic, lipaemic and icteric samples on anti-TG assay. Stability of anti-TG on storage were evaluated by comparing fresh anti-TG samples and samples stored at 3 different storage temperatures (4°C, -20°C and -80°C) on day 3 and day 12 of storage. *Results:* The within-run coefficient variations (CV) for low and high QC were 5.2% and 3.0%, whilst the between-day coefficient variations (CV) for low and high QC were 7.0% and 11.5%, respectively. The percentage deviation from the true value was 3.5% and 12.7% for low and high QC, respectively. Anti-TG showed variable recovery from 89.8% to 117.0% on day 3 and day 12 of storage. *Conclusion:* Roche Cobas e411 immunoassay has demonstrated acceptable within-run precision for anti-TG assay. The inaccuracies are acceptable as their deviations from the target value are within  $\pm 10\%$  and within  $\pm 2SD$ . It is recommended that samples for anti-TG are analyzed immediately as variable recovery was obtained on storage.

#### P5. Gestational hypertension and the pregnancy outcomes: A case-control study

Jabrullah AH<sup>1</sup>, Norhafizah M<sup>1</sup>, Malina O<sup>2</sup>, Andi Anggeriana AA<sup>3</sup>, Wan Hamilton WH<sup>4</sup>, Rohani A<sup>3</sup>

<sup>1</sup>Department of Pathology, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia; <sup>2</sup>Department of Medical Microbiology and Parasitology, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia; <sup>3</sup>Department of Obstetrics and Gynaecology, Faculty of Medicine and Health Sciences Universiti Putra Malaysia, Clinical Campus Hospital Kuala Lumpur; <sup>4</sup>Department of Obstetrics and Gynaecology, Serdang Hospital.

**Background:** Gestational hypertension (GH) is the most common hypertensive disorder in pregnancy. Various maternal and foetal complications can develop due to this disease. The purpose of this study was to study the frequency of adverse maternal and foetal outcomes in women who developed GH and to compare with normotensive pregnant women. *Methods:* Pregnant women who attended outpatient clinics at General Hospital Kuala Lumpur and Hospital Serdang were selected and divided into case (GH) and control (healthy normotensive) groups. Inclusion criteria were pregnant women who were between 20–40 weeks of gestation, aged between 18–40 years and singleton pregnancy. Exclusion criteria were multiple gestations, essential hypertension prior pregnancy, significant medical history (e.g. diabetes, cardiovascular disease), and other significant obstetric problems during pregnancy. Outcome variables were analyzed using contingency chi-square ( $\chi^2$ ) or two-tailed fisher test at significant level of  $p < 0.05$ . *Results:* A total of 118 patients, consisting of GH cases ( $n=54$ ) and normotensive control ( $n=64$ ) pregnant women were studied. Pre-eclampsia developed in 8 (15%) of GH women. Compared to the control group, the cases (GH) had higher rates of various adverse outcomes namely low birth weight, small for gestational age, meconium-stained liquor and admission to neonatal intensive care unit ( $p < 0.05$ ). The other outcomes like poor progress, caesarean delivery, instrumental deliveries, low APGAR score and neonatal jaundice were also increased in GH group however the value did not reach statistical significance ( $p > 0.05$ ). *Conclusion:* Gestational hypertension is associated with various adverse obstetric outcomes to the mother and foetus as demonstrated in this study. Further studies with a match case-control design with a larger scale involving broader parts of the country are required to provide population-based registry.

**P6. Post allogeneic HSCT chimerism analysis: A comparison between indel real-time PCR and VNTR microfluidic chip based assay**

Kamariah Ibrahim, Chong Soo Sin, Ariffin

*University Malaya Cancer Research Institute, Faculty of Medicine, University of Malaya, Kuala Lumpur*

**Background:** To ensure successful engraftment following haematopoietic stem cell transplantation, monitoring chimerism status of donor and recipient entities is vital. The standard method for chimerism monitoring by using short tandem repeats (STR) markers and the cost is quite exorbitant. Variable number of tandem repeats (VNTR) and insertion-deletion polymorphisms (indels) are alternative choices in which these are DNA polymorphic markers that have been characterized widely in the human genome. We aim to compare the accuracy, sensitivity and feasibility of VNTR and indel in post HSCT setting. **Methods:** DNA from twenty-eight donor-recipient pairs who underwent allo-HSCT was initially screened for informative VNTR and indel markers. We adopted six VNTR and six indel panels for screening purposes. Once informative markers were attained, a total of 100 post HSCT samples of various time points underwent quantitative chimerism analysis using indel real-time PCR and VNTR microfluidic chip electrophoresis platforms. **Results:** The Pearson correlation between the two methods was  $r = 0.8038$  (95% CI 0.72, 0.8638) ( $p < 0.001$ ). Bland-Altman data showed the standard deviations, which differed between the two methods (indel%-VNTR%) were 95% limit of agreement -29.46 to 42.91. The mean level of chimerism was 86.96% (vntr) while 80.23% (indel) and there was no significant difference ( $p > 0.05$ ) between the two. We selected four pairs of samples to determine the accuracy and the Pearson  $r$  ranges from 0.999 to 0.923. Sensitivity for indel was 0.1% while VNTR was 5%. **Conclusion:** VNTR and indel markers are promising tools to monitor haematopoietic engraftment with the fact that both are economical and feasible. Although VNTR is less sensitive than indel, both methods should complement each other. This will allow accurate management and treatment interventions for successful HSCT outcome.

**P7. CD55 and CD59 expression of erythrocytes and leukocytes in normal adult population by flow cytometry analysis**

Siti Hawa AA<sup>1</sup>, Asmah H<sup>2</sup>, Sivagengei K<sup>1</sup>, Nozi MZ<sup>1</sup>, Wan AS<sup>1</sup>, Hamidah NH<sup>1</sup>

<sup>1</sup>Department of Pathology, National University of Malaysia Medical Centre (UKMMC); and <sup>2</sup>Faculty of Allied Health Sciences, UKM, Kuala Lumpur.

**Background:** Paroxysmal Nocturnal Haemoglobinuria (PNH) is a clonal haematopoietic disorder characterized by deficiency in surface proteins that utilize glycosyl phosphatidylinositol molecule for attachment to the plasma membrane. Currently, flow cytometry analysis using anti-CD55 and anti-CD59 antibodies to evaluate CD55 and CD59 antigen expression on blood leukocytes and erythrocytes permits rapid confirmation of PNH diagnosis. To our knowledge, the normal reference ranges of CD55 and CD59 expressions have not been established for the Malaysian population. The aim of the study was to determine the expression of CD55 and CD59 in erythrocytes and leukocytes of normal adults in UKMMC. **Methods:** A total of 320 healthy adults; 153 males and 167 females, aged 16-65 years consisting of 219 Malays, 75 Chinese and 26 Indians from UKMMC were included in this study. Peripheral blood was taken for analysis with informed consent. In this cross-sectional study, the CD55 and CD59 expressions in erythrocytes and leukocytes were analyzed by flow cytometry using monoclonal antibodies. Statistical analysis was done using SPSS ver. 16. **Results:** Data showed that CD55 expression was higher in leukocytes compared to the CD59 expression. Meanwhile for erythrocytes, the expression of CD59 was higher compared to the CD55 expression. However, there was no significant difference in the expression of both markers among races and gender. The range for CD55 expression in erythrocytes was 80.17 - 171.62 and leukocytes was 277.41 - 975.09. The ranges for CD59 expression in erythrocytes and leukocytes were 67.21 - 212.18 and 100.23 - 444.10 respectively. **Conclusions:** This range could be used as a reference in our laboratory to diagnose and monitor patients with PNH. However, data from a bigger normal population is needed to further support these findings.

## P8. Characterization of human embryonic stem cells by pluripotent marker expression

Khoo Tze Sean<sup>1</sup>, Noor Hamidah Hussin<sup>1</sup>, Then Sue Mian<sup>2</sup>, Muhammad Abd Jamil Md. Yassin<sup>1</sup>, Syed Zulkifli Syed Zakaria<sup>2</sup>, Maha Abdullah<sup>3</sup>, Norfilza Mohd. Mokhtar<sup>2</sup>, Sharifah Akmal Syed Husain<sup>1</sup>, Leong Chooi Fun<sup>1</sup>, A Rahman A Jamal<sup>2</sup>

<sup>1</sup>Unit of Haematology, Department of Pathology, National University of Malaysia (UKM) Medical Center; <sup>2</sup>UKM Medical Molecular Biology Institute (UMBI), Kuala Lumpur; <sup>3</sup>Department of Pathology, Universiti Putra Malaysia, Selangor.

**Background:** Human embryonic stem (hES) cells are pluripotent cells derived from inner cell mass of 5-day-blastocyst-stage embryo. Human embryonic stem cells, being pluripotent in nature, is able to differentiate into all cell types in a human body. Besides, hES cells is also characterized by indefinite self renewal. Undifferentiated hES cells express surface markers SSEA-4, SSEA-3, TRA-1-81, TRA-1-60, and intracellular transcription factor *Oct-4*, but not differentiation marker, SSEA-1. Apart from that, hES cells in its undifferentiated state, express all *Oct-4*, *Nanog*, *FoxD3*, *Tdgfl*, *Rex-1*, and *Sox-2* genes. Characterization of hES cell lines are important, as a tool to identify the real stem cell population and its differentiated progenies. The objective of this study was to characterize hES cell line, by immunocytochemistry staining, flow cytometry approach, as well as Reverse Transcription-Polymerase Chain Reaction (RT-PCR). **Methods:** hES cells were stained with fluorochrome-conjugated antibodies specific to a panel of pluripotent markers, OCT3/4, SSEA-1, SSEA-3, SSEA4, TRA-1-60 and TRA-1-81, with human embryonal carcinoma cells(NTERA-2) serving as positive control. hES cells were then analyzed by both imaging and flow cytometry approach. Besides, RNA was extracted from hES cells, converted to cDNA by reverse transcription followed by amplification for 40 cycles with primers specific for *Oct-4*, *Nanog*, *FoxD3*, *Tdgfl*, *Rex-1*, and *Sox-2* genes. **Results:** Results showed hES cells were positive against OCT3/4, SSEA-3, SSEA-4, TRA-1-60 and TRA-1-81, but negative for SSEA-1. RT-PCR results showed that all *Oct-4*, *Nanog*, *FoxD3*, *Tdgfl*, *Rex-1*, and *Sox-2* genes were expressed in hES cells. These observations are typical for undifferentiated hES cells. **Conclusion:** In conclusion, hES cells were successfully maintained at its undifferentiated state, and remained pluripotent.

## P9. Cord blood expansion in matrix and liquid media

Azrina NA<sup>1</sup>, Hamidah NH<sup>1</sup>, Maha A<sup>4</sup>, Muhd Jamil MY<sup>2</sup>, Jamal AR<sup>3</sup>

<sup>1</sup>Department of Pathology, <sup>2</sup> Department of Obstetrics and Gynaecology, National University of Malaysia (UKM); <sup>3</sup>Medical Biology Institute, UKM Medical Centre, Kuala Lumpur; <sup>4</sup>Department of Pathology of Universiti Putra Malaysia, Selangor

**Background:** Cord blood (CB) is an important source of haematopoietic stem cells (HSCs) and increasingly banked for its therapeutic potential. The main CB limitation is that a single CB unit contains a limited number of HSCs and hence restricts its application in the adult population. Therefore, *ex vivo* expansion of CB HSCs is crucial to generate enough HSCs that are clinically relevant for this population. There have been reports on innovations of the CB expansion, however the optimal expansion conditions have not been fully determined. This study aims to compare CB HSCs growth and marker expression in matrix and liquid media. **Methods:** CB HSCs were purified using immunomagnetic beads and characterized using immunophenotyping. They were expanded in matrix and liquid media with addition of cytokines. CB HSCs growth and marker expression were examined between passages. **Results:** Our preliminary data showed that growth rate of CB HSCs in matrix media was higher than in liquid media. The expression of CD133 and CD34 HSC markers were also higher in the cells that have been expanded in the matrix media. The expression of both markers was found to decrease over passages in both media as expected. **Conclusion:** Expansion of CB HSCs appeared to be more effective in matrix media than in liquid media. The probable explanation for the difference could be due to the structural difference. The

three-dimensional structure of the matrix mimics the *in vivo* microenvironment of bone marrow which supports cell-to-cell contact and/or production of some growth factors. This indicates that matrix media may offer some advantages in the *ex vivo* expansion of CB HSCs and further study is needed to elucidate this finding.

#### **P10. DNA ploidy analysis in childhood acute lymphoblastic leukaemia by flowcytometer**

Ezalia E <sup>1</sup>, Hamidah NH <sup>2</sup>, Rahman J <sup>2</sup>, Hishamshah I <sup>3</sup>, Juraidah E <sup>2</sup>, Sivagengei K <sup>2</sup>, Nozi MZ <sup>2</sup>, Sukri WA <sup>2</sup>

<sup>1</sup>Institute for Medical Research; <sup>2</sup>National University of Malaysia; <sup>3</sup>General Hospital Kuala Lumpur

**Background:** DNA aneuploid has important implications in the treatment and prognostication of childhood acute lymphoblastic leukaemia (ALL). The flowcytometry technique provides a promising approach in the analysis of DNA ploidy due to its rapid analysis and reliable results. In our present study, we have used flowcytometry for the analysis of DNA ploidy in newly diagnosed childhood ALL cases from Hospital Kuala Lumpur and Hospital Universiti Kebangsaan Malaysia. This study also aims to correlate the status of DNA ploidy with the clinical and laboratory parameters of these childhood ALL cases. **Methods:** Bone marrow and peripheral blood specimen of these cases were assessed for response to the induction chemotherapy at day seven, fourteen and twenty eight and also at three months post-chemotherapy. We used peripheral blood or bone marrow aspirate specimen that were collected in EDTA for DNA ploidy analysis. The specimens were stained with DNA-specific dye, propidium iodide prior to the analysis by flowcytometer. Optimization of the method was performed by BDTM DNA QC particles kit. Twenty one cases of childhood ALL were analyzed from August 2007 to November 2008. The obtained DNA indexes for these cases were then compared with their cytogenetic analysis. **Results:** Our results showed that only one patient had DNA aneuploidy with hyperdiploid DNA index of 1.48, while the rest of the patients were diploid. Thus, we were unable to perform correlation study between DNA ploidy status and other prognostic factors. **Conclusion:** In conclusion, the analysis of DNA ploidy by flowcytometry among childhood ALL has been successfully optimized. However, as the population of this study was small, no correlation with DNA ploidy status and other prognostic factors was able to be obtained and thus, further study with more cases are needed.

#### **P11. Investigation of the role of Alpha Haemoglobin Stabilizing Protein (AHSP) in HbE/beta-thalassaemia patients in Malaysia**

Wai Feng Lim<sup>1</sup>, Lai Kuan Teh<sup>1</sup>, Tze Yan Lee<sup>1</sup>, Voon Kin Chin<sup>1</sup>, Karthipan Sharon Nisha<sup>1</sup>, Elizabeth George<sup>1</sup>, Jameela Sathar<sup>2</sup>, Gin Gin Gan<sup>3</sup>, Mei I Lai<sup>1</sup>

<sup>1</sup>Faculty of Medicine and Health Sciences, Universiti Putra Malaysia; <sup>2</sup>Thalassaemia Clinic, Hospital Ampang; <sup>3</sup>Haematology Clinic, University Malaya Medical Centre.

**Background:**  $\beta$ -thalassaemia is a quantitative reduction or absence of  $\beta$ -globin chain synthesis due to  $\beta$ -globin gene mutations leading to excessive unpaired  $\alpha$ -globin chains precipitates which causes membrane damage, thus ineffective erythropoiesis. HbE/ $\beta$ -thalassaemia patients have a remarkable variability in clinical severity due to globin chain imbalance and effects of other modifiers. Alpha haemoglobin stabilizing protein (AHSP), an abundant erythroid protein was discovered in 2002, that binds specifically to free  $\alpha$ -globin chains preventing its precipitation. Studies suggested that partial or full loss of AHSP may exacerbate  $\beta$ -thalassaemia phenotype in humans. In 2006, Lai *et al.* has shown that a certain allele of AHSP gene could contribute to the discordant phenotype of  $\beta$ -thalassaemia in families with similar  $\beta$  globin genotypes. We investigated the role of AHSP in HbE/ $\beta$ -thalassaemia patients in Malaysia. **Methods:** Peripheral blood samples from 109 patients were collected. Full blood count analysis and HPLC were carried out on peripheral blood. Patients with

transfusions less than three months were excluded. In the end, 38 selected samples without underlying iron deficiency or co-inheritance of alpha-thalassaemia or extra alpha genes were genotyped with ARMS PCR for beta-globin mutations followed by typing of common AHSP sequence variants by tetra-primer ARMS PCR. TaqMan<sup>®</sup> quantitative RT-PCR was employed for AHSP expression analysis and the results were correlated to the severity of HbE/ $\beta$ -thalassaemia. **Results:** AHSP expression among 38 HbE/ $\beta$ -thalassaemia patients varied up to 1.52-log differences which were positively correlated to RBC ( $p=0.040$ ) while negatively correlated to MCH ( $p=0.006$ ), MCV ( $p=0.066$ ) and HbF ( $p=0.039$ ). The significant correlation between AHSP and MCH and MCV shows that AHSP increases when there are more unpaired  $\alpha$ -globin chains and smaller RBC volume. When HbF acts as a compensatory factor in  $\beta$ -thalassaemia, AHSP plays a lesser role. **Conclusion:** AHSP could be a potential modifier to modulate the HbE/ $\beta$ -thalassaemia patients' phenotype.

## P12. Development of a non-viral technique to transfect therapeutic gene into human mesenchymal stromal cells

P-L Mok<sup>1</sup>, S-K Cheong<sup>2</sup>, C-F Leong<sup>3</sup>, K-H Chua<sup>3</sup> & A Othman<sup>3</sup>

<sup>1</sup>PPUKM-MAKNA Cancer Centre, Universiti Kebangsaan Malaysia; <sup>2</sup>Faculty of Medicine & Health Sciences, Universiti Tunku Abdul Rahman; <sup>3</sup>Faculty of Medicine, Universiti Kebangsaan Malaysia.

**Background:** Mesenchymal stem cells are attractive cell target as a vehicle for gene delivery. Properties contributing to their attractiveness include (1) ease of isolation, (2) robust growth potential, (3) amenability to gene transfer, (4) low immunogenicity, and (5) capability to migrate to injury site. The objective of this study was to develop a non-viral method to transfect erythropoietin gene into human Mesenchymal Stem Cells (MSC) and to test the capability of the transfected MSC to deliver erythropoietin protein in the *in vivo* system. **Methods:** Two types of transfection were tested in this study, i.e nucleofection and lipofection. Plasmid pRL-CMV encoding for luciferase gene was used to study the transfection efficiency and viability following transfection. The transfected cells were tested for capability to proliferate, maintain their surface markers and differentiate into the adipocytes and osteogenic lineages. A double stranded linear DNA vector, namely Minimally Defined Gene Expression (MIDGE) carrying erythropoietin (EPO) gene was then transfected into MSC. The protein was detected by *in situ* immunocytochemical staining and the amount secreted into the supernatant was measured by ELISA. The EPO transfected cells were then transplanted in the nude mouse model. **Results:** Nucleofection with pulsing programme U23 has yielded lower cell viability ( $24.8 \pm 7.0$  %) following 24 hours of transfection compared with lipofection with different ratio of DNA ( $\mu\text{g}$ ) to Lipofectamine 2000 ( $\mu\text{l}$ ) ( $43.8 \pm 8.5$  % to  $60.6 \pm 5.2$  %). The difference however was not significant ( $p>0.05$ ). Nucleofection resulted in a significantly higher ( $p<0.001$ ) transfection efficiency ( $26.1 \pm 5.6$  %) compared with lipofection ( $0.4 \pm 0.1$  % to  $0.6 \pm 0.1$  %). The single nucleofected cell was isolated and expanded. The single cell could form a clone and was found to maintain the strong expression of CD90 and CD105. The cells could also differentiate into the adipocytes and osteogenic lineage. Cells transfected with MIDGE encoding EPO could maintain a stable expression of EPO protein up to 55 days. A few of the cells were also found to strongly express EPO protein by immunocytochemical staining even after 3 months following nucleofection. Preliminary study involving implantation of EPO-transfected cells into a nude mouse showed that the EPO improved hemoglobin and hematocrit level two weeks post-implantation. Human EPO was also detected in the mouse blood sample. **Conclusion:** MIDGE vector is superior and efficient than the plasmid expression system in delivering the gene into human MSC by nucleofection. MIDGE vector is also relatively safer than the viral or plasmid expression system because it eliminates the unwanted eu- and prokaryotic genes that might be detrimental to the host recipient. Transfected cells could also stably carry and deliver the EPO protein in the mouse model.

**P13. Amplification Refractory Mutation Systems (ARMS) versus Reverse Dot Blot Hybridization (RDBH) in screening Beta Thalassaemia among Malays**Lai Kuan Teh<sup>1</sup>, Elizabeth George<sup>1</sup>, Mei I Lai<sup>1</sup>, Rozita Rosli<sup>1</sup>, Mary Anne Jin Ai Tan<sup>2</sup><sup>1</sup>Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Serdang, Selangor, Malaysia;<sup>2</sup>Faculty of Medicine, University of Malaya Medical Centre, Kuala Lumpur, Malaysia

**Background:** Beta thalassaemia is one of the common genetic disorders in Malaysia. It is a haemoglobin synthesis disorder with either reduction or absence of beta-globin chains production. About 4.5% of the population are heterozygous carriers of this disorder. Carrier couples or partners have 25% risk of having a child with homozygous beta thalassaemia in each pregnancy. Definitive diagnosis can be done with a number of DNA techniques incorporating polymerase chain reaction (PCR) techniques using primer-specific probes complementary to the mutations. **Methods:** In this study, the amplification refractory mutation system polymerase chain reaction (ARMS-PCR) and reverse dot blot hybridization (RDBH) were applied in parallel in patients clinically diagnosed as beta thalassaemia carriers. Both methods were tested to screen the six common mutations according to our previous knowledge of the spectrum of common beta-thalassaemia mutations in Malays. The following mutations were CD 26 (CAG→AAG), IVS I-5 (G→C), IVS I-1 (G→T), CD 19 (A→G), CD 8/9 (+G), CAP+1 (A→C). **Results:** The protocol based on reverse dot blot hybridization (RDBH) technology was able to screen for few common beta-thalassaemia mutations simultaneously. Diagnosis in mutation detection among Malays can be done with a lower cost and less labour-intensive. It is also easy to differentiate between heterozygous, homozygous or compound heterozygous state by using the RDBH technique. Mutations identified using ARMS-PCR was found tedious and labour-intensive as every mutation need to be identified by two separate reactions for wild type and mutant. **Conclusion:** RDBH technique is rapid and convenient in genotype characterization especially in a diagnostic laboratory offering prenatal diagnosis for carrier couples. It also will improve in patient management.

**P14. Observation of dendritic cell morphology under Light, Phase-Contrast or Confocal Laser Scanning microscopy.**Yuen-Fen Tan<sup>1</sup>, Chooi-Fun Leong<sup>2</sup>, Soon-Keng Cheong<sup>3</sup><sup>1</sup> Cell Banking Unit, PPUKM-MAKNA Cancer Centre, Kuala Lumpur, Malaysia; <sup>2</sup> Department of Pathology, UKM Medical Centre, Kuala Lumpur, Malaysia; <sup>3</sup> Faculty of Medicine and Health Sciences, University Tunku Abdul Rahman, Malaysia.

**Background:** Dendritic cells (DC) are professional antigen presenting cells of the immune system. It can be generated *in vitro* from peripheral blood monocytes supplemented with GM-CSF, IL-4 and TNF-alpha. During induction, DC will increase in size and acquire multiple cytoplasmic projections when compared to their precursor cells such as monocytes or haematopoietic stem cells which are usually round or spherical. **Methods:** Morphology of DC was visualised by conventional light microscopy after staining or phase-contrast inverted microscopy or confocal laser scanning microscopy. We describe the morphological appearances of DC captured using the above-mentioned techniques. **Results:** We found that confocal laser scanning microscopy yielded DC images with greater details but the operating cost for such a technique is high. On the other hand, the images obtained through light microscopy after appropriate staining or phase contrast microscopies were able to produce acceptable images for identification purpose. **Conclusion:** Morphological identification is just one of the methods to characterise DC. Other identifications for DC such as phenotypic expression markers and functionality tests such as mixed leucocyte reactions are essential.

**P15. Effects of *Morinda citrifolia* on platelet aggregation**

Sabariah MN, Mohd Arif AK, Zainina S, Eusni RMT, Faridah I

*Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Malaysia*

**Background:** *Morinda citrifolia* Linn. (MCL) is a plant widely used as food and medicine worldwide. It is a native plant from Southeast Asia to Australia and is cultivated in Polynesia, India, Central and northern South America. The fruit juice is in high demand in alternative medicine for different kinds of illnesses such as arthritis, diabetes, high blood pressure, menstrual difficulties, headaches, cancers, gastric ulcers, poor digestion, atherosclerosis, blood vessel problems and heart disease. Some of the diseases are associated with blood platelet activity. In this study, the properties of MCL water extract act as anti-platelet aggregation *in-vivo* was evaluated. **Methods:** Experimental animals, Sprague Dawley rats weighing 200-300g were used. The rats were treated daily for 2 weeks with *Morinda citrifolia* Linn. extracts (0, 7.5, 75, 750 mg/kg bodyweight) orally. The bleeding time and *in-vivo* aggregation were performed after treatment period. **Results:** The administration of MCL extracts prolonged bleeding time and inhibited platelet aggregation without interfering the platelet amount. These effects could be related to the presence of polyphenolic compounds in the extracts. **Conclusion:** These findings suggested that *Morinda citrifolia* Linn. extract may contribute some benefit as a supplement to prevent blood platelet related diseases.

**P16. Acquired von Willebrand Syndrome preceded Systemic lupus erythematosus: A case study**Sabariah MN<sup>1</sup>, Faridah I<sup>1</sup>, Zainina S<sup>1</sup>, Faraizah AK<sup>2</sup>

<sup>1</sup>*Department of Pathology, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia;*  
<sup>2</sup>*National Blood Center of Malaysia.*

**Background:** Acquired von Willebrand syndrome (AvWS) is a rare bleeding disorder that mimics the congenital form of von Willebrand disease (vWD) in both laboratory findings and clinical presentations. Haematological abnormalities are common in systemic lupus erythematosus (SLE). It is unusual to diagnose AvWS preceded SLE. **Method:** We described, a 46- year-old Malay gentleman, who was investigated for prolonged APTT; discovered prior to surgery. There was no known bleeding diathesis in either the patient or his family. He claimed to have recurrent episodes of epistaxis and gum bleeding for a few months prior to his presentation. **Result:** Analysis of haemostatic parameters showed a normal complete blood count and prothrombin time (PT) with prolongation of APTT, reduced level of factor VIII, von Willebrand factor ristocetin cofactor (vWF:Rco), von Willebrand factor antigen (vWF:Ag) and ristocetin-induced platelet aggregation (RIPA). Further evaluation showed he was positive for antinuclear antibody, and anti-double stranded DNA but was negative for rheumatoid factor. His clinicopathological findings were improved with corticosteroid treatment. **Conclusion:** Clinical suspicion of associated rare disorder in patient with autoimmune conditions should be given importance to enhance the optimal evaluation in perioperative management.

**P17. Reduction of Interleukin-6 mRNA in human bone marrow-derived mesenchymal stem cells post siRNA transfection**

Hoon Koon Teoh<sup>1,2</sup>, Pei Pei Chong<sup>2</sup>, Maha Abdullah<sup>2</sup>, Zamberi Sekawi<sup>2</sup>, Chooi Fun Leong<sup>3</sup>, Soon Keng Cheong<sup>4</sup>

<sup>1</sup>PPUKM-MAKNA Cancer Center, Universiti Kebangsaan Malaysia; <sup>2</sup>Faculty of Medicine & Health Sciences, Universiti Putra Malaysia; <sup>3</sup>Faculty of Medicine, Universiti Kebangsaan Malaysia; <sup>4</sup>Faculty of Medicine & Health Sciences, Universiti Tunku Abdul Rahman

**Background:** RNA interference (RNAi) is a powerful gene regulatory mechanism that selectively silences mRNA expression in various organisms. In the lab, chemically-synthesized small interfering RNA (siRNA) is used to perform the same function. In this present study, the inhibitory effect of interleukin-6 (IL-6) siRNA in human bone marrow-derived mesenchymal stem cells (BM-MSC) was investigated post transfection. **Methods:** BM-MSC was transfected with 100 pmol of IL-6 siRNA using 5 µl Lipofectamine 2000 (Invitrogen). siRNA-Lipofectamine complexes were added into wells containing 1 x 10<sup>5</sup> cells each. Untransfected BM-MSC at similar plating density was used as control. BM-MSC total RNA was extracted 48 h and 72 h post transfection using the RNeasy plus micro kit (Qiagen) and reverse-transcribed to generate cDNA. Real Time PCR was carried out in duplicates using Taqman Universal PCR Master mix and FAM-labelled IL-6 and β-actin Taqman probes. β-actin was used as endogenous control. Data were analyzed and IL-6 mRNA levels were determined using the comparative C<sub>T</sub> method. Supernatants from BM-MSC were also collected at 48 h and 72 h and IL-6 levels were assayed using ELISA. A luminescent-based viability assay using CellTiter Glo (Promega) was also carried out to assess the effect of IL-6 siRNA on BM-MSC viability. **Results:** Real Time PCR data analysis showed a 2-fold reduction of IL-6 mRNA 72 h post transfection when compared to control BM-MSC. However, no suppression of IL-6 protein level was detected in the culture supernatant post transfection. Lastly, cell viability assay showed that increasing concentration of IL-6 siRNA did not affect the viability of BM-MSC with post transfection cell viability remained above 80% for all tested siRNA concentrations (20 pmol – 100 pmol). **Conclusion:** Current results showed that IL-6 siRNA transfection successfully reduced IL-6 mRNA levels in BM-MSC with no adverse effect on the cell viability.

**P18. Transfection of human bone marrow-derived mesenchymal stem cells with hIFN-γ gene**

Lee-Chuen Liew<sup>1</sup>, Maha Abdullah<sup>2</sup>, Chooi-Fun Leong<sup>3</sup>, S.A.W. Fadilah<sup>3</sup>, Soon-Keng Cheong<sup>4</sup>

<sup>1</sup>PPUKM-MAKNA Cancer Centre, Universiti Kebangsaan Malaysia; <sup>2</sup>Faculty of Medicine & Health Sciences, Universiti Putra Malaysia; <sup>3</sup>Faculty of Medicine, Universiti Kebangsaan Malaysia; <sup>4</sup>Faculty of Medicine & Health Sciences, Universiti Tunku Abdul Rahman

**Background:** Human Mesenchymal Stem cells (hMSC) are multipotent progenitor cells that possess high proliferative capacity and are capable of multilineage differentiation. MSC also exhibit direct immunosuppressive ability both *in vitro* and *in vivo*, suggesting that they are suitable vehicle for targeted delivery and local production of gene products for therapeutic purposes. In this study, isolated bone marrow-derived mesenchymal stem cells (BM-MSC) were characterized and the *in vitro* expression of human interferon-gamma (hIFN-γ) gene in nucleofected BM-MSC was studied. **Methods:** Isolated BM-MSC were characterized through morphology, immunophenotyping, and differentiation assay. Expanded BM-MSC were transfected with pORF-hIFNγ plasmid DNA by nucleofection. ELISA was used to determine the levels of hIFN-γ protein expressed *in vitro*, while the mRNA expression of hIFN-γ in nucleofected BM-MSCs was determined by Real Time-Polymerase Chain Reaction. **Results:** BM-MSC were successfully isolated and shown to display the biological properties of MSC. BM-MSC were successfully transfected. hIFNγ mRNA and protein were shown to be expressed in transfected BM-MSC. **Conclusion:** Transfected BM-MSC with pORF-hIFNγ plasmid were shown to express hIFN-γ mRNA and protein *in vitro*.

**P19. The number of freeze-thaw cycles in generating complete tumour lysate**Wei-Yi Ng<sup>1</sup>, Maha Abdullah<sup>2</sup>, Chooi-Fun Leong<sup>3</sup>, S.A.W. Fadilah<sup>3</sup>, Soon-Keng Cheong<sup>4</sup>

<sup>1</sup>PPUKM-MAKNA Cancer Centre, Universiti Kebangsaan Malaysia; <sup>2</sup>Faculty of Medicine & Health Sciences, Universiti Putra Malaysia; <sup>3</sup>Faculty of Medicine, Universiti Kebangsaan Malaysia; <sup>4</sup>Faculty of Medicine & Health Sciences, Universiti Tunku Abdul Rahman.

**Background:** Tumour lysate-pulsed dendritic cells are able to elicit tumour-specific T-cell response against different malignancies. Freeze-thaw is one of the most commonly used methods to facilitate breakage of cell membrane and release of intracellular contents. This study aims to determine the minimum number of freeze-thaw cycles needed to generate tumour lysate. **Methods:** Murine acute myeloid leukaemia cell line C1498 was prepared in 1ml of phosphate buffered saline. The cell suspension was frozen with liquid nitrogen and thawed at 37°C in a water bath. Non-rupture cells were determined by trypan blue exclusion assay. These procedures were repeated until 10 cycles. **Results:** The majority of cells were ruptured after three freeze-thaw cycles and complete rupture of cell was achieved after five freeze-thaw cycles. **Conclusion:** Results indicate that a minimum of three freeze-thaw cycles was sufficient to cause the majority of cells to rupture and complete rupture was achieved with five freeze-thaw cycles.

**P20. Relationship between vascular endothelial growth factor-C (VEGF-C) expression with induction failure in childhood acute lymphoblastic leukaemia**Siti Shahrum MS<sup>1</sup>, Hamidah NH<sup>2</sup>, Arni T<sup>3</sup>

<sup>1</sup>Haematology Unit, Pathology Department, Hospital Kuala Lumpur; <sup>2</sup>Haematology Unit, Department of Pathology, National University of Malaysia; <sup>3</sup>Histopathology Unit, Pathology Department, Hospital Kuala Lumpur.

**Background:** Acute lymphoblastic leukemia (ALL) is a malignant proliferation of lymphoblasts involving bone marrow and peripheral blood (Conter et al. 2004). It is the most common leukaemia of childhood and the second most common cancer of childhood behind nervous system tumours (Redaelli et al. 2004; Liang & Pui 2005). Vascular endothelial growth factor-C (VEGF-C) is a secreted glycoprotein that plays key roles during embryonic and postnatal lymphangiogenesis. Nowicki et al. (2006) demonstrated that the expression of VEGF-C was found to be significantly associated with ALL treatment failures (both failed inductions and relapses). The aim of this study was to determine the immunohistochemical expression of VEGF-C in childhood ALL; the relationship between VEGF-C expression and induction failure and risk of relapse; and the association between VEGF-C status and established prognostic indicators of ALL, namely white blood count, sex, immunophenotype, central nervous system disease at presentation and age. **Methods:** Two hundred and three bone marrow trephine biopsy samples of childhood ALL diagnosed and treated at Hospital Kuala Lumpur and Universiti Kebangsaan Malaysia Medical Centre from January 2000 until June 2007 was included in this study. The trephines were stained immunohistochemically with VEGF-C. Slides were assessed blindly by three investigators. Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) programme version 12. **Results:** The expression of VEGF-C in childhood ALL was 7.4 %, lower than that reported in previous journal by Nowicki et al. (2006). This study showed no relationship between VEGF-C expression and induction failure and risk of relapse. There was also no correlation between VEGF-C status and established prognostic indicators of ALL, namely WCC, sex, immunophenotype, CNS disease at presentation and age. This study showed that immunohistochemical staining of VEGF-C is not helpful in predicting induction failure and risk of relapse in childhood ALL.

**P21. Generation of mesenchymal stem cells expressing Interleukin-12**Yap Fei Ling<sup>1</sup>, Leong Chooi Fun<sup>2</sup>, Ammu Radhakrishnan<sup>1</sup>, Cheong Soon Keng<sup>3</sup>*<sup>1</sup>Faculty of Medicine, International Medical University, Kuala Lumpur; <sup>2</sup>Faculty of Medicine, Hospital Universiti Kebangsaan Malaysia, Kuala Lumpur; <sup>3</sup>Faculty of Medicine, Universiti Tunku Abdul Rahman, Selangor.*

**Background:** Mesenchymal stem cells (MSCs) are considered to be a promising platform for cell and gene therapy for a variety of diseases. Work over the past 30 years has resulted in a greater understanding of the biology and therapeutic applications of MSCs. The cell population known as human mesenchymal stem cell is usually isolated from the mononuclear fraction of a bone marrow aspirate and subsequently grown as the cell population that adheres to culture dishes. IL-12 plays a pivotal role in the generation of Th1-type immune responses and exerts a pronounced antitumor and anti-angiogenic activities. In this study, we are modifying normal MSCs to express and secrete the therapeutic cytokines, IL-12. These modified MSCs that produce high concentrations of antitumor cytokine are useful as delivery vehicles directly within the tumor mass, which will help to suppress tumor growth. **Methods:** Human IL-12 cDNA gene which comprises both of the subunits p35 and p40, bridged by a linker was constructed into a bacterial plasmid, pBLAST42. Purified IL-12 plasmid was stably transfected into MSCs and expanded under the selection of blasticidin. Aliquots of culture supernatant from IL-12 transfected MSCs were harvested and measured for IL-12 secretion using human IL-12p70 ELISA kit. A modified IL-12 ELISPOT assay was also developed for the direct detection of individual IL-12 secreting MSCs and visualized using AEC coloring system. **Results:** The IL-12 transfected MSCs constitutively expressed and secreted IL-12 into the culture supernatant as determined by ELISA. About 450 pg of IL-12 was expressed per 10<sup>5</sup> cells per day. These results were in consistent with the ELISPOT assay. There were no significant changes on cell morphology and immunophenotypic characteristics of IL-12 transfected MSCs compare with untransfected MSCs. These transfected cells also retained its differentiation properties. **Conclusion:** MSCs can be transfected with the IL-12 gene and MSCs expressing IL-12 were generated by its prolonged secretion.

**P22. Identification of stem cell and cell cycle associated genes in potential markers of prognostic value in AML**Ang Pei Shen<sup>1</sup>, Rajesh Ramasamy<sup>1</sup>, Sharmili Vidyadaran<sup>1</sup>, Leong Chooi Fun<sup>2</sup>, Cheong Soon Keng<sup>3</sup>, Seow Heng Fong<sup>1</sup>, Maha Abdullah<sup>1</sup>*<sup>1</sup>Dept of Pathology, Faculty of Medicine and Health Sciences, UPM; <sup>2</sup>Dept of Pathology, Universiti Kebangsaan Malaysia Medical Centre; <sup>3</sup>Faculty of Medicine & Health Sciences, Universiti Tunku Abdul Rahman.*

**Background:** Acute myeloid leukaemias are characterized by a high degree of genetic aberration resulting in dysregulated genes. Some dysregulated genes are good candidates as molecular markers and/or therapeutic targets. Many cell processes are targets of transformation including cell cycle, apoptosis, and metabolic pathways. Recently, cancer stem cells were implicated as a potential candidate in poor prognosis. Array methods have identified large number of genes highly expressed in cancers and potentially useful as prognostic markers. Validation of its prognostic value and understanding its natural function are important downstream work. Previously, we subtracted two libraries of genes (good and poor) potentially useful to predict duration of survival in AML patients. **Method:** We determined expression of these markers on normal peripheral blood mononuclear cell (PBMC) and normal fibroblast and cord blood stem cells at 48 hours of culture (to represent cycling cells) and 72hr of culture (non-cycling cells) using conventional PCR method. **Results:** We observed 21/37 of good prognostic genes expressed >1.3-fold higher than housekeeping genes in normal PBMC. Only 4/51 of the poor prognostic genes were expressed in PBMCs. The majority

of the markers in fibroblasts were expressed at similar levels with normal PBMC as determined after normalization against PBMC. Nevertheless, 10/37 and 10/51 good and poor genes, respectively, were increased 1.3-fold or higher. Of these 2/37 and 5/51 were observed to be down-regulated while 7/37 and 19/51 mostly of other genes were observed up-regulated when non-cycling fibroblasts were normalized against fibroblasts in cycling state. A search on available databases showed they consisted of both known and unknown genes. A larger number of both good (21/37 and 19/37) and poor (26/51 and 29/51) prognostic genes were expressed higher in stem cells (obtained from a cord sample) in both the cycling and non-cycling state, respectively. Similar to fibroblast, some genes were also observed to be down-regulated and others up-regulated when cells changed from cycling to non-cycling state. Some of these genes were identified to be important in cell cycle progression and adaptation to nutrient poor environment. Many of the known genes from the poor prognostic pool were identified to be significant as poor prognostic markers in leukaemia or other cancers. **Conclusion:** Genes from the good prognostic pool were more 'normal' as they were expressed in normal cells. Many were also involved in cell cycle progression. While none of the genes from the poor prognostic pool were identified as stem cell markers, many have potential as they are unknown genes and highly expressed on this stem cell sample.

### P23. Expression of potential prognostic markers of AML in leukaemia cell lines

Ang Pei Shen<sup>1</sup>, Ngiew Shin Foong<sup>1</sup>, Noor Hamidah Hussin<sup>2</sup>, Cheong Soon Keng<sup>3</sup>, Zainina Seman<sup>1</sup>, Rajesh Ramasamy<sup>1</sup>, Sharmili Vidyadaran<sup>1</sup>, Seow Heng Fong<sup>1</sup>, Maha Abdullah<sup>1</sup>

<sup>1</sup>Dept of Pathology, Faculty of Medicine and Health Sciences, UPM; <sup>2</sup>Dept of Pathology, Universiti Kebangsaan Malaysia Medical Centre; <sup>3</sup>Faculty of Medicine and Health Sciences, Universiti Tunku Abdul Rahman

**Background:** Acute myeloid leukaemia (AML) results from disruption of normal haemopoiesis leading to accumulation of immature myeloid progenitors in the bone marrow. It consists of a heterogeneous group of disease and treatment outcome is characterized by a high rate of drug resistance and relapse. Current prognostic markers include cytogenetic studies signifying the important role of gene defect in determining disease character. In our previous study, we isolated two libraries of genes with the potential as markers to predict patients of good (39 genes) and poor (53 genes) prognosis. **Method:** We determined the expression of these markers on six leukaemic cell lines of various lineages (BV173, HL60, Jurkat, K562, RS4;11, Reh) and compared them with expression on cultured fibroblast and stem cells using conventional PCR method. Expression levels were normalized against normal PBMC. **Results:** MTS assay determined IC50 values for the cell lines to be 16-31ng/ml, 280-330ng/ml, 25-40ng/ml, 45-65ng/ml, 16-31ng/ml and 16-31ng/ml, respectively. The ratio of good and poor prognostic genes expressed in the cell lines were 11/37 and 9/51, 8/37 and 7/51, 8/37 and 16/51, 8/37 and 15/51, 7/37 and 13/51 and 27/37 and 34/51, respectively. Using Pearson's correlation test we observed a significant ( $p < 0.05$ ) and strong positive correlation in genes expressed in Jurkat, RS4;11 and Reh and cultured cells. When good and poor prognostic genes were analyzed separately, we found in good prognostic genes, a significant and high positive correlation of BV173, Jurkat, K562 and Reh cells with cultured cells, the highest with stem cells at 48 hr culture ( $R^2=0.747$ ), fibroblast at 48hr ( $R^2=0.927$ ), fibroblast at 48 hr ( $R^2=0.793$ ) and stem cell at 48 hr culture ( $R^2=0.488$ ), respectively. For poor prognostic genes, significant correlations were observed only for Jurkat ( $R^2=0.942$ ), RS4;11 ( $R^2=0.947$ ) and Reh ( $R^2=0.881$ ). These were the best correlations and were with fibroblast at 48hr. No significant correlation was observed for the HL60 cell line. **Conclusion:** Based on expression patterns, BV173 may have a better prognosis since it expressed a higher number of good prognostic genes while the other cell lines may be inclined to a poorer prognosis as each expressed a higher number of poor prognostic genes. Correlation studies with normal fibroblast and stem cells in culture suggested BV173 and K562 had more similarities to good prognostic features while Jurkat, RS4;11 and Reh had stronger associations with poor prognostic genes. These conclusions however do not correlate with their IC50 values. The prognosis for HL60 could not be indicated with these genes.

**P24. Evaluation of the appropriateness in the usage of fresh frozen plasma in a local district hospital**

Zainina S<sup>1</sup>, Sabariah MN<sup>1</sup>, Faridah I<sup>1</sup>, Chew CY<sup>#</sup>, Mawaddah Z<sup>2</sup>, Norain M<sup>2</sup>, Soon LS<sup>2</sup>

*Department of Pathology<sup>1</sup>, Faculty of Medicine and Health Sciences<sup>1,2</sup>, Universiti Putra Malaysia.*

**Background:** Fresh frozen plasma (FFP) has been used to treat a variety of medical conditions. Despite the availability of the guideline on its usage, misuse of it in hospital practices is rising. The objectives of this study were to evaluate the appropriateness of FFP usage in Hospital Kajang. **Method:** A retrospective cross sectional study was conducted, whereby seven hundreds and eleven blood request forms were retrieved and review from Hospital Kajang Blood Bank records in year 2008. The guidelines set by British Council for Standardization in Haematology (BCSH) were used as the standard. **Result:** Appropriate usage of FFP were observed highest among patient age group of 30-39 years old (28.2%), diagnosed with acute disseminated intravascular coagulopathy and bleeding (54.1%) and mainly from intensive care unit (56.4%). Highest inappropriate usage of FFP was observed among patient age group of 50-59 (16.1%) and diagnosis categorised as 'others' (47%). There were significant associations between appropriateness of FFP usage with age group ( $\chi^2=41.069$ ,  $p < 0.001^*$ ), diagnosis ( $\chi^2=429.149$ ,  $p < 0.001^*$ ) and department ( $\chi^2=35.860$ ,  $p < 0.001^*$ ). **Conclusion:** In conclusion, inappropriate usage of FFP in the local district hospital was still high. Therefore, training programs should be implemented together with the establishment of written guidelines and monitoring of usage by haematologists.

**P25. Mesenchymal stem cells inhibit proliferation of lymphoid origin haematopoietic tumour cells by inducing cell cycle arrest**

Rajesh Ramasamy, Vahid Hossienpour Sarmadi, Chih Kong Tong, Sharmili Vidyadaran, Maha Abdullah and Heng Fong Seow

*Immunology Unit, Department of Pathology, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia*

**Background:** We have previously shown that mesenchymal stem cells (MSC) inhibit tumour cell proliferation, thus promising a novel therapy for treating cancers. However there is insufficient data to elucidate the molecular mechanisms that mediates this anti-proliferative activity. Therefore, we have explored the inhibitory effect of MSC on cancer cell cycle status and their respective signalling pathways. **Methods:** Adult human bone marrow aspiration was utilised to generate MSC and their immunophenotype profile and mesodermal differentiation ability were confirmed by flow cytometry and differentiation assays respectively. Lymphoid origin haematopoietic tumour cells BV173 and Jurkat cell lines were purchased from ATCC and maintained in 10% foetal bovine serum supplemented RPMI media. Cell cycle analysis was performed using flow cytometry and western blotting. **Results:** In the presence of MSC, tumour cell proliferation was profoundly inhibited in dose dependent manner as measured by <sup>3</sup>H-thymidine uptakes and mainly mediated by cell-to-cell contact. Further investigation on tumour cell cycle revealed that, MSC induce an arrest in G<sub>0</sub>/G<sub>1</sub> phase of cell cycle of tumour cells. In order to dissect the cell cycle mechanism, cyclin molecules that govern cell cycle progress, their relevant kinases and kinase inhibitors have were investigated. The result showed a generalised pattern of inhibition exerted by MSC on all tumour cells. Mainly the expression of cyclin D1, D3, A, E, PCNA and ERK signalling molecules were significantly reduced in the presence of MSC. CDK4 enzymes that control transition of G<sub>1</sub>-S check point were reduced indicating cell cycle arrest at G<sub>1</sub>. **Conclusion:** Our results showed that, MSC exerted anti-proliferative effect is specifically targets the cell cycle through the cell cycle check point. The generalised tumour cell inhibition by MSC could be potentially exploited to treat various tumours. However, this anti-proliferative activity needs to be tested and verified with primary tumour cells for their better understandings.

**P26. Awareness of tuberculosis as a resurgent disease and its risk of transmission in the pathology laboratory**Gurjeet Kaur <sup>1,2</sup>, Aishah Knight Abd. Shatar <sup>1</sup>, Hasmah Hussin<sup>1</sup><sup>1</sup>Advanced Medical and Dental Institute, <sup>2</sup> Institute for Research in Molecular Medicine, Universiti Sains Malaysia, Penang, Malaysia.

**Background:** Tuberculosis (TB) disease is re-emerging in the country and many medical practitioners may be unaware of this. Personnel in a pathology laboratory handle various tissue specimens fixed in 10% buffered formalin with an assumption that all infectious organisms are inactivated in it. We aimed to review the risk of TB transmission from lymph node specimens sent to the pathology laboratory. **Methods:** Five cases of TB and suspicious TB lymphadenitis were identified from pathology records in 2009-2010. Patient demographics and clinical information were obtained from request forms. **Results:** All patients had presented with cervical lymph node swelling. Clinical information stated in request forms identified 4 females with no known medical illness, aged between 17-50 years, and a 35-year old HIV positive male. Only the HIV patient had a history of fever and cough, and a provisional diagnosis of TB lymphadenitis. One other patient had prolonged cough with loss of appetite and weight stated. Gross examination and processing were carried out in the usual manner with no special precautions. Lymph node histopathology of the 4 'healthy' patients revealed characteristic caseating granulomatous inflammation without demonstration of acid fast bacilli (AFB). The HIV patient had vague granulomas in lymph node with evidence of AFB. **Conclusion:** Most of the suspicious TB lymphadenitis cases did not have a provisional diagnosis of TB on the request form. The lack of clinical suspicion of TB in 'healthy' individuals, compounded with a false sense of safety among pathology personnel and lack of well referenced guidelines on safe handling of formalin-fixed tissue possibly infected with TB should be of concern. There is scarce data suggesting a risk of TB and nontuberculous mycobacteria transmission from infected formalin-fixed tissue, however it is recommended that pathology specimens be handled under a hood and laboratory personnel use personal protective gear when handling suspected TB-infected tissues.

**P27. High expression of cyclooxygenase-2 in high grade human prostate adenocarcinoma**

Rohaizad MR, Norhafizah Mohtarrudin, Chong PP, Malina Osman

Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Selangor, Malaysia.

**Background:** Prostate adenocarcinoma is one of the most common forms of malignancy that occurs in the Malaysian male population. Inflammation has been identified in many researches so far to play a key role in the process of carcinogenesis. Inflammation is responsible in prompting angiogenesis, enhancing cellular motility and increase resistance to apoptosis. Cyclooxygenase-2 (COX-2) is an enzyme that converts arachidonic acid into pro-inflammatory prostaglandins and other eicosanoids. Additionally, COX-2 is highly expressed in a wide number of human cancers including prostate adenocarcinoma. The goal of this study was to determine the expression level of COX-2 in different types of prostate tissues and its association with clinicopathological data. **Methods:** Paraffin-embedded prostate tissues (n = 263) were obtained from Pathology Department of Hospital Kuala Lumpur with mean age of 64.54. The tissues consist of 63 normal prostate, 100 each for Benign Prostatic Hyperplasia (BPH) and prostate adenocarcinoma (PCA). The COX-2 expression was performed by standard immunohistochemistry method. The anti-human COX-2 monoclonal rabbit primary antibody was used in a dilution of 1:100. For each sample, the extent and intensity of staining with COX-2 antibody was graded in a scale from 0 to 4+. Staining was classified as 0 - none, 1 to 2 - weak and 3 to 4 - strong expression. Next, to confirm the accuracy of staining, we further analysed selected samples by semiquantitative reverse transcriptase PCR (RT-PCR). **Results:** 56/100 PCA samples were shown strong COX-2 expression, in comparison to BPH, which is 16/100 samples. Weak COX-2 expression was seen in all 63 of normal samples. In

RT-PCR analysis, COX-2 expression was detected in high Gleason score 8 and 9 with 2.17 and 2.01 fold respectively higher compared with the normal tissue. BPH displayed only 1.04 fold higher than normal tissue. **Conclusion:** From the results of this study, we suggest that COX-2 overexpression may play a role in progression of prostate adenocarcinoma. Therefore, COX-2 expression might be useful to be evaluated further as a potential therapeutic target for prostate cancer.

#### P28. Cyclooxygenase-2 expression in renal cell carcinoma

Norhafizah M<sup>1</sup>, Hairuszah I<sup>1</sup>, Shiran MS<sup>1</sup>, Razana MA<sup>1</sup>, Razmin G<sup>2</sup>

<sup>1</sup>Department of Pathology, Faculty of Medicine and Health Sciences, University Putra Malaysia, 43400 UPM Serdang, Selangor; <sup>2</sup>Department of Pathology, Hospital Kuala Lumpur, Jalan Pahang, 50586 Kuala Lumpur

**Background:** Cyclooxygenase-2 is key enzyme in conversion of arachidonic acid to prostaglandin. It stimulates malignant cell proliferation and angiogenesis, and it decreases apoptosis. Renal cell carcinoma (RCC) is the commonest type of renal tumour with high metastatic rate. In about one-third of patients, the cancer has already metastasized at the time of diagnosis. The aim of this study was to determine the expression of COX-2 in RCC and its possible association with the tumour grades. **Methods:** 40 cases of histologically diagnosed RCC who underwent nephrectomy in Hospital Kuala Lumpur were retrieved from the department archives. The paraffin embedded tissues were cut at 4 µm thick and were immunohistochemically stained with COX-2 monoclonal antibody (DAKO, Denmark). **Results:** 40 RCC cases were included in this study. There were 27 (67.5%) men and 13 (32.5%) women with median age of 56.2 years (range: 16-74 years). Ethnic Malays formed 17 (42.5%) cases; Chinese 15 (37.5%) cases and Indian 8 (20%) cases. There were 9 (22.5%) grade 1 RCC, 21 (52.5%) grade 2 RCC, 10 (25%) grade 3 RCC and none grade 4 RCC. 24 (60%) RCC cases showed increased COX-2 expression and 16 (40%) RCC were immunonegative. All the immunopositive cases showed cytoplasmic membrane positivity and 2 cases showed both cytoplasmic and membranous staining. There was significant correlation between grade of RCC and COX-2 expression ( $p=0.008$ ). **Conclusion:** The findings suggest that COX-2 may be associated with progression of RCC and its aggressive behaviour. COX-2 has a prognostic value and potential as a non-invasive therapy in RCC. The results also provide a baseline data for further research on therapeutic intervention in renal cell carcinoma.

#### P29. Immunohistochemical analysis of HER-2 in *Helicobacter pylori*-associated chronic gastritis and gastric cancer in Malaysian patients

Nurulhafizah Samsudin<sup>1</sup>, Hairuszah Ithnin<sup>1</sup>, Razana Mohd Ali<sup>1</sup>, Maizura Ithnin<sup>2</sup>

<sup>1</sup>Faculty of Medicine and Health Sciences, Universiti Putra Malaysia; <sup>2</sup>Malaysian Palm Oil Board

**Background:** HER2 gene, a potential proto-oncogene, encodes for 185-kDa HER-2 protein. The HER-2 protein is involved in signal transduction pathways leading to cell growth, proliferation, and differentiation. Abnormal HER-2 expression has been described in a wide variety of malignancies including gastric cancer. However, the patterns of HER-2 alterations and its association with outcome variables in gastric cancer and *Helicobacter pylori*-associated chronic gastritis, a precursor for gastric cancer, are still not well established in Malaysian patients. **Methods:** Formalin-fixed paraffin embedded tissues from 59 patients with *H. pylori*-associated chronic gastritis and 31 patients with gastric adenocarcinoma were stained using immunohistochemical methods for the evaluation of HER-2 protein expression. Slides were scored semi-quantitatively based on staining intensity and distribution positivity. SPSS 17.0 was used for statistical analysis. **Results:** 9% (5 out of 59) of *H. pylori*-associated chronic gastritis cases were scored as HER-2 positive. On the other hand, of the 31 patients with gastric adenocarcinoma, 94% (15 out of 16) of patients with

intestinal type adenocarcinoma are HER-2 positive, whereas, 71% (12 out of 17) of patients with diffuse type adenocarcinoma are HER-2 positive. In addition to that, when compared with their respective adjacent normal gastric mucosa, the *H. pylori*-associated chronic gastritis ( $P < 0.05$ ), intestinal type adenocarcinoma ( $P < 0.05$ ), and diffuse type adenocarcinoma ( $P < 0.05$ ), showed significant differences in HER-2 expression. **Conclusion:** Increase of HER-2 protein expression occurs commonly in intestinal type adenocarcinoma and diffuse type adenocarcinoma in gastric cancers. Our results support the proposed role of HER-2 as a potential proto-oncogene, which is commonly over-expressed in cancer. However, the HER-2 overexpression in *H. pylori*-associated chronic gastritis which happens less commonly suggests a need for further study on the association of this marker between gastric cancer and its precursor.

### P30. Labelling of double antigenic markers and DNA using automated Stainer System

Chandramaya Sabrina Florence, Rahimah Rahmat, Noraidah Masir

*Department of Pathology, Faculty of Medicine, Universiti Kebangsaan Malaysia, Kuala Lumpur*

**Background:** Immunohistochemical labelling is widely used in routine diagnosis and research to study protein expression in tissues and cells. Double immunolabelling in contrasting colours using two monoclonal antibodies allow localization of two different antigens to be detected in tissue sections. In addition, detection of DNA by *in-situ* hybridization (ISH) can be combined with immunohistochemistry (IHC) to detect both protein expression and genetic abnormalities on a single section. However, these procedures are time consuming since they are carried out in sequence. In this study, we report the results of double labelling using IHC, and IHC combined with silver *in-situ* hybridization (SISH) performed using automated stainer system. The aim of this study is to determine the requirements, the quality of staining and the reproducibility of the techniques using automated stainer. **Methods:** Double-IHC and IHC-SISH staining techniques were performed using the automated stainer Ventana BenchMark XT (Tucson, Arizona, USA) on paraffin-embedded tissue sections of normal tonsil as well as prostate and breast carcinoma. **Results:** Double-IHC staining techniques gave clear results to detect two antigenic markers present in distinct cell populations or at different cellular locations within a single cell. The IHC-SISH staining clearly demonstrated the protein expression and SISH signals on the same tissue sections. **Conclusion:** The double-IHC staining using automated stainer system offers an effective means of detecting two antigenic markers on the same tissue with much less technical time. The staining was reproducible and free of background. In addition, we showed that detection of DNA by ISH can be combined with IHC labeling. This opens the possibility of using these techniques for analyzing histopathological samples for more accurate diagnosis.

### P31. Pathological study of Dermatitis Herpetiformis association with Coeliac Disease among Iraqi patients

Muhamed T Osman<sup>1</sup>, Sanaa A Al-Nasiry<sup>2</sup>, Balsam I Taha<sup>3</sup>, Makki H Fayadh<sup>4</sup>, Luay A Muhamed<sup>5</sup>, Ghada Al-Duboni<sup>6</sup>

<sup>1</sup>Department of Pathology, Faculty of Medicine, Universiti Teknologi MARA, 40450 Shah Alam, Selangor, Malaysia; <sup>2</sup>Department of Pathology, College of Medicine, University of Baghdad, Baghdad, Iraq; <sup>3</sup>Department of Pathology, Specialized Surgery Hospital, Medical City, Baghdad, Iraq; <sup>4</sup>GIT & Liver Diseases Hospital, Medical City, Baghdad, Iraq; <sup>5</sup>Jenin General Hospital, Baghdad, Iraq; <sup>6</sup>Department of Basic Sciences, College of Dentistry, University of Baghdad, Baghdad, Iraq.

**Background:** Coeliac disease (CD) is not the only autoimmune condition related to eating gluten. Autoimmune reactions to gluten can also take the form of an extremely itchy, burning, stinging skin rash called dermatitis herpetiformis (DH). An association between DH and CD has been recognized

for a long time in many different populations. Objectives: This study was carried out for the first time in Iraq to (a) estimate the prevalence of DH among Iraqi coeliac patients (b) describe HLA class II genotypes predictive of CD in dermatitis herpetiformis patients. **Methods:** A total of 314 Iraqi patients in whom coeliac disease was suspected on clinical grounds, underwent (1) serologic tests of coeliac disease with antiendomysial and anti-tissue transglutaminase antibodies by using indirect immunofluorescence technique and ELISA respectively, (2) oesophagogastroduodenoscopy with intestinal biopsies to confirm CD diagnosis histopathologically. All coeliac patients underwent HLA class II genotyping. Moreover patients with skin lesions underwent skin biopsies and direct immunofluorescent technique to confirm DH diagnosis. **Results:** The diagnosis was documented CD in 226 patients only (155 children and 71 adults). Among coeliac patients there were only 8 were diagnosed as DH (3 children 1.93% and 5 adults 7.04%) while 1 case confirmed DH among non coeliac patients. There was highly significant association between DQ2 antigen and both dermatitis herpetiformis and coeliac patients with ratio of (76.2%) and the remaining ratio (19%) was DQ8 antigens, while DR3 and DR5/7 antigens frequencies yielded no evident association with ratio of (4.8%) and (0%) respectively. **Conclusion:** The study confirmed the association between dermatitis herpetiformis and coeliac disease but less than expected ratio among Iraqi population in comparison with other populations.

### **P32. Glypican 3 expression in ovarian malignancy: An immunohistochemical study of 33 cases**

Razana M Ali<sup>1</sup>, Norhafizah Mohtarrudin<sup>1</sup>, Anita Abd Rahman<sup>2</sup>, Razmin Ghazali<sup>3</sup>

<sup>1</sup> Department of Pathology, Faculty of Medicine and Health Sciences, UPM Serdang 43400 Selangor;

<sup>2</sup> Department of Community Health, Faculty of Medicine and Health Sciences. UPM Serdang 43400 Selangor; <sup>3</sup> Histopathology Unit, Department of Pathology, Hospital Kuala Lumpur.

**Background:** Glypican 3 (GPC3) is a heparin sulphate proteoglycan which plays a role in the regulation of cell proliferation and survival through apoptosis induction. Its expression is down regulated for tissues from ovary, breast or mesothelium during tumour progression and over expressed in hepatocellular carcinoma and yolk sac tumours. It is currently regarded as a potential target for immunotherapy in GPC3 positive neoplasm. In this study, we sought to determine the expression of GPC3 in all types of ovarian malignancy. **Methods:** A total of 33 cases of ovarian malignancy were selected from the archived tissue blocks. The majority of cases were of epithelial type (n=32, 97%) and one miscellaneous type (n=1, 3%) i.e. malignant mixed mullerian tumour (MMMT). Sections from each representative blocks were subjected to immunohistochemistry with GPC3 antibody (clone IG12 Santa Cruz Biotechnology) in a dilution of 1:150. Cytoplasmic and/or membranous staining of neoplastic cells was evaluated as focally positive (1+, 5-10%), positive (1+ to 3+, >10%) and negative (0 or <5%). **Results:** 19/33 (58%) cases showed positive expression of GPC3 ranging from 1+ to 3+ staining intensity. The GPC3 is expressed in 4/7 (57) cases of endometrioid adenocarcinoma, 2/4 (50) mucinous adenocarcinoma, 7/13 (54) serous adenocarcinoma, 2/2 (100) cases of mixed clear cell and endometrioid adenocarcinoma, 2/3 (67) of clear cell carcinoma and 1/1 (100) each for MMMT and mixed malignant Brenner and serous adenocarcinoma (100). There are no significant relationship between the GPC3 expressed ovarian cancer with grade and stage (p=0.232, p=0.927). **Conclusion:** GPC3 expression is seen in most epithelial types of ovarian malignancy included in this study except the adenocarcinoma NOS. Strong expression is seen in some of the clear cell carcinoma, serous adenocarcinoma and MMMT. Our results suggest that the expression in some of the epithelial ovarian cancer could indicate a potential candidate for immunotherapy and larger study sample and a more variety of cases are needed for a more significant result.

**P33. Expression of basal markers CK14 and EGFR in triple negative invasive ductal breast carcinoma**

Isa M R and Norazizah M

*Pathology Department, UKM Medical Centre, Cheras, Kuala Lumpur, Malaysia*

**Background:** Triple-negative breast cancers are breast cancers that are defined by negative expression of estrogen and progesterone receptors and Her2 with aggressive clinical behavior and currently lack effective targeted therapies. Basal-like breast cancers represent a subset of breast cancers derived from gene expression profiling studies with significant overlap with triple-negative breast cancers. Its phenotype has been characterised immunohistochemically by various basal-like markers including CK5/6, CK14, p63, EGFR, SMA and others. **Methods:** We performed immunostaining for basal markers CK14 (Clone LL002, Dako, 1:100) and EGFR (Clone H11, Dako, 1:150) on 229 pan-grade invasive ductal carcinomas (IDC) accessed from the files of the Pathology department, UKMMC according to published protocols. Results were correlated with clinico-pathological variables obtained from archival histopathology reports that include hormonal status, tumour size and grade and axillary lymph node status. **Results:** Thirty-five of 229 IDC cases (15%) were triple negative. Basal markers CK14 and EGFR were expressed in 14 cases (6.1%) and 16 cases (7.0%) respectively. However, using them as a two-antibody panel, 18 of 229 IDC cases (7.9%) or 51.4% of triple negative breast cancers expressed basal markers. Majority of basal phenotype tumours affected patients aged >50 years, had tumour sizes of 2-5 cm with negative axillary lymph node status and occurred in grade 3 tumours. **Conclusion:** The incidence of triple-negative breast cancer in our study at 15% is within the range of 10%-17% generally reported in the literature. The incidence of basal-like breast cancers at 51% too fall within a wider range of 50%-90% reported depending on the antibody panel used. The variability reflects the lack of consensus in defining the immunophenotype of basal-like breast cancer.

**P34. Trends in cardiovascular disease mortality in Hospital Kuala Lumpur from year 1999-2008**Norhafizah M<sup>1</sup>, Logeswary B<sup>1</sup>, Lee CY<sup>1</sup>, Nur Afikah MS<sup>1</sup>, Norsarah S<sup>1</sup>, Malina O<sup>2</sup>, Rohayu SA<sup>3</sup>, Mohd Shah M<sup>3</sup>

<sup>1</sup>Department of Pathology, <sup>2</sup>Department of Microbiology & Parasitology, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor; <sup>3</sup>Department of Forensic, Hospital Kuala Lumpur, Jalan Pahang, 50586 Kuala Lumpur

**Background:** Cardiovascular disease (CVD) is a leading cause of morbidity and mortality globally. In Malaysia, CVD are among the top 10 principal causes of death. The objective of this study is to determine trends in CVD related mortality in Hospital Kuala Lumpur and the socio-demographic distributions. **Methods:** Mortality data was obtained from Forensic Department of Hospital Kuala Lumpur. All mortality cases registered in the department from 1<sup>st</sup> January 1999 to 31<sup>st</sup> December 2008 were included in this study. However those registered in 2003, 2006 and 2007 were omitted due to incomplete data. The underlying cause of death was determined using ICD-10 (chapter IX, block I00-I99). CVD was further classified into ischaemic heart disease, acute rheumatic fever, chronic heart disease, hypertensive heart disease, pulmonary heart disease and others (congenital heart disease, cardiac arrest, arrhythmia, myocarditis, infective endocarditis, cardiomyopathy and cardiogenic shock). **Results:** Of 25738 mortality cases, 3563 (13.84%) were due CVD. From 1999 to 2008, CVD related mortality showed inconsistent trends. The mortality rates vary from 11.4% to 18.1% with the highest mortality occurred in 2008. Of all the CVD related mortality cases, ischaemic heart disease was the main cause of death (67.57%). Mortality due to CVD mainly occurred among males (69.5%), Malays (43.2%) and above 60 years old (50.7%). **Conclusion:** Mortality due to CVD, mainly ischaemic heart disease remains as a major health issues. Unfavourable trends in the mortality due to CVD warrant efficient health care system and should aim at younger population to effectively address the problem. Improvements in population risk factors and in medical management of patients with CVD are essential to ensure substantial decline of the mortality cases due to CVD.

**P35. Primary central nervous system lymphoma with underlying aetiology related to Epstein Barr virus and Human Herpes Simplex virus 8**

Norizal Mohd Noor<sup>1</sup>, Patricia AC<sup>2</sup>, Wong KT<sup>2</sup>

<sup>1</sup>Faculty of Medicine, Universiti Teknologi MARA, Selangor; <sup>2</sup>Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia

**Background:** Primary central nervous system lymphoma (PCNSL) are among the least common pathological diagnosis of all primary brain tumour worldwide. Despite being uncommon, it is notoriously known for being least favourable with very low survivorship. Commonly occurs among elderly male. Usual location is in supratentorial region and associated with focal neurological deficit. Common histological classification is Diffuse Large B cell Lymphoma. The main treatment for this tumour is combined chemotherapy and radiotherapy. However, the response and survivorship of this tumour are still unpredicted. **Methods:** This is a retrospective cohort study of patients diagnosed with PCNSL in University Malaya Medical Centre (UMMC) from 2002 to 2009. The sample size of this study is 31 cases. All case noted were reviewed and the respective H&E slides, including previously performed immunohistochemistry stain, observed by 1 certified pathologist and a trainee. Further stain was performed on the paraffin block using mouse anti-LMP1 monoclonal antibody (DAKO Corp) and anti-HHV8 monoclonal antibody (DAKO Corp). **Results:** The incidence of PCNSL has increased 4 folds in the past 8 years (2002-2009). Majority are in elder age group with median of 56.3 years old. Most patients are male (58.4%) predominantly Malays (45.2%). The most common location is basal ganglia/paraventricular and parietal region (22.6%). All subjects are HIV negative and most presented with focal neurological deficits. Solitary (61.3%) and homogeneity (87.1%) are among common radiological feature. The main classification of diagnosis is Diffuse Large B Cell Lymphoma (80.6%) with many characteristic feature; (CD20,96.8%; Angiocentricity,71%; Parenchymal infiltration,28%; Necrosis, 58.1%). Association with LPM1 were prominent (51.6%) but not with HHV8 (6.5%). The median survival is 5 ± 1.39 months. The only significant predictive indicator for survivorship is treatment response. **Conclusion:** There was notable increment of cases diagnosed with PCNSL but no histological predictor significant for the prognosis of the survivorship.

**P36. Analysis of  $\beta$ -catenin expression in chronic gastritis and gastric cancer in Malaysian population**

Tay TC<sup>1</sup>, Norhafizah M<sup>2</sup> and Hairuszah I<sup>2</sup>

<sup>1</sup>Institute of Bioscience, Universiti Putra Malaysia; <sup>2</sup>Faculty of Medicine and Health Sciences, Universiti Putra Malaysia

**Background:** Gastric cancer is diagnosed as a mortality causing malignancy worldwide. In Malaysia, it is the 7th most common cancer affecting the local population. It has been shown that gastric cancer can be a curable disease if it is diagnosed at an early stage. Many studies have been carried out to identify early markers associated with gastric cancer but unfortunately none has been considered useful for early diagnosis of this disease. Recent reports indicate that  $\beta$ -catenin expression may be increased in gastric cancers, and  $\beta$ -catenin mutations have been identified in intestinal type gastric cancer. However, the expression and mutation of the  $\beta$ -catenin have not been studied in relation to the changes underlying the progression and formation of metastasis in gastric cancer. In this study,  $\beta$ -catenin was studied in order to determine whether alteration in  $\beta$ -catenin occurs prior to the full development of gastric cancer and can be detected at precursor lesions such as chronic gastritis. **Methods:** Immunohistochemical staining of  $\beta$ -catenin was performed on 51 chronic gastritis samples, 41 *Helicobacter pylori*-associated chronic gastritis samples and 33 gastric cancer samples to analyse the expression levels of this protein in those samples. Mann Whitney U Test was used to analyse the results of immunohistochemical staining. **Results:** By using Mann Whitney U Test, it was

shown that the P value of the expression between chronic gastritis and gastric cancer was 0.003, whereas the P value of the expression between *Helicobacter pylori*-associated chronic gastritis and gastric cancer was 0.007. **Conclusion:** There are significant differences of the  $\beta$ -catenin expression between chronic gastritis and gastric cancer as well as between *Helicobacter pylori*-associated chronic gastritis and gastric cancer. Further studies such as Western Blotting need to be carried out to further confirm the expression of  $\beta$ -catenin before we can conclude  $\beta$ -catenin as an early diagnostic marker of gastric cancer.

### **P37. Vascular endothelial growth factor (VEGF) expression in renal cell carcinoma (RCC)**

Norhafizah M<sup>1</sup>, Shidee N<sup>1</sup>, Rosila AB<sup>1</sup>, Koey PM<sup>1</sup>, Malina O<sup>2</sup>, Suryati MY<sup>3</sup>

<sup>1</sup>Department of Pathology, <sup>2</sup>Department of Microbiology & Parasitology, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor; <sup>3</sup>Department of Pathology, Hospital Kuala Lumpur, Jalan Pahang, 50586 Kuala Lumpur

**Background:** Vascular endothelial growth factor (VEGF) facilitates angiogenesis and has been correlated with advanced tumour stage. In renal cell carcinoma (RCC), the tumour grade as determined in microscopic sections is an important prognostic factor. This study was conducted to determine the expression of VEGF in renal cell carcinoma (RCC) and the possible association with the tumour grades. **Methods:** 40 cases of histologically diagnosed RCC who underwent nephrectomy were retrieved from departmental archives. The patients' ages ranged between 16 to 74 years with a mean of 56.2 years. There was a male preponderance with M: F= 2.1:1. Ethnic Malays formed 17 (42.5%) cases; Chinese 15 (37.5%) cases and Indian 8 (20%) cases. The formalin fixed, paraffin-embedded tumour tissue blocks of each case were selected and cut at 4  $\mu$ m sections. The sections were immunohistochemically stained with VEGF (DAKO Monoclonal mouse anti-human VEGF clone VG1). RCC cases that demonstrated VEGF expression in more than 25% of malignant cells were considered immunopositive and the intensity was evaluated. **Results:** Of 40 RCC cases, 28 (90%) RCC were immunopositive for VEGF. In grade 1 RCC (n=9; 22.5%); 11.1% (n=1) showed no staining, 22.2% (n=2) weak staining, 11.1% (n=1) moderate staining and 55.6% (n=5) strong staining. In grade 2 RCC (n=21; 52.5%); 4.8% (n=1) showed no staining, 14.3% (n=3) weak staining, 47.6% (n=10) moderate staining and 33.33% (n=7) strong staining. In grade 3 RCC, (n=10; 25.0%); 20.0% (n=2) showed no staining, 30.0% (n=3) weak staining, 10.0% (n=1) moderate staining and 40.0% (n=4) strong staining. Statistical analysis demonstrated no correlation between VEGF expression and tumour grade ( $p=0.39$ ,  $r=-0.14$ ). **Conclusions:** VEGF was expressed in high percentage of RCC, suggesting a possible role of VEGF in the pathogenesis of RCC. These data suggests that VEGF inhibitors may be useful for prevention or therapy of RCC in humans.

### **P38. Bilateral lung lesions with breast metastasis: A case report**

Nor Salmah Bakar<sup>1</sup>, Norra Harun<sup>2</sup>, Ng Teck Han<sup>3</sup>

<sup>1</sup>Pathology Discipline, Faculty of Medicine, Universiti Teknologi MARA; <sup>2</sup>Department of Pathology, Hospital Tengku Ampuan Afzan; <sup>3</sup>Faculty of Medicine, International Islamic University.

**Background:** Development of metastasis to the breast from lung cancer is very rare. **Methods:** We present a 22-year-old lady who had multifocal and bilateral lung lesions, metastasize to the left breast. Physical examination, CT scan and cytologic and histology study on breast biopsy were performed. **Results:** Patient was a 22-year-old lady presented with history of cough for 4 months prior to the hospital admission. The cough was productive with greenish sputum on and off. Occasionally, she also noted small amount of blood in her sputum. Other associated symptoms include hoarseness of voice, fatigue, loss of appetite and loss of weight. She was anaemic and found to have cervical lymphadenopathy, vocal cord palsy and right facial nerve palsy. Computed Tomography Scan of

neck and thorax revealed multiple neck and mediastinal lymph nodes and multiple lung nodules which were seen in both lung fields. The left breast lump was identified on admission. She underwent series of cytologic and histologic examination of the neck swellings and the left breast lump. Both lesions showed an adenocarcinoma; with the left breast lesion appeared rather poorly differentiated but exhibited focal positivity for mucin stain. Both neck and left breast lesions were positive for Cytokeratin 7(CK7), and TTF1, while negative for CK20, Estrogen receptor (ER), Progesterone receptor (PR) and thyroglobulin. **Conclusion:** Thorough morphologic assessment together with support from panels of immunohistochemical stains and special stain are able to indicate the possible primary source of malignancy. It is important to distinguish a primary breast cancer from a metastasis to the breast, as the therapeutic planning and the outcome between them are different.

### **P39. Toxoplasmosis among immunocompromised patients at a teaching hospital in Kuala Lumpur**

Amal R Nimir<sup>1</sup>, Amizah Othman<sup>2</sup>, Noor Hayati Mohd Isa<sup>2</sup>

<sup>1</sup>Department of Pathology, Cyberjaya University College of Medical Sciences- Malaysia; <sup>2</sup>Department of Parasitology, Universiti Kebangsaan Malaysia .

**Background:** Seroprevalence of toxoplasmosis in different populations may vary according to different environments, social customs and habits. This study was designed to measure the seroprevalence of toxoplasmosis among patients with different malignancies and to ascertain the association between common risk factors and disease transmission. **Methods:** Cross-sectional study from January-April 2009. Four Oncology wards in Hospital Universiti Kebangsaan Malaysia (HUKM) were selected as the site for undertaking the present study. The survey involved 129 patients with different malignancies. Information was gathered by using study subject information sheet and a standardized structured questionnaire. *Toxoplasma* was screened by a standard ELISA commercial kit in accordance with the manufacturer's instructions and performed at the Department of Microbiology, HUKM Kuala Lumpur. A result of > 51 IU/ml of anti-*Toxoplasma* (IgG) antibody was regarded as positive, indicating latent or pre-existing *Toxoplasma* infection. A result of > 51 IU/ml of anti-*Toxoplasma* (IgM) antibody was regarded as positive, indicating recently acquired *Toxoplasma* infection. **Results:** Total number of seropositive patients was 54 (67.6%), the mean age was 51 year (range 15-88 year). *Toxoplasma* IgG positivity was highest among Malaysian (32%). Male to female ratio was almost equal. There was a statistically significant difference in seropositivity between patients living in rural areas compared to those living in urban areas, positive history of consumption of undercooked meat and/or blood transfusion ( $p < 0.05$ ). **Conclusion:** In conclusion, these findings give some support to *Toxoplasma* screening program and health education, including promotion of a healthy lifestyle exclusively in seronegative patients in order to prevent seroconversion and the incidence of clinically evident opportunistic infection.

### **P40. Clinical and microbiological characteristics of *Stenotrophomonas maltophilia* in a tertiary care hospital in Malaysia**

Chong Seng Shit<sup>1</sup>, Salasawati Husin<sup>2</sup>, Vasantha Kumari Neela<sup>1</sup>, Rukman Awang Hamat<sup>1</sup>

Department of Medical Microbiology and Parasitology, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia<sup>1</sup>; Department of Medical Microbiology and Immunology, Faculty of Medicine, Universiti Kebangsaan Malaysia Medical Centre<sup>2</sup>.

**Background:** *Stenotrophomonas maltophilia* is a non-fermentative Gram-negative bacterium that has been recognized as an important nosocomial pathogen with increasing frequency in recent decades, especially in immunocompromised and clinically debilitated patients. Despite *S. maltophilia* is considered as a low-virulent and opportunistic microorganism, its intrinsic resistance to multiple

antibiotic agents, including carbapenems and an increasing trend of antimicrobial resistance have led to many treatment failures and high mortality rate. Infections with this pathogen commonly manifest as bacteraemia, although it can cause a wide spectrum of other infections as such as pneumonia, endocarditis, urinary tract infection, ocular infection, and wound infection. However, to our knowledge very little information is available regarding the clinical and microbiological characteristics of *S. maltophilia* infections in Malaysia. **Methods:** Fifty-four clinical isolates of *S. maltophilia* was collected from University Kebangsaan Malaysia Medical Centre (UKMMC) in between March 2009 to March 2010. Susceptibility profiles for eleven antimicrobial agents (amikacin, cefepime, cefoperazone-sulbactam, ceftazidime, ciprofloxacin, gentamicin, imipenem, meropenem, piperacillin-tazobactam, polymyxin-B, co-trimoxazole) were determined by the NCCLS disc diffusion method for non-fermentative bacteria. Data from medical records of all patients harboring *S. maltophilia* were retrospectively analyzed especially on nature of sample collections, previous antibiotic used, clinical conditions, and placement of ward. **Results:** Fifty-four patients had positive cultures of *S. maltophilia*. The most frequent isolation site was in blood specimens (44.44%), followed by tracheal aspirates (29.63%), nasopharyngeal aspirates (7.41%), wound infections (7.41%), arterial lines (3.70%), cerebrospinal fluid (3.70%), sputum (1.85%), and urine (1.85%). Meanwhile, fifty percent of the isolates were from ICU, high dependency unit (5.56%), medical ward (29.63%), and surgical ward (14.81%). Antibiogram pattern showed that all *S. maltophilia* isolates are susceptible to co-trimoxazole, and polymyxin-B. Meanwhile, they are totally resistant to imipenem and meropenem. **Conclusion:** We conclude that most of the *S. maltophilia* isolated from ICU patients may be due to the usage of invasive medical devices and broad spectrum antibiotics. Meanwhile, the resistance characteristic of *S. maltophilia* to most of the currently available antibiotics emphasizes the importance of continued local surveillance of antimicrobial resistance.

#### P41. Molecular characteristics of multi-drug resistant *Escherichia coli*

Rohaidah Hashim<sup>1</sup>, Norazah Ahmad.<sup>1</sup>, Salbiah Nawi<sup>2</sup>, Hanna E. Sidjabat<sup>3</sup>, David L. Paterson<sup>3,4,5</sup>

<sup>1</sup>Bacteriology Unit, Institute for Medical Research, Kuala Lumpur; <sup>2</sup>Selayang Hospital; <sup>3</sup>University of Queensland Centre for Clinical Research, Brisbane, Queensland 4029, Australia; <sup>4</sup>Microbiology, Pathology Queensland Central Lab., Brisbane, Queensland 4029, Australia; <sup>5</sup>Division of Infectious Diseases, Royal Brisbane and Women's Hospital, Brisbane, Queensland 4029, Australia.

**Background:** Extended Spectrum Beta-lactamases (ESBL) producing organisms are the leading cause for nosocomial infection. Recently, CTX-M genotyped ESBL are becoming the predominant type of ESBL. *E. coli* is generally divided into 4 main phylogenetic groups (A, B1, B2 and D). Pathogenic strains comprise B2 and D groups while others are commensal strain. We conducted this study to determine the genes responsible for the resistant phenotype and to determine the phylogenetic groups of the isolates. **Methods:** Phenotypic and genotypic methods were used to investigate 19 strains of multi-drug resistant *E. coli* isolated from Selayang Hospital. We performed antimicrobial susceptibility testing, screening and confirmation for ESBL production on all the isolates. The extracted DNA was subjected to PCR using specific primer pairs for *bla*<sub>SHV</sub>, *bla*<sub>CTX-M</sub>, *bla*<sub>CTX-M-15</sub> and *bla*<sub>CMY</sub> genes. Phylogenetic grouping of the strains were determined using phylogenetic group multiplex PCR assay and detection of the pandemic O25b-ST131 *E. coli* was made using O25pabBspe primers as previously described. **Results:** CTX-M gene was found in 66.6% (n=10) of isolates, SHV-gene 66.6% (n=5) and CMY-gene 20.0%. From the strains harbouring CTX-M gene, 60% (n=6) were CTX-M-15-type ESBL producer. One isolate harbored both SHV and CTX-M genes and two isolates co-producing both CMY and CTX-M-15 genes. Group D was the most predominant phylogenetic group, 60% (n=9), followed by B2, 26.6% (n=4) and B1, 6.6% (n=1). Amongst the CTX-M type beta-lactamases, three isolates had B2 phenotype and the rest were from group D. All isolates of CTX-M-15 type beta-lactamases belonged to group D. A total of four isolates (26.6%) were determined as O25b-ST131 *E. coli* clone of which 2 isolates were typical CTX-M O25b-ST131 and the other two, SHV producers. **Conclusion:** CTX-M ESBLs were the predominantly expressed gene amongst *E. coli*. Despite our limited ability to further type the genes,

CTX-M-15 would appear to be the most common type of beta-lactamases. Our isolates comprised of strains with phylogenetic groups B1, B2 and D. We have identified the *E.coli* clones belonging to the phylogenetic group B2, phylogenetic subgroup I, to the multilocus sequence type (MLST) 131 and exhibited a specific O25 type (O25b) hence providing the evidence that this virulent strain which has been reported worldwide also exist in Malaysia.

**P42. Detection of toxoplasmosis in environmental samples at a wet market of a capital city centre**

Amal R Nimir<sup>1</sup>, Tang Cher Linn<sup>2</sup>, Noor Hayati Mohd Isa<sup>2</sup>

<sup>1</sup>Department of Pathology, Cyberjaya University College of Medical Sciences; <sup>2</sup> Department of Parasitology, Universiti Kebangsaan Malaysia

**Background:** The local Chow Kit market is the largest wet market in the city of Kuala Lumpur. It is very close to the biggest government hospital in the city centre. However, the level of cleanliness in this area is always questionable and a matter of concern. The aim of this study was to identify the prevalence of *T. gondii* oocyst in water samples used by hawkers in that market and tissue cysts in rats' brains captured from the same area. **Methods:** Water samples were taken to Parasitology laboratory in Universiti Kebangsaan Malaysia and sugar flotation concentration method was used. Supernatant microscopical examination was performed then after. Seven hundred fifty two slides were screened for the presence of *T. gondii* oocyst. Hundred rats wandering in the same area were also captured by the hawkers using the mousetrap. After each animal was sacrificed, electric microtome sectioning of the rats' brain was used to cut serial sections of 5 $\mu$  in thickness. The de-waxed tissue sections were stained by the progressive Haematoxylin and Eosin (H&E) stain for microscopical examination. A total of 1000 slides were screened under the light microscope to detect the presence of *T. gondii* brain cyst. **Results:** All the water samples found to be negative for *T. gondii* oocyst. Out of one hundred rats captured, three rats found to possess *T. gondii* cysts in their brain. **Conclusion:** Water samples reflect minimum or no possibility of solid food contamination, while the 3% of positive brain cysts influence the researchers to broad their investigations for future projects.

**P43. Zero prevalence of intestinal cryptosporidiosis among children with different types of malignancies in a tertiary hospital in Malaysia**

Lubna M Elbishti<sup>1</sup>, Ngah Zasmy Unyah<sup>2</sup>, Amal R Nimir<sup>3</sup>, Rukman Awang Hamat<sup>4</sup>

<sup>1, 2, 4</sup> Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia; <sup>3</sup>Faculty of Medicine, Department of Pathology, Cyberjaya University College of Medical Sciences

**Background:** *Cryptosporidium parvum* is an opportunistic parasitic agent that has a world-wide distribution. The disease caused by this parasite can be severe and very difficult to manage in immunocompromised patients especially in children with malignancy. However, data on immunocompromised children in Malaysia is very much lacking. The aim of this study was to estimate the prevalence of cryptosporidiosis in children and types of malignancies. **Methods:** A cross-sectional study was conducted over a 5-month period from November 2009 until March 2010 in Institute of Pediatrics, Hospital Kuala Lumpur. A self administered questionnaire was used and medical records were obtained. Stool samples were examined for the *Cryptosporidium* oocyst by using two different techniques i.e. Modified Ziehl-Neelson stain and Immunochromatographic (ICT) assays (RIDA-Quick *Cryptosporidium* R-Biopharm, Germany). **Results:** Sixty stool samples were collected from children with different types of malignancies that comprised acute lymphoblastic leukemia [(50%)], brain tumor [(13.3%)], lymphoma (10%), acute myeloid leukemia (6.6%), retinoblastoma (5%), Wilm's tumor (3.3%), hepatoblastoma (3.3%), acute lymphocytic leukemia

(1.6%), acute myeloblastic leukemia (1.6%), osteosarcoma (1.6%), juvenile myelomonocytic leukemia (1.6%) and pleuropulmonary blastoma (1.6%). All samples were negative for *Cryptosporidium* oocyst. 19 (31.1%) of those patients had history of admitted to other wards while 12 (20%) had history of admitted to a day-care center and history of contact with animals. In terms of personal hygiene practices 11(18.3%) did not washed their hands before and after eating, or after going to the toilet. In addition, 10 (6%) had history of swimming in the swimming pool and 3 (5%) had history of drinking tap water) **Conclusion:** In this present study, the prevalence of cryptosporidiosis was 0% in children with malignancies. A large multicenter study is needed to establish the prevalence and characteristics of cryptosporidiosis in these immuno-compromised children.

#### **P44. Emergence of *ampC* gene among cefepime-resistant *Stenotrophomonas maltophilia* in two tertiary care hospitals in Malaysia**

Chong Seng Shit<sup>1</sup>, Salasawati Husin<sup>2</sup>, Suhaila Md Hanapiah<sup>3</sup>, Vasantha Kumari Neela<sup>1</sup>, Rukman Awang Hamat<sup>1</sup>

<sup>1</sup>Department of Medical Microbiology and Parasitology, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia; <sup>2</sup>Department of Medical Microbiology and Immunology, Faculty of Medicine, Universiti Kebangsaan Malaysia Medical Centre; <sup>3</sup>Department of Pathology, Tengku Jaafar Hospital, Seremban.

**Background:** *Stenotrophomonas maltophilia* is a non-fermenting Gram-negative bacillus that has been recognized as an important cause of nosocomial infection, especially in immunocompromised patients, resulting in significant morbidity and mortality. This is further complicated by the intrinsic nature of these bacteria against multiple antimicrobial agents, including carbapenems and aminoglycosides. Meanwhile, rapid emergence of antibiotic resistance against two and third-generation cephalosporins has made cefepime as an alternative agent used in resistant infections. However, this has put cefepime at risk of resistance. Indeed, isolation rates of cefepime-resistant *S. maltophilia* (CRSM) in Malaysian hospitals have been increasing every year since the year 2000. High morbidity and mortality rates were also observed in immunocompromised patients with co-morbid illnesses. The capability of as an opportunistic pathogen causing various nosocomial infections in immunocompromised patients has been demonstrated. **Methods:** Clinical isolates of *S. maltophilia* from University Kebangsaan Malaysia Medical Centre (UKMMC) and Seremban Hospital were identified by species-specific PCR (SS-PCR) based on 23s rRNA gene. The antibiotic susceptibility test (AST) and E-test (AB bioMerieux) were conducted to determine its minimum inhibition concentration (MIC) of cefepime according to the Clinical and Laboratory Standards Institute (CLSI) agar dilution method for aerobic, non-fermentive organisms. New primers were designed which targeted to full sequence of *ampC* gene in *S. maltophilia* for sequence analysis purpose. Results were sequenced and analyzed. **Results:** Clinical isolates of *S. maltophilia* were verified by SS-PCR with the amplification of a 531 based-pair product. Each of hospitals mentioned above isolated one CRSM recently. However, their genetic morphology is the same. Sequence analysis of *ampC* gene amplicons (1152 bp) of sensitive strain shown is similar to NCBI database sequence. However, point mutations were found in both intermediate and resistant strains from UKMMC and Seremban Hospital. **Conclusion:** The finding suggests that point mutation in *ampC* gene of *S. maltophilia* causing it to be resistant to cefepime. Cloning and expression will be conducted in future for amino acids sequence analysis purpose.

**P45. Intestinal microsporidiosis in children with malignancy in Malaysia: Prevalence and clinico-epidemiological characteristics.**

Nur Raihana I<sup>1</sup>, Rukman AH<sup>1</sup>, Malina O<sup>1</sup>, Norhayati M<sup>2</sup>, Fatmah MS<sup>2</sup>, Anisah N<sup>2</sup>, Eni Juraida AR<sup>3</sup>

<sup>1</sup>Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Selangor; <sup>2</sup>Department of Parasitology & Entomology, UKM Medical Centre; <sup>3</sup>Institute of Pediatrics Hospital Kuala Lumpur, Malaysia.

**Background:** Intestinal microsporidiosis is caused by Microsporidia-obligate intracellular protozoans of the phylum Microspora, which commonly affect people with HIV/AIDS. Recently, infection with this microsporidian has been described in other immuno-compromised individuals and also immuno-competent hosts. However, intestinal microsporidial infections have been rarely reported in HIV-negative patients and in a limited number of case reports associated with elderly, children and in patients who receive immunosuppressive drugs. **Methods:** Seventy-five stool samples were collected from children who had malignancies in an Institute of Pediatrics, HKL from November 2009 until March 2010. Each sample underwent a Modified Chromotrope-Kinyoun staining technique for the detection of microsporidian spores and visualized under light microscopy with high magnification (x100). Clinical data was retrieved from patient's medical records. **Results:** Out of 75 samples, 8 (10.7%) were positive for microsporidian spores. Of this, 7 (75%) and 1 (25%) were from Malays and Indians, respectively. Female children with cancers were the main group affected with microsporidiosis as compared to male children [6 (75%) vs. 2 (25%)]. The median age was 2.25 years (interquartile range: 1.43 to 8.35 years). In terms of type of malignancies, microsporidian spores were found in 6 children with leukemia (75%). This was followed by lymphoma [1, (12.5%)] and retinoblastoma [1, (12.5%)]. Moreover, 5 (62.5%) patients with microsporidia had diarrhea and 3 (60%) of them had cramping abdominal pain as well. In addition, 6 (75%) out of 8 patients had fever more than 38.5°C. **Conclusion:** Microsporidia are also prevalent in other immuno-compromised groups such as in children with malignancy. Stools stained with Modified Chromotrope-Kinyoun (MCK) stain can be used as a screening tool for intestinal microsporidiosis in immuno-compromised children.