POSTER PRESENTATIONS

P001. Purification and characterization of -D-mannosidase from human serum

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The human serum proteins are a mixture of simple proteins, glycoproteins, lipoproteins and high activities of glycosides enzymes viz. -mannosidase, -galactosidase, N-acetyl- -D-glucosaminidase and acid phosphatase. -mannosidase (EC:3.2.1.24), a glycoprotein with 10.6% carbohydrate was purified (13846 fold purification) to Human serum, by gel filtration on sephadex G200 and affinity chromatography on Con A-CL-seralose. The enzyme had the molecular mass of 354813 Dal and 423790 Dal by gel filtration and SDS-PAGE, respectively. The optimum temperature and pH for enzyme activity were found to be 40°C and 4.2. The Vmax and Km value for the enzyme were 101 and 2.75 mM for p-nitrophenyl -D-mannopyranoside. Chemical modification studies revealed the involvement of tryptophan for enzyme activity. The enzyme was found to depend on the presence of Zn²⁺ and Mn²⁺.

P002. Non enzymatic glycation of Lp(a) in diabetic patients increase damage of tissue under hyperglycemia condition

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Lipoprotein (a)[Lp(a)]; is a major and independent risk factor for cardiovascular disease. The pathogenicity of Lp(a) as a risk factor may depend upon its Lysine binding site(LBS) activity. It is suggested that non enzymatic glycation of Lp(a) resulting from high plasma glucose level found in diabetic patients may be one of the factors contributing to the severity of this disease. Aim: Because it is suggested that glycation increases the Lysine Binding Site properties of Lp(a). In the present study the aim was to compare the lysine binding site activity of Lp(a) which is separated from diabetic serum with normal human serum. Method: 100 ml serum was collected from diabetic patients and also 100 ml serum was collected from healthy people. Lp(a) was separated and its electrophoretic mobility was studied, then Lysine binding site properties of Lp(a) from diabetic serum were compared with native Lp(a) by using lysine sepharose affinity chromatography. Results: It was found that glycation has increased the negative charge of Lp(a) as monitored by electrophoresis, also increased the affinity of Lp(a) for Lysine sepharose affinity column chromatography. Therefore Glycation affected on lysine binding properties of Lp(a), so that the proportion of Lp(a) Lysine positive was increased. Conclusion: Nonenzymatic glycation of Lp(a) may contributes premature atherogenesis of patients with diabetes mellitus by Increasing its LBS activity and diverting lipoprotein catabolism from non-atherogenic to atherogenic pathways and causing damage to the tissues.
P003.  Effect of vitamin C on nonenzymatic glycation of Lp(a)
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Lp(a) is a cholesterol rich plasma lipoprotein discovered by Kare Berg in 1963. Experimental studies have implicated high levels of plasma Lp(a) with increased risk of atherosclerotic cardiovascular disease. Increased Lp(a) in plasma is an independent risk factor for premature cardiovascular disease. Lp(a) resulting from high plasma glucose levels found in diabetes to be one of the factors contributing to the severity of this disease. Nonenzymatic glycation of Lp(a) may contributes premature atherogenesis of patients with diabetes mellitus by diverting lipoprotein catabolism from non-atherogenic to atherogenic pathways. **Aim of study:** It is suggested that non enzymatic glycation of Lp(a) resulting from high plasma glucose levels found in diabetes to be one of the factors contributing to the severity of this disease. Since glycation of proteins alters both their structure and function, in this study the effect of vitamin C on Lp(a) glycation was studied. **Methods:** 100 ml of serum contain 100 mg/dl of Lp(a) was incubated with 200 m M/L glucose at 37°C for 7 days under sterile conditions. Sodium azide (0.02%) was added to prevent bacterial growth. Throughout EDTA presents at a concentrations > 0.5 mM to minimize Lp(a) oxidation. On days 1 to 7 Lp(a) was removed and dialyzed against 1L of PBS at 4 °C in the dark. Glycated Lp(a) was separated from non glycated one by boronate affinity chromatography and the percentage of glycated Lp(a) was determined. Lp(a) was also incubated at the same condition with 200 mM/L glucose and 0.2 mg/100 vitamin C, glycated Lp(a) was determined. **Results:** Glucose uptake by Lp(a) was linear as a function of time. The percentage of glycated Lp(a) was 4.3% in the glycated serum without Vitamin C, and in the presence of vitamin C was 3%. It was found out that glycation of Lp(a) in the present of Vitamin C was significantly decreased. **Conclusion:** We could suggest that supplementation of vitamin C can inhibit nonenzymatic glycation of Lp(a) in diabetic patient and protect tissues from damage under hyperglycemia.

P004.  A comparative microscopic study of human and nonhuman long bone histology
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The need to identify human from nonhuman in fragmented skeletal remains has attracted many researchers to develop various techniques for histological measurements. This project focuses on the study of microstructural parameters in human and non-human (mammal) long bones, of which the main objective is to develop a histological technique to distinguish human from nonhuman, based on the Malaysian population. The materials consist of 64 human and 65 animal bones, which are collected from the mortuary and zoos in Malaysia. The bone thin section is prepared by using a method, modified from Caropresso (2000). The procedure comprises sawing the bone, defatting in diethyl-ether, embedding in epoxy resin, mounting, sectioning to 30 µm, grinding, polishing, and mounting with a cover-slip. Measurement of microstructural parameters is done by using a transmitted light microscope, and an image analyser. The microstructural parameters assessed are cortical thickness, medullary diameter, osteon count, osteon diameter, Haversian canal (HC) diameter, osteon area, HC area, osteon perimeter, HC perimeter, and Haversian lamella count. From the results, the microstructural parameters have shown a significant difference between human and nonhuman (P<0.01). A discriminant equation is produced with a correct classification of 94.6% for all cases, 100% for human and 89.2% for animal (P<0.01). In conclusion, the technique has offered distinct advantages over currently available histological techniques for human and nonhuman differentiation. This has significant implications in the assessment of fragmentary skeletal and forensic population samples. Further research would be needed to validate the technique for a larger population sample.
P005. Determination of relationship between tuberculosis and vitamin 3 deficiency among hospitalized patients in Razi Hospital, Ahwaz, Iran, 2004-2005

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Previous vitamin D3 deficiency has effect on the induction and development of patients infected with M. tuberculosis to tuberculosis. In this case-control study, 45 patients with tuberculosis were selected. Forty five age, sex, and season-matched volunteers without past medical history of tuberculosis or chronic cough were selected. All patients and control groups were evaluated by measurement the level of vitamin D3 by RIA (radioimmunoassay) method. All results were analyzed by t-test and analyze & variance in spss11/1. The mean and SD of the level of vitamin D3 were (M=12.25, SD=9.98) and (M= 24.68, SD=19.22) among patients and control, respectively. Thirty nine (86.66%) patients and 26(57.77%) controls had level of vitamin D3 below 20ng/ml. Twenty eight (62.22%) patients had tuberculosis in warm seasons; (summer, P=0.007) & (fall, P=0.02) & (winter, P=0.08). There was significant association between previous vitamin D3 deficiency and tuberculosis. Also, tuberculosis was frequently occurred in warm seasons.

P006. Microdeletions of Y-chromosome in infertile males referred to Mehr Infertility Center in Rasht City of Iran

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Introduction: Male factor infertility accounts for about half of the cases of couple infertility. Recent extensive molecular studies have revealed that there are 4 regions in azoospermia factor locus (AZF) designated as AZFa, AZFb, AZFc and AZFd on the long arm of the Y chromosome and gene deletions in these regions have been shown to be pathogenically involved in male infertility associated with azoospermia or severe oligospermia.

Methods: 50 infertile men who referred to Mehr infertility institute in Rasht were included in this study. Semen analysis was done in each case to determine the spermatogenic statues and azoospermia and severe oligospermia men were selected. Genomic DNA was extracted from blood lymphocytes of these patients and was amplified by STS-PCR method to determine the presence of micro deletions in AZF locus. 34 STS primers including 2 controls were selected to identify micro deletions of Y chromosome on each subject.

Results: twenty six of fifty cases (52%) showed deletion of at least one of the STS Marker. 17 cases (34%) had deletion in one STS. Four oligospermia individuals (8%) had deletion in 2 STS site. Three azoospermia individuals (6%) had again deletion in 2 STS site, but in different STSs. One case had three deletions in three STS site and finally one individual had seven deletions in AZF locus.

Conclusion: Significant advances in treatment have enabled infertile males to achieve fatherhood; male infants conceived through assisted reproductive. Techniques have inherited the same Y chromosome microdeletions as their fathers, so it is important to screen men who are at risk of Y chromosome microdeletions, as this will determine if counseling is needed prior to starting infertility treatment.
P007. Effects of Iranian Vipera lebetina venom on coagulation and purification of Factor X activator

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Vipera lebetina is one of the poisonous snakes on the Iran. Vipera lebetina venom is a complex mixture of components, many of which are to be proteins possessing enzyme activities. Our studies of the crude venom have demonstrated the existence of both coagulant and anticoagulant effects of haemostasis system. In absence of calcium no coagulation of plasma was observed and the presence of calcium, it showed coagulant activity at low concentration and anticoagulant activity at high concentration. On further analysis by a purified systems it was concluded, that, it contains factor X activation activity. A factor X activator was purified from the venom by sephadex G-100 gel filtration and two steps of DEAE-cellulose (DE-52) column ion exchange chromatography. It showed a single protein band in sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) in non-reducing condition. The mol.wt was estimated to be 78 kDa by SDS-PAGE. The activator activated factor X to Xa in presence of calcium ions. It could not activate prothrombin, plasminogen nor had any effect on fibrinogen and thus appeared to act specifically on factor X. The activator has no amidolytic activities toward factor Xa substrate S-2222, Thrombin substrate S-2238, and plasmin substrate S-2251. It had no arginin esterase and caseinolytic activities on substrates benzoylarginine ethylester (BAEE) and casein, respectively.

P008. Association between ACE gene insertion/deletion polymorphism with metabolic syndrome in Iranian type 2 diabetic patients

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Background: Because ACE insertion/deletion (I/D) polymorphism has been shown to be associated with diabetes, hypertension, coronary artery diseases, and diabetic nephropathy, the aim of this study was to investigate whether ACE gene I/D polymorphism is associated with metabolic syndrome in Iranian subjects with type 2 diabetes. Methods: A total of 170 patients with type 2 diabetes (119 type 2 diabetics with MS and 51 type 2 diabetics without MS) and 91 control subjects were studied. The ACE I/D polymorphism was determined by polymerase chain reaction (PCR) utilizing specific primers. The definition and criteria of metabolic syndrome used in this study matched those proposed in the 1998 World Health Organization classification. Results: Of 170 patients with type 2 diabetes, 119(70%) fulfilled the criteria for metabolic syndrome. The prevalence of ACE genotype in control subjects with DD, ID, and II genotype was 13.2%, 47.3%, and 39.5%, respectively, and in patients with metabolic syndrome, it was 26.9%, 56.3%, and 16.8%, and in patients without metabolic syndrome, it was 21.6%, 62.7%, and 15.7%, respectively. The ACE I/D polymorphism was not significantly associated with the syndrome in patients with type 2 diabetes (P = 0.711). The frequency of DD genotype in the MS group was higher than that of the type 2 diabetic patients without MS(26.9% vs. 21.6%, P=0.447) and the control group (26.9 vs. 13.2%, P=0.02). The frequency of D allele in metabolic syndrome patients was 55% as compared to type 2 diabetic patients without metabolic syndrome 52.9% and control subjects 38.8%. Conclusion: It is concluded that the DD genotype and/or D allele of ACE gene may increase the risk for hypertension but not metabolic syndrome.
P09. Protective effect of ischemic preconditioning against hepatic ischemic injury during the course of liver resection in a rat model

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Ischemic preconditioning (IP) is a process by which a brief ischemic episode confers a state of protection against subsequent sustained long-term ischemia-reperfusion injury. The purpose of this study was to evaluate the protective effect of ischemic preconditioning on ischemic injury of the liver during the course of hepatic resection in a rat model. Three groups of 6 Wistar rats were examined; Group A: subjected to sham-operation, Group B (ischemia/reperfusion group I/R): animals subjected to left-lobe hepatic ischemia for 30 min followed by reperfusion and right lobe resection and Group C (ischemic preconditioning group IPC): subjected to 10 minutes to left-lobe hepatic ischemia followed by 10 minutes of reperfusion followed by the prolonged ischemia for 30 min followed by reperfusion and right lobe resection. After 10 days the animals were killed and the livers were excised for histopathological assessment of the degree of liver damage. Serum concentrations of lactate dehydrogenase (LDH), aspartate aminotransferase (AST), alanine aminotransferase (ALT), were measured as markers of hepatocyte injury at completion of the first laparotomy and at relaparotomy after 10 days. There were significantly higher levels of AST, ALT and LDH in ischemia/reperfusion group as compared to ischemic preconditioning group.

P10. Obstructive jaundice promotes intestinal barrier dysfunction and bacterial translocation: experimental study

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Although clinical and experimental studies have demonstrated a correlation between obstructive jaundice and the development of sepsis, the mechanism has not been fully elucidated. The aim of this study was to investigate the influence of biliary obstruction on bacterial translocation as a possible source of infection in cases of obstructive jaundice. Two groups of 12 Wistar rats were examined: rats subjected to common bile duct (CBD) ligation (group A) and rats subjected to a sham operation (group B). After 7 days blood samples were taken and liver, spleen, and mesenteric lymph nodes (MLN) from the ileocaecal area were removed, divided into small pieces and cultured. Quantitative culture results were determined by the number of colony-forming units (CFU) per ml homogenate. Bacterial translocation was defined as the presence of a positive culture of mesenteric lymph nodes, blood, liver and/or spleen. Samples for histopathological examination were taken from the mucosa of the ileum and There was no evidence of bacterial translocation to MLN, blood, spleen or liver detected in any of the 12 sham-operated control rats. In contrast, bacterial translocation was demonstrated in 8 of the 12 CBD ligated rats (P < 0.01). In all 8 cases in which translocation occurred, Escherichia coli were cultured from the mesenteric lymph nodes. There were no histological changes in the mucosal samples of the control animals. In the CBD ligated rats hyperemia, vacuolization, reduction of goblet cells, decreased mitotic activity and infiltration by lymphocytes and PMNLs were detected. Cases in which translocation occurred were significantly associated with decreased mitotic activity in the colon Obstructive jaundice in a rat model predisposes to bacterial translocation. This suggests a mechanism whereby jaundiced patients are susceptible to septic complication.
P011. Mutant p53 protein as a biomarker histopathological features and prognosis of hepatocellular carcinoma after surgical resection

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Background and Aim: Prognosis after hepatectomy for hepatocellular carcinoma (HCC) has been improved by early diagnosis, progress in the surgical techniques and perioperative management. However, even when curative resection is performed at a relatively early stage, a considerable number of patients develop early intrahepatic and/or extrahepatic recurrence postoperatively. This study analysed the correlation between the p53 mutations and the clinical, pathological features, tumor recurrence and patient survival after surgical resection of HCC. Patients and methods: Specimens from 20 HCCs and surrounding liver tissues from patients who underwent surgical resection were examined histopathologically and by immunohistochemistry for detection of p53 gene mutation. The association between p53 mutation and histopathological features of HCCs, as well as tumor recurrences and patient survival were evaluated. Results: p53 mutation was found in 9 out of 20 HCCs. p53 mutations were frequent in large, and poorly differentiated HCCs. Five out of 9 with p53 mutation showed microvascular invasions in contrast to none out of 11 without mutation. Hepatocellular carcinoma recurred in 6 out of 9 with p53 mutation, in contrast to only 2 out of 11 without mutation. The 1-year survival rate in patients with p53 mutation was significantly lower than those without. Conclusion: The detection of p53 mutation in HCC may provide additional and independent prognostic information for HCC and may serve as an indicator of high-risk patients for whom closer follow-up and aggressive adjuvant therapy may be required.

P012. Elastofibroma: a histochemical, immunohistochemical and ultrastructural study of three tumors

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Elastofibroma is a rare neoplasm. Recently, the clonal proliferation of CD34-positive fibroblasts in elastofibroma has been found in elastofibroma. In this article, we studied the pathogenesis of fibrosis in elastofibroma. Three tumors obtained from two patients were selected. One patient was a 57-year-old Japanese woman had a bilateral tumor and the remaining patient was a 83-year-old Japanese man. All tumors occurred in the infrascapular region. Macroscopically, the cut surface of all tumors showed an ill-defined and whitish mass with yellowish foci. Microscopically, the tumor consisted of collagen fiber bundles, abnormal elastic fibers and spindle cells suggestive of fibroblasts. Elastica-van-Gieson and Masson-Trichrome stain identified abnormal elastic fibers and abundant collagen fibers in elastofibroma, respectively. Immunohistochemically, fibroblasts were positive for CD34, but negative for alpha smooth muscle actin and h-caldesmon. Additionally, the cytoplasm of many fibroblasts was positive for TGF-beta 1 in all tumors. Ultrastructurally, some fibroblasts with abundant organelles in one tumor were observed in the adjacent area to amorphous elastic mass and bundles of collagen fibers. However, no myofibroblasts were also ultrastructurally identified in the tumor. Finally, our study supplies a further evidence that elastofibroma may show the proliferation of CD34-positive fibroblasts and contain no myofibroblasts, and fibroblasts activated by the autocrine mechanism of TGF-beta 1 may produce both abnormal elastic fibers and collagen fibers.
P013. **Bacteriologic findings in patients with chronic otitis media**

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**Background:** Chronic Otitis media (COM) and its complications are among the most common conditions seen by the otologist. Both aerobic and anaerobic organisms are responsible for infection of the middle ear. We studied the bacteriology of otic samples from patients diagnosed with chronic otitis media referred to Otorhinolaryngology Clinic. **Materials and Methods:** Samples for culture were taken from external meatus of 50 patients with chronic otitis media using a cotton swab. The samples were cultured on enriched differential and selective media and kept in both aerobic and anaerobic conditions. Conventional biochemical tests were then performed on isolated colonies. **Results:** Forty one out of 50 (82%) of the middle ear cultures were positive. We recovered 84 strains with 15 ears having two or more different aerobic and anaerobic bacteria identified, of which 74 (85.7%) were aerobes and 10 (14.3%) were anaerobes. The most prevalent aerobes were: 24 *Staphylococcus aureus* (32.4%), 16 *Pseudomonas aeruginosa* (21.6%), 14 *Entrobacter* species (18.9%), 6 *Proteus mirabilis* (8.1%), 6 *Diphtheroid* (8.1%), 4 *Klebsiella pneumoniae* (5.4%) and *Citrobacter freundii* and *Escherichia coli* 2 strains each (2.7%). Among the recovered anaerobes were species of *Peptostreptococci* (2), *Peptococcus* (6) and *Propionibacterium* (2). **Conclusion:** Our study is one of the extensive reports on both aerobic and anaerobic bacteria in chronic otitis media. We found that the prevalence and type of organisms we identified was different from those reported in previous studies.

P014. **Bacteriologic findings in patients with ocular infection and antibiotic susceptibility patterns of isolated pathogens**

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**Objective:** Isolation of common pathogens involved in ocular infection and their in vitro susceptibility to commonly used ocular antibiotics and the trends in antibiotic resistance developed by these pathogens was investigated. **Materials & Methods:** Corneal scrapings were obtained from 318 hospitalized patients and inoculated directly onto blood agar and Thioglycollate broth and were incubated. Subcultures were performed and the necessary biochemical tests were conducted and the organisms identified as per standard procedures. Antibiotic susceptibility was determined for all positive cultures and susceptibility of isolated pathogens to commonly used ocular antibiotics was examined. **Results:** Seventy various organisms were isolated. Gram positive cocci accounted for 47 (67.2%) of all bacterial isolates and gram negative bacilli for 23 (32.8%). Coagulase negative staphylococci (33%) and pseudomonas sp. (24%) were the most common isolated organisms. In susceptibility testing, Gentamicin had coverage against 35 (74.5%) of 47 gram positive cocci and 19 (82.6%) of 23 gram negative bacilli. tested isolates. The coverage of Tetracycline, Cephalotin and Ceftriaxon against gram positive cocci tested isolates were 61.7%, 55% and 53% respectively. All the tested isolates of gram positive cocci showed resistance to Cefotaxime and Penicillin. Ceftriaxon and Tobramycin had coverage against 17 (73.9%) and 14 (60.8%) of 23 gram negative bacilli tested isolates respectively. The coverage of Vancomycin against coagulase negative staphylococci was 100%. However all the isolates of *S. aureus* were resistant to Vancomycin. **Conclusion:** Susceptibility analysis revealed, the antibiotics with great coverage were Gentamicin (77.1% of 54 isolates) and Ceftriaxon (42% of 42 isolates). Both antibiotics had good coverage against gram-positive cocci which constitute the majority (67.1%) of ocular isolates in this study.
P015. Antibiotic and biocides resistance of MRSA isolated from ICU in a teaching Hospital in Al-Khobar

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The sensitivity of *Staphylococcus aureus* strains isolated from ICU at King Fahd Hospital of the University (KFHU), Al-Khobar, Saudi Arabia to antibiotics and biocides was examined. The in vitro efficacy of various antibiotics, antiseptics and disinfectants was studied against methicillin resistant *Staphylococcus aureus* (MRSA) and methicillin-sensitive *Staphylococcus aureus* (MSSA) standard strains and clinical isolates. The minimum inhibitory concentration (MIC) was determined. MRSA isolates produced beta-lactamase and were resistant to erythromycin. Most MRSA isolates showed intermediate sensitivity to gentamicin, based on MIC values. No vancomycin intermediate *Staphylococcus aureus* (VISA) strain was detected. The isolates were sensitive to rifampicin. MRSA and MSSA strain were equally sensitive to phenolic disinfectants, esters of para (4)-hydroxybenzoic acid and chlorhexidine. Quartenary ammonium compounds (QAC) showed higher MIC values for MRSA strains. Methicillin resistance was associated with resistance to metal ions (cadmium and organic mercury compounds).

P016. Expression of Galectin-3 and Galectin-7 in thyroid malignancy as potential diagnostic indicators

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It has been suggested that Galectin-3 (Gal-3) and Galectin-7 (Gal-7) are potential tumor markers for differentiating thyroid carcinoma from its benign counterpart. Galectins are the β-galactoside-binding proteins with Gal-3 being a redundant pre-mRNA splicing factor. They are supposed to be p53 related regulators in cell growth and apoptosis, being either anti-apoptotic or pro-apoptotic. Although the value of Gal-3 was being studied extensively there was a little knowledge upon expression of Gal-7 in thyroid malignancy. We initiated an immunohistochemical (IHC) expression of Gal-3 and Gal-7 on various thyroid lesions. A total of 95 cases were collected including 32 benign and 63 malignant thyroid lesions. These contained 37 cases of papillary thyroid carcinoma (PTC), 9 cases of papillary thyroid carcinoma follicular variant (PTCFV), 16 cases of follicular carcinoma (FCA), 1 case of anaplastic carcinoma (ACA), 14 cases of follicular adenomas (FA) and 18 cases of nodular goiter (NG). Formalin-fixed paraffin embedded thyroid tissues were stained for IHC expression of Gal-3 and Gal-7 using monoclonal anti-human Gal-3 antibody and anti-human Gal-7 antibody (R&D Systems Inc 1-800-343-7475). Gal-3 and Gal-7 expression were measured semi-quantitatively on their distribution and staining intensity. Gal-3 expression was significantly strong in cancer cases compared to non-cancer cases (P=0.000) whereas no significant difference was noted with Gal-7 expression (P=0.870). Our findings suggested that the IHC localization of Gal-3 is a useful marker in conjunction with routine H & E staining in differentiating benign from malignant thyroid lesions while there is no significant adjunct diagnostic value in Gal-7 for thyroid malignancy.
P017. Kikuchi lymphadenitis

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Kikuchi’s lymphadenitis (KL) is a self-limiting condition typically that can mimic high-grade lymphoma or the lymphadenitis of lupus erythematosus (SLE). It is reported that while fine needle aspirations were suspicious for malignant lymphoma, a final diagnosis of “Kikuchi’s lymphadenitis” was established histologically from excised lymph nodes. Others reported that surgical biopsy is unnecessary if diagnosis can be rendered by fine-needle aspiration cytology (FNAC). The objectives of this study is to evaluate the importance of cytologic features of Kikuchi’s lymphadenitis (KL) in FNAC smears, to identify the role of apoptotic genetic disorders in the pathogenesis of necrosis in KL and to identify the types of lymphocytes and the nature of plasmacytoid cells. Four cases of Kikuchi disease were received at Pathology Department, FNAC smears stained by papaniculou were examined. Histologic sections from blocks stained by H&E were examined. Immunohistochemistry was performed using a panel of monoclonal antibodies (CD20, CD3, CD68, CD8, CD4, bcl2 P53 and Granzyme B. Three Indian cases were females with average age 24.6 years and one Indian case was male, 17 years. Fever and cervical lymphadenopathy were the main presenting symptoms. Two cases (50%) were diagnosed as KL by FNAC smears; two were suspicious for necrotizing lymphadenitis. The main cytologic features were the phagocytic histiocytes containing eosinophilic debris. Also plasmacytoid cells were numerous. Karyorrhectic, granular necrotic debris was the main feature. Examination of histologic sections showed diffuse necrosis in the background, large mononuclears with phagocytosis, plasmacytoid cell. Immunohistochemically,CD68 positive cells were numerous, Plasmacytoid cells were CD68 positive, and CD20, CD3 negative. CD3, CD8 positive lymphocytes were more predominant than CD20 and CD4 respectively. The positively stained CD8 cells showed also positive staining with granzyme- B. In conclusion KL cannot be diagnosed only by FNA even after getting the clinical data. Plasmacytoid cells are of monocytic origin. The main lymphoid cellular infiltrate is by the cytotoxic T lymphocytes which release Granzyme-B protein (effector) that induces apoptosis in target cells.

P018. Electron microscopic aspects of goiter

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For over a century, non- toxic goiter has been looked upon as the simple consequence of iodine deficiency. This view is now no longer tenable. Indeed, many characteristics of goiter do not fit with the iodine deficiency concept. Nodular goiter is a frequent disease even in those countries where the population is never exposed to iodine shortage. To differentiate between the adenomatous goiter- common type and the variant type, which is reported to be due to hereditary abnormality in thyroglobulin synthesis or abnormal iodoprotein production. Forty cases of goiter, with average age of 50 years. Sections provided were stained by H&E for diagnosis. Immunohistochemistry using monoclonal antibody for thyroglobulin, Histochemical stain using Masson’s trichrome. For electron microscope, five fresh specimens of simple and multinodular goiter were included and compared with normal sections. Comparing with normal follicle, sections of goiter showed marked invaginations in nuclear membranes, chromatin condensation under nuclear membrane and visible nucleoli, excess rough endoplasmic reticulum, numerous secretory vesicles, swollen disrupted mitochondria, abundant secretory vesicles at the apical pole. The vesicular bodies are bigger and very numerous in multinodular goiter. It was found that there is only variation in distribution and appearance of microvilli in goiter in comparison with the normal follicular cells. The nuclei have big amounts
of euchromatin and some of them have dense round bodies. Immunocytochemistry: showed the intense positive staining for thyroglobulin in lumena and cytoplasm of the normal follicles. Light staining was in goitrous sections. Histochemistry: Masson’s trichrome showed that the perifollicular sheaths were differed in size and in shape according to the stage. In conclusion, it is essential to differentiate between the different types of goiter using ultrastructural and immunohistochemical methods in conjunction with the clinical findings as the follow up is different.

P019. **Antistreptococcal and antioxidant activity of essential oil from *Matricaria chamomilla***

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Streptococci have gained increasing attention as pathogens of public health importance owing to large numbers of outbreaks of streptococcal infections. Because of negative consumer perception of chemical drugs and development of drug resistance, attention is shifting towards natural alternatives. Particular interest has been focused on the potential application of plant essential oils. The objective of the present study was to determine antibacterial efficacy and antioxidant property brought about by essential oils from *Matricaria chamomilla* L. Disk diffusion and tube dilution methods were employed to evaluate inhibition, Minimal inhibitory (MIC) and minimal bactericidal (MBC) concentrations and bactericidal kinetics of the oil. *Streptococcus pyogenes, Streptococcus mutans, Streptococcus salivarius, Streptococcus faecalis* and *Streptococcus sanguis* were exposed to essential oils of *Matricaria chamomilla* L. The oil composition was analyzed by GC and GC/MS. Antioxidant activity was determined by â-carotene bleaching test. The oil from the above plant was found to be strongly antimicrobial. MICs/MBCs of *Matricaria chamomilla* L. oil determined for *Streptococcus pyogenes, Streptococcus mutans, Streptococcus salivarius, Streptococcus faecalis* and *Streptococcus sanguis* in terms of ìg/ml were 0.1/0.2, 0.5/1.5, 0.5/0.8, 4/7 and 0.5/1.1 respectively. The oil analysis lead to identification of 18 components of which the major ones were: guaiazulene (25.6%), (E)-â-faranesens (20.1%), chamazulene (12.4%), â-bisabolol oxide B (7.3%), â-bisabolol (7.3%), and hexadecanol (5.6%). In the â-carotene bleaching test, the oil gave the best inhibition result of 82.5% after 120 minutes. The antistreptococcal effects of *Matricaria chamomilla* L. oil is stronger at lower concentrations against various streptococci strains tested. It is concluded that low concentrations of *Matricaria chamomilla* essential oil could be considered as alternative antistreptococcal and anioxidant agent.

P020. **Drug resistance of isolated strains of *Pseudomonas aeruginosa* from burn wound infections to the antibiotics and disinfectants**

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Infection is the most common problem following burn injury. Selection and dissemination of intrinsic and acquired resistance mechanisms increase the probability of burn wound colonization by resistant species such as *Pseudomonas aeruginosa*. Multidrug resistant *Pseudomonas aeruginosa* has frequently been reported as the cause of nosocomial outbreaks of infection in burn units or as colonizers of the wound of burn patiants. To compare the activity of various antibiotics and disinfectants against clinical strains of *Pseudomonas aeruginosa*. One hundred strains of *Pseudomonas aeruginosa* were obtained as clinical isolates from burn wound infections. The antimicrobial activity of antibiotics were tested by disk diffusion method of Kirby-Baur. For disinfectants, 30 ìl of each disinfectants placed on strile blank disk and was used by disk diffusion method.
The frequency of resistant strains to kanamycin, tobramycin, amikacin, cefotaxime, carbenicillin, ceftazidime, ceftriaxone, cefazolin, cephalaxine and ceftriaxone were 100, 93, 95, 81, 84, 95, 100, 99, 100, 100 and 92 respectively. The means of diameter of inhibition zone for chlorhexidine (0.2%), povidone iodine (10%), cetrimide-C (3.5%), dekosept, hypochlorite (10%), micro 10+ (2%), deconex 53+ (2%) and ethanol (70%) were 14.4±1.9mm, 10.6±1.3 mm, 9.1±2.6 mm, 8.6±2.2 mm, 26.9±5.2 mm, 6.58±1.5 mm, 8.3±2.2mm and 6±0.0 mm respectively. The high frequency of resistance to antibiotics and sensitivity to a few disinfectants, were suggest to restrict the spread of *Pseudomonas aeruginosa* and to limit administration of these antibiotics and to use of hypochlorite and chlorhexidin as disinfectant, for prevention.

P021.  **Angiogenesis in colon cancer and correlation with clinicopathologic aspects**

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The basic pathogenic step in the process of tumor growth, invasion and metastasis, is tumor - induced angiogenesis. The aim of this study was to evaluate the angiogenesis in colorectal carcinoma by microvascular density (MVD) determination with IHC method and to determine if and how angiogenesis correlates with clinicopathologic parameters. Seventy cases, archival, paraffin embedded tissue of colorectal carcinoma of Omid hospital (Mashad,Iran) were selected. Microvessels were identified immunohistochemically, using monoclonal CD34 antibodies. CD34 positive staining was obtained in 62 out of 70 cases, and observed mainly in the cytoplasm of endothelial alls. Counting was performed at the invasive foci of tumor and not in granulation tissue or necrotic area. Two investigators examined the microvessel density by counting immunoreactive cells in the three hot spot area of tumor using a 400 X field in a blind fashion, then the median and mean value of MVD was determined and correlates with clinicopathologic parameters. Tumor - induced angiogenesis of colorectal carcinoma statistically correlated with histologic tumor grade (P=0.000). There was no significant correlation between intratumoral microvessel density and sex and age of patients, localization, stage and histologic tumor type. (P>0.1) Intratumoral microvessel density quantification in histologic specimens of colorectal carcinoma reflects the biological malignant potential of tumors and may be a useful additional prognostic factor. Microvessel density in tumor specimens may be valuable in stratifying patients in planning appropriate adjuvant and antiangiogenic therapy after surgery.

P022.  **Evaluation of MVD in biopsy specimens of ATL patients and comparison with normal cases by immunohistochemistry method**

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Adult T-cell leukemia-lymphoma (ATLL) is a rapidly progressive systemic illness with the causative agent ,the human T-cell lymphotropic virus type-1. It is endemic in the Japan, Africa, Brazil and recently north east of Iran, particulary the region of Mashhad. The role of angiogenesis is appreciated in development and prognosis of many malignancies. To examine a possible association between tumor angiogenesis and ethiopathogenic and prognostic role of it in ATL patients this study is performed. This study included 20 ATL patients between 2000-2006. Micro vessel density (MVD) were identified immunohistochemically , using monoclonal CD34 antibody .the MVD of tumoral tissues was defined by counting the number of microvessels in three hot spot , and the mean values indicated the Micro vessel density (MVD). Normal tissues as control group are noticed. There was statistically significantly increased MVD in tumoral tissue of ATL patients (Mean =17.78) in
comparison to normal tissues (Mean =3.54) [p=0.000]. In conclusion, intratumoral microvessel density quantification in histologic specimens of ATL patients reflects the biologic malignant potential and may be valuable in stratifying patients in planning appropriate adjuvant and antiangiogenesis therapy.

P023. Alveolar proteinosis in patient with myeloma and acute respiratory failure

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Pulmonary Alveolar Proteinosis (PAP) is an underestimated cause of a persistent pulmonary infiltrate in patients with haematologic malignancies. We report a case of progressive PAP in patient with multiple myeloma. Due to accumulation of paraprotein in the alveolar spaces, with the lethal outcome. On autopsy, areas of bilateral consolidation of the pulmonary parenchima was found. Microscopic examination showed alveolar accumulation of proteinaceous PAS positive material, which was positive for IgG paraprotein immunostaining. Ultrastructural analysis revealed coexistence of the bacterial infection. In this case, the rapid accumulation of intra-alveolar material led to acute respiratory failure (ARF). With this report we would like to emphasise the importance of the early PAP diagnosis as a potential cause of complications as ARF. To our knowledge pulmonary presentation or infiltration in myeloma is exceedingly rare, this being the fourth case of PAP in myeloma patient in the literature.

P024. Prevalence of hemoglobinopathies in Ahwaz City of Iran

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Haemoglobinopathies such as thalassemia and sickle cell disease are common genetic disorders with a high prevalence worldwide. These diseases are without clinical symptoms in heterozygotes, but in homozygotes needs blood transfusion and hospital care. Carriers of these diseases are at risk to have homozygote child. In our country prevalence of emoglobinopathies is high, it is therefore important to identify heterozygous carriers so that appropriate counseling may be given. The aim of this study was to determine the prevalence of hemoglobinopathies in Ahwaz. Diagnosis of haemoglobinopathies was according to the recommendation of ICSH guidelines for haemoglobinopathy diagnosis. For each sample 5 EDTA blood sample was taken, hematological indices (RBC, Hb, MCV, MCH) were determined by using Sismax coulter analyzer, Hb variants were etected and measured using CECIL HPLC with poly CAT 35x4.6 Cm column. 500 blood samples were collected. Of those 43 samples were MCV3.5 % (female: 0.66%, male: 0.66%) diagnosed as beta thalassaemia minor and 37 samples (8.16%) were diagnosed as Iron deficient, alpha thalassaemia or beta thalassaemia with normal or borderline HbA2. Hemoglobin variants for all samples showed that 7 samples had hemoglobin variants, including 4 HbS (0.88%) and 3 HbD (0.66%). In conclusion, high prevalence of thalassaemia and hemoglobin variants in Ahwaz emphasizing using of rapid and accurate methods such as HPLC for screening of all people especially newborn babies and pregnant women for haemoglobinopathies
P025. To study the effect of oxamate on the activity of LDH-C4 extracted from rat testes

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L-Lactate dehydrogenase (EC: 1.1.1.27) (LDH) catalyzes the interconversion of lactate and pyruvate with nicotinamide adenine dinucleotide as coenzyme. The homotetrameric LDH-C4 isoenzyme of LDH is present only in mature testes and spermatozoa. Oxamate inhibits sperm motility and glycolysis. Oxamate acts by inhibiting selectively the intracellular LDH-C4, thus affecting energy metabolism. Development of selective inhibitors for LDH-C4 can be completely justified because it could be useful as biochemical tools for further characterization of the metabolic and fertility implications of LDH-C4. Aim: In the present research the inhibitory effect of oxamate on LDH-C4 was studied. Methods: LDH-C4 isoenzyme from adult rat testes was extracted using: hemogenation, ammonium sulfate precipitation and DEAE Sephadex ion exchange chromatography. LDH-C4 activity was determined by recording the absorbance change at 340 nm produced by the oxidation of NADH. Oxamic acid is a competitive inhibitor of lactate dehydrogenase when the reaction goes to the reduction of pyruvate The inhibitory effect of 0-1 mM of oxamate on enzyme activity was studied. 100 fractions were collected from column chromatography. Fractions 7 to 21 had enzyme activity. Specific activity and fold of purification was 42.6 and 71% respectively. The inhibitory effect of oxamate was concentration dependent and at 0.1 and 1mM the inhibition was 65% and 100% respectively. In conclusion, LDH-C4 is related to metabolic process that provide energy for mobility and survival of spermatozoa. Due to the unique presence of LDH-C4 in spermatozoa and spermatogenic cells, LDH can be used as a target enzyme in antifertility studies.

P026. Determination of coagulase negative Staphylococcus species and antimicrobial susceptibility pattern isolated from patients referred to Golestan Hospital

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Coagulase negative Staphylococci (CoNS) are the important agents in nosocomial infection, and pathogenic agents in immunocompromised patients. Among 32 recognized species of CoNS S.epidermidis is majority. Recently resistant of CoNS against antimicrobial agents is increasing, especially against beta lactam groups. The aims of this study were isolation of species of CoNS from different organs of patients and determination of antimicrobial susceptibility. Two hundred and ten clinical specimens from different organs of patients were recognized as Staphylococci, were cultured on solid medium. The Gram staining and coagulase test were done in suspected colonies. Species of CoNS were recognized by nevobiocin and other biochemical tests. Of the 210 specimens were determinate as Staphylococci, 66.7% were shown as CoNS, and 33.3% as Staphylococcus aureus. The 16 species of all CoNS were recognized, that 68.65% of which sensitive to nevobiocine. The majority of the species of CoNS, were isolated from male patients (55.24%). The majority of species of CoNS was belonging to S.epidermidis (19.4%), and lower of isolated of CoNS were S.aureicularis, S.caprae and S.intermedius with 0.7% respectively. The isolated CoNS of the different clinical specimens were 51.49% of urine, 25.34% of blood stream, 9.7% of catheter, 8.2% of discharge, and 5.22% of the wounds. The antimicrobial susceptibility pattern were shown that the majority resistant of the CoNS belong to oxacilin (94.2%), and lower resistant belong to vancomycin (20.89%). We concluded that majority of CoNS were isolated from male, also the majority of isolated of CoNS belong to urine. The higher species of isolated CoNS were S.epidermidis. The higher resistant to oxacilin and the higher sensitive to vancomycin were shown in S.epidermidis.
P027. Relationship between specific anti-brucella antibodies in Wright’s test and detection of brucella DNA by PCR

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Since clinical presentation of brucellosis is nonspecific and show great variability, therefore, its diagnosis requires the isolation of the bacterium or detection of specific antibodies by serological tests. The sensitivity of the serological tests is low, so amplification of DNA by PCR is currently used for brucellosis diagnosis. A total of 60 serum samples were collected from brucellosis patients. Wright sero-agglutination test carried out for all sera, and PCR technique was performed involving specific primers for \( B. \) melitensis based on IS 711 in brucella chromosome. The DNA fragments were stained by ethidium bromide in gel electrophoresis and visualized by UV light. Based on results of serological Wright test, the level of antibodies was varied from 1/40 to 1/2560, which the majority of patients showed 1/320 and 1/640 antibody levels (39.9%). For these patients the PCR results were 11 cases (84.6%) and 9 cases (81.8%) respectively. All the patients with highest antibody levels (1/1280, 1/2560) were positive in PCR, while the lowest PCR positive samples were seen among patients with 1/40 antibody levels. Although the majority of patients with variable levels of antibody had a positive PCR, however due to prior antibiotic therapy and non-specific serological tests we still had a few PCR negative So PCR still can be used as a sensitive technique for detection of suspect cases of Brucellosis.

P028. Bilateral Leydig Cell tumor and male infertility: a case report

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Leydig cell tumor is a rare form of testicular neoplasm which comprises 1-3% of all testicular tumors and only about 3% of these tumors are bilateral. A few Leydig all tumor have been described in patients with klinefelter’s syndrome so far. The patient described in this case report was a 24 year-old man with chief complaint of infertility for one year. Physical examination, semen analysis, testes sonography and hormonal assay were done for him. Right side simple orchiectomy was performed for patient. In conclusion, this tumor is always benign in children and approximately 90% are benign in adults. Clinical presentation is testicular enlargement, gynecomastia, sexual activity disturbances such as decreased libido, infertility and azoospermia. We recommend complete exam and karyotype in patients with infertility and azoospermia.

P029. Extragonadal germ cell tumor and male infertility

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Over recent decades a possible decrease in sperm quality and an increase in the incidence of testicular cancer have been reported in many populations. Some recent findings, as cohort studies, showed an increased risk of testicular cancer in men with abnormal semen analysis. A 30 years old man referred to our clinic with chief compliant of infertility for 3 years. Spermogram revealed azoospermia and right extratesticular intrascrotal mass was detected by ultrasound examination. Right inguinal surgical approach showed intact small sized atrophic right testis and an intrascrotal mass. In microscopic
examination of the mass mixed germ cell tumor with teratoma, yolk sac and embryonal components
were reported. In conclusion, extragonadal germ cell tumors, like their testicular counterparts are
associated with primary germ cell defects. The higher incidence of antecedent infertility suggests
that either congenital or acquired primary germ cell defect contributes to defective spermatogenesis
and therefore, there is higher risk of cancer development in incompletely migrated germ cells. We
recommend complete evaluation of cancer in patients with infertility and azoospermia.

P030. Fine needle aspiration cytology of malignant peripheral nerve sheet tumor in the base
of the tongue

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Malignant peripheral nerve sheath tumors (MPNST) are rare in the head and neck, however benign
d schwannoma of this area are common. We described cytological features of MPNST arising in
the base of tongue which was treated by local excision and postoperative radiotherapy. To our
knowledge cytological findings of this tumor, in this anatomical location, is the first reported
case in the literature. A 65-year-old female patient presented with a swelling in the base of the
tongue for two weeks duration. She was subjected to fine needle aspiration of the mass was
reported as malignant spindle-cell tumor. Its neurogenic origin was confirmed on histopathology
and immunohistochemistry. In conclusion, fine needle aspiration cytology is a very useful, rapid
sensitive and inexpensive investigative procedure for the diagnosis of oral cavity tumors and can
guide surgeons in their plans. The cytological diagnosis of MPNST may be difficult. The correlation
between the the cytomorphologic features along with clinical correlation are necessary to increase
the diagnostic accuracy of MPNST on aspiration cytology.

P031. Nevoid basal cell carcinoma syndrome, a rare syndrome

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Nevoid basal cell carcinoma syndrome (NBCCS) is also known as Gorlin-Goltz Syndrome. The
autosomal dominant nevoid basal cell carcinoma syndrome (NBCCS) represents a series of multiorgan
abnormalities known to be the consequence of abnormalities in the PTCH gene. The approximate
prevalence is reported to be 1 case per 56,000-164,000 population. The disease is present (inherited)
at birth and most commonly manifests itself with either BCCs (usually multiple) or odontogenic
keratocysts presenting in the young age. Case Report: We report a 16 year old boy who came with
a swelling on the left side of mandible since 3 years ago. He did not have any symptoms during
mastication, no history of dental infection or trauma. In clinical examination, irregular arrangements
of teeth and disfigured jaw were seen. In dermatologic examination multiple small indurated pearly
papule with telangiectatic vessels around it, were present in face and lateral aspect of neck. One of them
that became ulcerated and crusted was excised. In dental panoramic radiography, there was multiple
well circumscribed radiolucency with smooth radiopaque margins in mandible with unerupted dental
component. These findings are confirmed in CT scan. Both maxillary sinuses were opened that these
cysts contain pertinacious whitish material and multiple small teeth. Pathologic findings of the cysts,
composed of uniformly thin epithelial lining, generally ranging from 8-10 layers thick. The luminal
epithelial cells are parakeratinized and produce corrugated profile. The skin tumor lesion is in favor
of basal cell carcinoma. So clinical manifestation of this case include multiple OKCs, facial bone defect and deformity and basal cell carcinoma, that are compatible with nevoid basal cell carcinoma syndrome (NBCCS).

**P033. The effect of post-burn local hyperthermia on reducing burn injury: The possible role of opioids**

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This paper studied the effect of post-burn local hyperthermia on burn induced injury. A second-degree burn injury was induced on the right and left flanks of Balb/c mice. Thirty-two burn wounds were divided into four groups. Opioid receptor blocking was done for groups 3 and 4 by intra-peritoneal administration of Naloxone (NLX) 30 min before the thermal injury. Local hyperthermia (45°C, 30 s) was applied only for the peripheral area of burn wounds of groups 2 and 4. Twenty-four hours after burn injury, the burned wounds were assessed for the level of iNOS (by immunohistochemistry) and the number of hair follicles (as an indicator of tissue injury). The wounds that received hyperthermia (group 2) had significantly more hair follicles (p < 0.001) compared to the control wounds (group 1). There was no significant difference between the number of hair follicles and acute inflammation of group 1 and group 3 (NLX + burn). Group 4 (NLX + burn + hyperthermia) had significantly fewer hair follicles compared to group 1 (p < 0.001), group 2 (p < 0.001) and group 3 (p < 0.001). The level of iNOS in groups 1, 3 and 4 was not significantly different but significantly more than group 2 (p < 0.001, p < 0.001 and p < 0.001, respectively). In conclusion, the results showed that local hyperthermia after second degree burn decreased the tissue injury and iNOS expression. It is also concluded that endogenous opioid response may have a key role in the above-mentioned effects of post-burn local hyperthermia.

**P034. Immunohistochemical expression of P53 protein in histologically favorable Wilms tumor and its relationship to tumor stage at presentation**

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Wilms tumor as the most common renal tumor of children has been associated with chromosomal abnormalities. Although a correlation between anaplasia and mutations of P53 tumor suppressor gene has been found in Wilms tumor, significance of these mutations in different clinical stages of favorable-histology Wilms tumor remains largely unresolved. The goal of this study was to determine the frequency of P53 expression in histologically favorable Wilms tumors and its correlation to tumor-stage at presentation. In this retrospective study 48 cases of confirmed Wilms tumor with favorable-histology were retrieved from the files of departments of pathology in 3 hospitals in Mashhad University of Medical Sciences between 1990 and 2004. Histological characteristics and clinicopathological staging were in accordance with National Wilms Tumor Study guidelines. P53 expression was determined by immunohistochemical method. For each section, the proportion of neoplastic cells exhibiting nuclear positivity was broadly quantified and their intensity of staining was charted based on visual impression by two pathologists. A total of 48 cases of histologically favorable Wilms tumor were assessed. 11 cases (23%) showed positivity for P53 which were 3 (27.3%) with stage II, 3 (27.3%) with stage IV, 2
(18.2%) with stage I, 2 (18.2%) with stage III and 1 case (9.1%) with stage V. The P53 immunopositivity was seen in 1-25% of tumor cells in 9 cases (18.8%), in 26 to 50% of tumor cells in 1 case (2.1%) and in >75% of tumor cells in the other one case (2.1%). The intensity of staining was moderate in 6 cases (12.5%), weak in 4 (6.3%) and strong only in one case (4.2%). The most common component with P53 immunoreactivity was blastemal in 11 cases (100%). In 4 cases (36.4%) there was also positivity in epithelial and in 2 cases (18.2%) in mesenchymal components. In conclusion, we found no correlation of P53 immunoreactivity and its intensity to tumor stage at presentation in individuals with histologically favorable Wilms tumors (P-Value=0.66, P-Value=0.52 respectively).

**P035. Investigation of adults patients with acute lymphoblastic leukemia at the Shafa Hospital Ahvaz**

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Acute lymphoblastic leukemia usually occurring in all age groups, and is the most common form of acute leukemia in children. This disorder often present with symptoms of fatigue, fever, bleeding and acute infection. Generalized lymphoadenopathy, splenomegaly and hepatomegaly are common findings. Classification was made according to the FAB (L1, L2, L3). The aim of this investigation was study of patients with ALL at Shafa Hospital of Ahvaz. In the present study, all patients with the diagnosis of ALL, admitted to the Shafa Hospital, Ahvaz hematology & oncology department, from 2001-2005 were reviewed. The age range of patients was 15-77 years. The majority (67%) of patients were between 15-25 years. There were 42 male (60%) and 28 female (40%), and male/ female ratio was 1.5/1. The most common form was L2, which occurred in 60 percent of the patients. Leukocyte >100 x 10^9/L was seen in 50% of cases and also platelet level. In conclusion, the results of present study indicated that L2 was the most common types in the patients.

**P036. Ameloblastic fibroblastic fibrosarcoma of the maxillary sinus in an infant: a case report with long term follow up**

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Ameloblastic fibrosarcoma (AFS) or ameloblastic sarcoma is an extremely rare odontogenic neoplasm. The authors report ameloblastic fibrosarcoma (AFS) in the maxillary sinus of a 4-month-old boy. The tumor composed of odontogenic epithelium, resembling that of ameloblastoma and a mesenchymal part exhibiting features of fibrosarcoma. We also found some areas with deposition of dentinoid material closely adjacent to ameloblastic epithelium. Although AFS has been occurred in a wide age range, this is the first report of this tumor in infancy with long term follow up.
P037. Paediatric renal neoplasm – A clinicopathological study in Pathology Departments of Dr Sheik and Imam Reza Hospital during 15 years

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Pediatric renal tumors represent approximately 7% of all childhood cancers and are completely different from those occurring in adults. The aim of this study was to make an analysis of clinical and pathological characteristics of these tumors. In this descriptive study all of the pediatric patients diagnosed as having renal neoplasm in departments of pathology of Dr Sheikh children hospital and Imam Reza hospital from 1991-2006 were evaluated. Fifty-two patients including 27 boys and 25 girls with mean age of 40.63 months were studied. Tumors involved each kidney in 24 (45.3%) and were bilateral in 5 (9.4%) patients. Abdominal mass was the commonest clinical symptom and sign. Congenital anomalies were presented in 6 (14.6%) patients. Histopathological examination showed Wilms tumor in 46 (86.8%), cystic partially differentiated nephroblastoma in 2 (3.8%), mesoblastic nephroma in 2 (3.8%) rhabdoid tumor of the kidney in 1 (1.9%), metanephric adenofibroma and low-grade tubulopapillary carcinoma in 1 (1.9%) and clear cell sarcoma of the kidney in 1 (1.9%) patient. 11 (20.8%) cases were in stage I, 16 (30.2%) stage II, 13 (24.5%) stage III, 8 (15.1%) stage IV and 5 (9.4%) stage V. Although Wilms tumor is the commonest renal neoplasm in childhood there are also recently described entities such as metanephric tumors and juvenile renal carcinoma that must be considered in histopathological evaluation of a pediatric renal neoplasm. Role of molecular and cytogenetic methods is increasing for classification and treatment of childhood renal neoplasms.

P038. Study of incidence of urinary tract infections in patients referred to Emam Khomeini Hospital in Alshter City during six months in 2005

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Urinary tract infection (UTI) is symptomatic or asymptomatic colonization of pathogenic microorganisms and the most prevalence of bacterial infections in human. Nowadays, his infections and antimicrobial resistance against related to pathogens are one of the most important health problems in the world that occurs in populations. In this study, the incidence of UTI’s during six months in patients which referred to Emam Khomeini Hospital in Alshter City were examined by considering type and quantity of bacteria in men and women. The related macroscopic and microscopic tests and urinary culture were performed, then the results compare together. After these steps, the antibiogram lest for each organism was separately executed. The results indicated that among 1266 samples refered to Emam Khomeini Hospital in Alshter city, 327 cases (25/82%) showed positive culture and women had the most positive culture (68/19%). Infectious organisms in this manner were in clued E.coli 172 cases (52/60%), Staph epidermidis 119 cases (36/40%), Enterobacter 7 cases (2/14%), Proteus 5 cases (1/52%) and Klebsiella one case (0/3%). The most effective antibiotics in this manner were Ciprofloxacin, Nitrofurantoin and Gentamycin and the least effective was Methoxazole. The positive cultures had more WBC, RBC, EP cells, mucus, bacteria and gravity weights.
P039.  Shigellosis and study of prevalence of shigella in bacillary dysentery patients referred to Imam and Golestan Hospitals in Ahwaz during 2000-2001

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Shigella is a gram negative bacillus that belongs to Enterobacteriaceae family. Human is the only know reservoir of shigella species. It can cause bacillary dysentery in the worldwide distribution especially tropically regions. Transmission of this infection is oral fecal and usually human to human directly. 200 million cases are reported annually with dysentery with 0.5 million mortality. Samples were obtained from 1075 patents with dysentery in Imam and Golestan Hospitals in Ahwaz during one year. Stool samples were collected and cultured in selective and differential media including S>S agar, Mac Conkey and enrichment media of selenit broth. The strains were identified by using biochemical tests and the shigella antisera. Therefore, epidemiological study and identification of predominant species are necessary for shigellosis. Among 1075 patients, 21 case (1.95%) were found to be infected with Shigella. The 9 case (0.8%) women and 12 case (101%) were men. Shigella species frequency were identified as indicated below: 16 strains (76%) of serogroup B (S.flesneri), 2 strains (9.5%) of serogroup D (S.sonnei), 1 strain (4.7%).fo serogroup A (S.djsenteriae) and 2 strains (9.5%) of serogroup C (S.boydii). Our results indicated that prevalence of shigellosis is higher in men than women and S.flexneri the most predominant etiologic agent that similar to developing country in contrast S.sonnei and then S. flexneri is the most predominant etiologic agent in US. Therefore, more epidemiologic studies for predominant species identification are necessary.

P040.  Pleuropulmonary blastoma: A report of three cases

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Pleuropulmonary blastoma (PPB) is a rare and aggressive tumor that is emerging as a distinct entity of childhood disease. It is characterized by mesenchymal elements (including undifferentiated blastoma and often cartilaginous, rhabdomyoblastic, or fibroblastic differentiation) and epithelium-lined spaces. PPB may be exclusively cystic (type I), solid (type III) or both solid and cystic (type II). We present the clinical and histopathologic features of 3 PPB cases, diagnosed and managed in our medical center during the past 10 years.

P041.  Rapid preparation of tissues sections by microwave and their comparison with routine method

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Microwave energy which is produced from electricity via magnetron which was found almost 60 years ago in vastly applied in technology and laboratories, especially in pathology field. The investigation in this project was focused on time and cost saving, since processing tissues takes 24 hours, the report consequently being delayed one working day. Hence initiating an applicable method applying microwave oven for a rapid tissue processing because the purpose of this project. This semi-trial study
was accomplished in 1383 (2004-2005) at department of pathology of Imam Reza hospital in Mashhad (Iran). 60 specimens from different parts of the body were verified under routine and microwave method within similar conditions. Each pair slides of similar specimen were compared in a similar technique and, quality of microscopic slides were evaluated by two pathologist. Every stage of histoprocessing was initiated with microwave calibration and standardized approaches were ultimately collected up and, results were verified in internal of 70 minutes. Collected information were arranged by descriptive statistics and affluence distribution tables. In verifying 60 above mentioned slides and the comparison of every pair slides, their quality consisting of clarity of nucleus, cytoplasms and their components were quite similar and, ever more demanding processed by microwave. This technique can completely be applied in pathology laboratories in case of accurate calibration and arrangement for time saving of tissue processing.

P042.  
Alport syndrome: ultrastructural study of 26 cases
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Alport’s syndrome (Hereditary nephritis with deafness) is a familial uncommon disease that ultra structural studies are gold standard method of it’s diagnosis. We studied 26 Iranian’s Patient’s suspicion to Alport’s syndrom by Electron microscopy and reported the findings. We observed 19 men and 7 women (male to female ratio was 2.7), the arrange age was 11.5 years (range 2.5-75 Years). The commonest findings were irregularity of the glomerular basement membrane with splitting, lamination and foot process effacement. Thining in basement membrane was reported and one case had disruption of capillary walls in study by only light microscopy. All of patient, had mesangial hypercellularity diagnosis. Immunofluorescence studies didn’t show any deposits of Immunoglobulin and complement component. Definite diagnosis must be confirmed by ultrastructural findings.

P043.  
Familial lecithin cholestrol acyltransferase deficiency: a case report
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Familial lecithin cholestrol acyltransferase deficiency is an uncommon autosomal recessive disorder from a heritable defect in esterification of plasma cholestrol. in 1968 The disease, at first was described by Gjone and Norum in Norway . This is the first report of LCAT deficiency in Iran. Our case is a 38 years old woman.Her disease was manifested by presence of lower extremities edema, proteinuria corneal opacities, increased plasma cholestrol and hemolytic anemia. Suspision of disease was based on renal biopsy, which revealed mesangial expansion, capillary wall widening with clusters of foamy cells in mesangium. Immunofluorescence study was nonspecific, but specific findings of electron microscopy showed deposition of lipid in glumerular basement membran and mesangium. The diagnosis was confirmed by low HDL-cholestrol concentration, decrease of LCAT activity in plasma and positive familial history of the disease.

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Study of tumor proliferative activity may provide a predicting parameter to estimate biologic aggression and a subsequent prognosis that has been evaluated in many malignancies. We have selected Renal cell carcinoma in this study. To determine tumor proliferative activity, Ki67 antibody was applied and results were compared with apoptosis, applying P53 antibody by immohistochemical staining. Specimen of 30 patients who underwent radical nephrectomy for RCC were selected for histopathology and immunohistochemically study. Applying two different grading systems (S & H, Fuhrman) to calibrate average nucleoli diameter and tumor grading on all specimen. Tissues immersed in paraffin were immunohistochemically stained applying (MIB-1) ki67 monoclonal and P53 antibodies, then statistical analysis were practiced. The average nucleoli diameter of tumours was significantly bigger than a normal nucleus. Tumor grading is corresponding with the average nucleus diameter. Positive reaction to ki67 and P53 antibodies in tumors were increased comparing to control group. In conclusion, no considerable relationship between age/sex and tumor grading was observed. Average diameter dimensions of tumor cell nucleoli has direct correspondence with tumor grading positive reaction ratio to antibodies (versus ki67 and P 53) increases along with tumor grading. Existing coherency coefficient between ki67 and P53 proves strong relationship between P53 oncogene and multiplying tumor cells rate.

P046. Tissue pathology and hematological profile in a population of Malaysian HIV patients

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To describe the tissue pathology and hematological profile of serologically positive HIV patients identified at Infectious disease Clinic in General Hospital, Ipoh from 1999 to December 2006. Review and analysis of materials obtained from FNAC, biopsy, trephine, bone marrow aspiration and blood were carried out after identification of these patients via computerized data base, case notes and HP reports. Appropriate ethical approval was taken before the study and microphotographs were obtained. Ninety six samples from 59 HIV patients (43 males and 16 females) from a total of 498 HIV patients (11.84%) were examined and analyzed. The mean age 41.17 ± 1.412 with peak range in 31-40 years. Ethnically 35 were Chinese (59.3%), 11 Malays (18.6%), 9 Indians and 4 Thai nationals. 64% had sexual relationship as risk factor, 18.6% had IVDU and in 10% risk factors were not identified. Forty six patients (78%) were in HARRT while 13 (22%) had defaulted or had not started HARRT. Association of hepatitis B virus and HCV in our patients were in the range of 10.2% (6 cases) and 16.9% (10 cases) respectively while more than 60% of our patients had CD4 count less than 200/ cumm.3 Seventy three percent (11/15 cases) of our skin biopsy had inflammatory lesion while only 4 cases (26.7%) had infective lesion of which 3 had histoplasmosis and 1 had candidal fungal infection. 50% of 10 patients who had FNAC of lymphnodes had lesions consistent with mycobacterium tuberculosis followed by chronic nonspecificlymphadenitis, granulomatous and reactive follicular hyperplasia. Bone marrow aspiration and trephine biopsy on 10 patients had reactive marrow in 10% and 20% each of hypercellular marrow with erythroid hyperplasia, myelofibrosis, hypocellular marrow and ITP respectively. While majority (89.9%) of peripheral blood smears (44/49 cases) had anemia followed by leucopenia (22 cases), leucocytosis (15 cases) and pancytopenia (9 cases). Prevalence of opportunistic infections and malignancies in our study in low due to effectiveness of HARRT however hematological abnormalities still persists.
P047. Immunohistochemical expression of autophagy-related mTOR and Beclin-1 protein in breast cancer tissues, a preliminary study

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Autophagy is a process that involves degradation and recycling of proteins and intracellular components in response to starvation, stress and chemicals. It has been suggested that unrestrained autophagic proteolysis lead to autophagic cell death or Type II cell death, which limits cell-autonomous growth. Decreased levels of autophagy have been associated with tumor development, especially breast cancers. The mTOR gene which belongs to the family of phosphotidylinositol kinase-related kinases, is a key regulatory component that controls the induction of autophagy. Increased level of the mTOR is known to inhibit the autophagy process and thus leads to tumor development. Beclin-1, a tumour suppressor gene was found to be monoallelically deleted in a high percentage of ovarian, breast and prostate cancers (based on the 17q21 and gene mapping studies) and low expression of Beclin-1 is associated with a reduction in autophagic vacuole formation. Currently, there are no known relationships between these two proteins in relation to autophagy process and breast cancer. The aims of the study were to assess the expression of protein products of mTOR and Beclin-1 in human breast cancer tissues, to estimate their possible co-expression and to correlate the results of the immunohistochemical analysis with various clinicopathological parameters. Immunohistochemical staining with anti-mTOR and anti-beclin-1 antibody was carried out in adjacent normal breast tissues and cancerous breast tissues of 24 patients, mostly diagnosed with invasive ductal carcinoma, infiltrating ductal carcinomas or invasive medullary carcinomas. These breast tissues were obtained from Hospital Kepala Batas and Hospital Seberang Jaya, Pulau Pinang. Preliminary results showed that the mTOR protein is highly expressed in the breast cancer tissue samples, indicating possible reduced levels of autophagy in these tissues. The Beclin-1 protein of both adjacent normal tissues and cancerous tissues appeared to be equally expressed in all samples, indicating that the Beclin-1 tumor suppressor gene is functional in these breast cancer patients. Patients with high level of mTOR kinase expression may be good candidates for treatment using mTOR kinase inhibitors, some of which were already in the later phase of clinical trials. The relationships between the expression of Beclin-1 and mTOR and clinicopathological parameters are further discussed.

P048. Forensic Anthropology

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The main problem facing by the Forensic Pathologist when receiving the human remains and/or completely or partially skeletonized body is the body is covered with all the decomposed soft tissues which will hampering the Forensic Pathologist from examining the bones in details. This presentation is regarding the method and procedures on cleaning, drying and storage of these human remains and/or completely or partially skeletonized body in order to prepare them for the following post mortem examination. This method is adopted by our laboratory since year 2006 and found to be effective. I’ll focus on the chemical solution used in cleaning the bones, which is a mixture of hydrogen peroxide and sodium bicarbonate. This solution will detach the soft tissues and clean the bone and at the same time, will preserve the bone in its ante mortem condition so that it can be examined in a more detail and accurate condition.
P049. Lupus anticoagulant and anti-cardiolipin antibody in SLE with secondary APLAS

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Systemic Lupus Erythematosus (SLE) patients with antiphospholipid antibody syndrome (APLAS) have a high frequency of thrombosis and missed abortions. This makes it essential to be able to predict which subset of patients is likely to develop thrombotic events. This study evaluates the use of minimum, cost effective and freely available tests to identify the at risk patients. **Aim:** To study the 1) prevalence of various antiphospholipid antibodies in SLE patients with suspected secondary antiphospholipid syndrome(APLAS). 2) To detect the usefulness of activated partial thromboplastin time(APTT), dilute Russel viper venom time(dRVVT), kaolin clotting time(KCT) and anticardiolipin antibodies in the detection of APLAS in SLE. 3) To correlate between the major clinical manifestations and laboratory parameters. **Methods:** 30 SLE patients with suspected secondary APLAS based on their clinical manifestations were evaluated in the study. APTT, dRVVT, KCT and anticardiolipin antibodies were analysed. These were compared with clinical features and the results were statistically analyzed. **Results:** 40% patients presented with history of either arterial or venous thrombosis. History of recurrent abortions was present in 17% patients. The prevalence of Lupus anticoagulant and anticardiolipin antibodies in patients of SLE with secondary APLAS was 12% and 17% respectively. All the patients who had Lupus anticoagulant had either arteriovenous thrombosis or recurrent abortions as compared to 72% patients with anticardiolipin antibodies. The dilute Russel viper venom time and kaolin clotting time proved to be much more specific tests and the anticardiolipin antibody test was more sensitive.

P050. A simple spectrophotometric micromethod to estimate serum copper

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In this study we standardized a simple procedure to estimate serum copper. Guanidine hydrochloride was used to release ceruloplasmin bound copper and determined using bathocuproine disulphonate disodium salt (BCDS). Our proposed micromethod correlated well with the method of zak et al and shown good recovery and precision. Our spectrophotometric micromethod is simple, requires very less sample and coloring reagent, and can be adopted in any clinical laboratory setups.

P051. Leukotriene B4 acts as a survival factor in Hodgkin and Reed-Sternberg cells in classical Hodgkin’s lymphoma.

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Membrane phospholipids are rapidly metabolized to various proinflammatory mediators via the cyclooxygenase or lipoxygenase pathways. Many of the eicosanoids thus produced can act as potent autocrine and paracrine regulators of physiological cell functions and in tumor cell growth and survival. Leukotriene B4 (LTB4), a product of 5-lipoxygenase and LTA4 hydrolase, is a well-known potent chemoattractant for neutrophils. In-vitro and in-vivo studies have suggested that treatment of
pancreatic cancer cells with a LTB4 receptor antagonist, LY293111, can inhibit tumor growth. Similarly, anaplastic large cell lymphoma cells treated in-vitro with the LY293111 antagonist were reported to undergo cell cycle arrest and apoptosis. Classical Hodgkin’s Lymphoma (cHL) is characterized by the minority presence of Hodgkin and Reed Sternberg (HRS) cells amongst a heavy infiltrate of inflammatory cells. While HRS cells are known to produce a variety of cytokines and chemokines that modulate the inflammatory and immune-suppressive background in cHL, little is known about LTB4 expression in cHL. The aims of this study are to determine (1) whether LTB4 is expressed in cHL and (2) its role in HRS cell survival. Frozen or paraffinized sections prepared from archival patient materials diagnosed with cHL were immunostained for LTB4 and its receptors, BLT-1 and BLT-2, expression. Cultured HRS cell-lines were serum starved and treated with LTB4 receptors antagonists for 72 hrs and cell survival assessed by Alamar Blue Proliferation assay. Analysis revealed moderate to strong expression of LTB4 and its receptor on HRS cells in cHL. HRS cells also upregulated LTB4 receptors expression in response to serum stress conditions in-vitro. Furthermore, treatment of HRS cells with LTB4 receptors antagonists induced significant HRS cell death as compared to vehicle-treated controls. Taken together, our data support the hypothesis that LTB4 may be an important autocrine survival factor for HRS cells.

P052. Evaluation of HbA\textsubscript{1c} assay on Primus PDQ automated analyzer

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**Objectives:** To evaluate the performance of HbA\textsubscript{1c} assay on Primus PDQ (Primus Corporation, USA) analyzer with respect to precision, recovery and comparability with Cobas Integra 800 (Roche Diagnostics, Germany) and Bio-Rad D10 (Bio-Rad Laboratories, USA) automated analyzers. **Methods:** Primus PDQ (PDQ) employs High-Performance Liquid Chromatography (HPLC) boronate affinity chromatography methodology. Bio-Rad D10 (D10) uses HPLC cation-exchange chromatography methodology. Cobas Integra 800 employs an immunoturbidimetric method. All these three methods are according to IFCC reference standardization. Precision studies were performed using two patients’ samples and two levels of commercial control sera. Recovery was assessed using a patient’s sample with low HbA\textsubscript{1c} level admixed, in various ratios, with another sample containing high HbA\textsubscript{1c} level. Sixty patient samples analyzed on the PDQ were compared to D10 and Cobas Integra 800. **Results:** The assay time for PDQ was 2 minutes and the throughput was 30 samples per hours. The intra-run coefficient of variations (CVs) on patients’ samples were 0.5% (mean HbA\textsubscript{1c}=6.4%) and 0.6% (mean HbA\textsubscript{1c}=11.6%). The inter-run CVs on patients’ samples were 1.0% (mean HbA\textsubscript{1c}=6.4%) and 0.8% (mean HbA\textsubscript{1c}=11.6%). The intra-run CVs were 1.1% and 0.7% for level 1 (normal) and level 2 (abnormal) controls respectively. The inter-run CVs were 1.7% and 0.8% for normal and abnormal controls respectively. Mean recovery was 99.7%. The PDQ showed good correlation with D10 (PDQ = 0.96D10+0.52; r=0.99) and Cobas Integra 800 [Cobas 800] (PDQ= 1.02Cobas800-0.37; r=0.99). **Conclusion:** The HbA\textsubscript{1c} assay on PDQ was precise with CVs < 2.5% and correlated well with D10 and Cobas Integra 800.
P053. Evaluation of a urinary metanephrines reagent kit: An automated approach
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Introduction: Determination of urinary metanephrines aids in the investigation of patients with suspected phaeochromocytoma. The Bio Rad Urinary Metanephrines Reagent Kit measures total normetanephrine and metanephrine in urine by HPLC. It features automated sample preparation and injection. Objective: To evaluate the Bio Rad Urinary Metanephrines Reagent Kit. The kit was evaluated in view of improving assay reliability and specificity as compared to the manual method based on cation exchange chromatography and spectrophotometry used in UMMC. Method: Performance was evaluated for precision, linearity, accuracy, sensitivity and detection limit according to NCCLS protocols. Analytical precision was evaluated using commercial controls and patient sample. Accuracy was evaluated by assessing recovery. Linearity was determined using aqueous standards. Results: The within-run CV for metanephrine and normetanephrine was 1.9% and 2.4% (low control); 4.2% and 3.5% (high control); 3.8% and 3.3% (patient sample), respectively. The between day precision was 3.8% and 4.3% (low control); 5.5% and 3.7% (high control) for metanephrine and normetanephrine, respectively. The linearity curve showed metanephrine and normetanephrine to be linear with concentration to at least 1600µg/L and 2000µg/L, respectively. Analytical recovery averaged 102% for metanephrine and 95% for normetanephrine. Sensitivity of 23µg/L normetanephrine and 10µg/L metanephrine was confirmed. The detection limit was 3.3µg/L for metanephrine. Conclusion: The performance characteristics were satisfactory and compares well with that reported by Bio Rad. Laboratories should look into automation as it improves assay reliability, facilitates handling of larger number of samples and once established, can be reliably performed by less experienced technical staff.

P054. Performance evaluation of Arkray Adams HA-8160 HbA\textsubscript{1c} analyzer
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Objectives: To evaluate the analytical performance of Arkray Adams HA-8160 HbA\textsubscript{1c} analyzer which based on cation exchange HPLC method and its correlation with HbA\textsubscript{1c} assay on Cobas Integra 800 which based on immunoturbidimetric method. Methods: For precision study, patient samples with medium (mean = 6.6%) and high (mean = 12.9%) HbA\textsubscript{1c} values were assayed 20 times in a single run concurrently with control materials (high and low level) in duplicate. Samples with medium (mean = 6.5%) and high (mean=10.7%) HbA\textsubscript{1c} values and control materials were run by HA 8160 in duplicate twice a day in 5 consecutive days for between run study. For recovery study, 2 samples each with high and low value were selected and mixed in ratios of 1:3, 1:1 and 3:1 were analyzed by HA 8160. 60 samples were analyzed by both Cobas Integra 800 and HA-8160 for method comparison study. 10 uraemic samples and 10 thalassaemic samples were assayed on Cobas Integra 800 and HA 8160 for interference study. Results: Within run CVs were 0.6% and 0.7% for medium and high value samples respectively, 0.6% and 0.7% for low and high level control respectively. Between run CVs were 0.5% and 0.4% for medium and high value samples respectively, 0.5% and 0.6% for low and high level control respectively. The mean recovery was 100.1%. Good correlation between the 2 methods (Adams=1.05 Cobas -0.11, r=0.98) was observed. Both methods showed low HbA\textsubscript{1c} value (mean of 5.7% and 5.8% for Cobas Integra and HA 8160 respectively) in interference study. Conclusions: The Arkray Adam HA-160 HbA\textsubscript{1c} analyzer had performed within target CV of <2.5% and showed good correlation with Cobas Integra 800.
P055. Evaluation of HbA\(_1c\) Assay on Bio Rad D10 Analyzer in our Clinical Diagnostic Laboratory

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**Objective:** To evaluate the analytical performance of BioRad D10 HbA\(_1c\) analyzer (HPLC method) and compare it with Roche’s Cobas Integra HbA\(_1c\) assay (immunoturbidimetry method). **Materials and Methods:** Within-run imprecision was done by assaying controls (2 levels) and patient samples [medium (mean = 6.3%) and high (mean=10.5%) level] 20 times within the same run while between-run imprecision was conducted by assaying aliquots of controls (2 levels) and patient samples (medium and high levels) in duplicates twice a day for 5 consecutive days. Sixty patient’s samples were assayed on both analyzers concurrently for comparison study. Recovery was established by obtaining neat low and high values of patients’haemolysates, mixing them in ratios of 1:3, 1:1 and 3:1 and assaying the mixtures in duplicates. Expected HbA\(_1c\) levels were calculated to obtain recovery. Ten uraemic and then thalassaemic venous samples were assayed on the analyzers on the same day to assess interference. **Results:** The D10’s within-run coefficient of variation (CV) for control 1 and 2 were 0.5% (mean = 5.9% and 10.0% respectively). Within-run CV for patients’ sample were 1.1% (mean = 6.0%) and 1.4% (mean=10.5%) respectively. The between-run CV for control 1 and 2 were 1.0% (mean =6.4%) and 0.7% (mean = 10.0%) respectively while patients’ sample CVs were 0.3% (mean = 6.4%) and 1.2% (mean 10.5%) respectively. Both analyzers correlate well (r=0.99) and the D10 showed good recovery (mean = 101.7%). Generally, both analyzers had lower HbA\(_1c\) results with uraemic and thalassaemic samples but D10 has the advantage of displaying HbF peak/value on the chromatogram and alerting on low haemoglobin (Hb) samples enabling lab staffs to confidently verify results based on the acceptance criteria set by the manufacturer. **Conclusion:** D10 exhibited acceptable imprecision with CV <2.5%, correlates well with Cobas Integra and has good recovery with an added feature of HbF peak/value availability and low Hb value alert to allow more accurate result verification.

P056. Aspirin resistance as measured by platelet aggregometry in patients on prophylactic aspirin

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Objectives are to identify aspirin non responsiveness in patients on aspirin prophylaxis by platelet aggregometry with ADP (10 micromol) and arachidonic acid (AA) (.5 millimole). Correlate platelet aggregation responses with clinical risk factors for vascular events. Evaluate spectrum of platelet responsiveness to ADP. 20 patients on varying doses of aspirin (50mg, 75mg, 150mg) for primary or secondary prophylaxis for vascular events were studied. ADP induced aggregation was measured on Chronolog 490 Optical Aggregation System. 60 normal patients were controls. In the present study mean of ADP aggregation in normal was taken as 60%. Aspirin resistance was defined as > 60% ADP induced aggregation with >20% aggregation for AA. Partial responders were those in whom only one criterion fulfilled. Aspirin responsiveness was depicted by < 60% aggregation with ADP and < 20% with AA. Responders were characterized based on % of ADP aggregation into 2 groups – 1) aggregation between 30 – 60% and 2) <30% aggregation. Aspirin resistance was found in 2 (10%), partial resistance in 5 (25%) and aspirin responsiveness in 13 (65%). Aspirin resistance was seen in both sexes with mean age of 66 years in lower doses of aspirin (75mg). Partial responsiveness was seen more in males with 100% association with hypertension. In aspirin sensitive patients where ADP
induced aggregation was <30% or between 30 – 60%, 72 % were non smokers. Diabetes mellitus and hypertension were associated in 71% patients. Patients with recurrent vascular events (25%) were seen more in elderly (>70years). There was higher incidence in smokers (60%) with 40% having aspirin resistance or partial resistance. 4/7 (57%) patients on low dose (50 – 75mg) were either resistant or partially resistant. Normal aggregation varies according to population characteristics in a geographical location. ADP and AA induced aggregation by aggregometry is a useful diagnostic tool in investigating aspirin resistance.

**P058. Kikuchi lymphadenitis cytology, histopathology correlation and immunohistochemical study**

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Kikuchi’s lymphadenitis is a self-limiting condition typically that can mimic high-grade lymphoma. It is reported that while fine needle aspirations were suspicious for malignant lymphoma, a final diagnosis of “Kikuchi’s lymphadenitis” was established histologically from excised lymph nodes. Others reported that surgical biopsy is unnecessary if diagnosis can be rendered by fine-needle aspiration cytology (FNAC). Objectives are to evaluate the importance of cytologic features of Kikuchi’s lymphadenitis (KL) in FNAC smears, to identify the role of apoptotic genetic disorders in the pathogenesis of necrosis in KL and to identify the types of lymphocytes and the nature of plasmacytoid cells. Four cases of Kikuchi disease were received at Pathology Department, FNAC smears stained by Papaniculou were examined. Histologic sections from blocks stained by Hx&E were examined. Immunohistochemistry was performed using a panel of monoclonal antibodies (CD20, CD3, CD68, CD8, CD4, bcl2, P53 and Granzyme-B). Three Indian cases were females with average age 24.6 years and one Indian case was male, 17 years. Fever and cervical lymphadenopathy were the main presenting symptoms. Two cases (50%) were diagnosed as KL by FNAC smears; two were suspicious for necrotizing lymphadenitis. The main cytologic features were the phagocytic histiocytes containing eosinophilic debris. Also plasmacytoid cells were numerous. Karyorhectic, granular necrotic debris was the main feature. Examination of histologic sections showed diffuse necrosis in the background, large mononuclears with phagocytosis, plasmacytoid cells. Immunohistochemically, CD68 positive cells were numerous, Plasmacytoid cells were CD68 positive, and CD20,CD3 negative. CD3, CD8 positive lymphocytes were more predominant than CD20 and CD4 respectively. Numerous lymphocytes showed positive staining with granzyme-B. KL cannot be diagnosed only by FNA even after getting the clinical data. Plasmacytoid cells are of monocytic origin. The main lymphoid cellular infiltrate is by the cytotoxic T lymphocytes which release Granzyme-B protein (effector) that induces apoptosis in target cells, while no rule of apoptotic genes bcl2 or p53.
P059. Solid pseudopapillary tumour of the pancreas: report of four cases from Manipal

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Recently, pathologists have been showing great interest in the rare, distinct variant of pancreatic neoplasm namely solid pseudopapillary tumour (SPT). The obvious reason being that it carries a much better prognosis with surgical excision being curative. SPT is an unusual pancreatic neoplasm of low malignant potential mostly seen in young women. Since it has a characteristic light microscopic picture, diagnosis is usually not a problem. Immunohistochemistry is very puzzling as it shows both exocrine and endocrine differentiation. From the years 2002 to 2006, we encountered 4 patients with SPT of pancreas. All were young females with a median age of 19.8 years. All presented with upper gastrointestinal symptoms and mass abdomen. Ultrasound / CT scan showed heterogenous solid and cystic mass arising from the pancreas. CT guided FNAC was done for 2 cases which were reported as papillary cystic neoplasm of the pancreas. Surgery was done for all – debulking for one case due to local infiltration, Whipple’s procedure for two cases and chemotherapy followed by surgery for one case. Grossly, the tumours were encapsulated with solid, cystic and haemorrhagic areas. Microscopically, tumour cells were arranged in the form of solid sheets, pseudopapillae and microcysts with areas of haemorrhage and necrosis. With histopathology and supported by immunohistochemistry, all were diagnosed as solid pseudopapillary tumour of the pancreas. One patient was lost for follow up and the others are alive, doing well with no metastases.

P060. Phospholipase a2 activity in fractions separated from Iranian Vipera lebetina venom

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Snake venoms are complex mixtures containing many different biologically active proteins and peptides. These proteins are very specific for their molecular targets and stable in vitro and in vivo. Phospholipase A2 (PLA2) is an enzyme which hydrolyses phospholipids at sn-2 position, releasing fatty acids and lysophospholipids. Some venom contains a number of PLA2 isoforms. PLA2s are major components in snake venom. They display a wide range of biological effects including neurotoxic, myotoxic, cytotoxic, edema producing and antitumoral effects. Vipera lebetina is one of the most poisonous snakes in Iran. Our studies on the crude venom have demonstrated the existence of PLA2 activity in the suspension of egg yolk substrate. Phospholipase A2 acted on lipoproteins in egg yolk and produced lysolecithin. The produced lysolecithin is able to solubilizing the egg yolk suspension. The venom of Vipera lebetina was separated into five fractions, using gel filtration chromatography on sephadex G-100 equilibrated with 20mM ammonium acetate buffer PH= 6.8. These fractions were tested for PLA2 activity. This activity was detected in PII and PIII. It was more prominent in PII. The specific activity of PII was about 2 times higher than that of crude venom.
P061. The status of cathepsin D expression in primary breast cancer and its correlation with prognostic factors

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The breast cancer is the most common malignancy in women. Even according to the new studies this is concluded that the breast cancer in women is the most essential and important oncology’s disease. This malignancy is a suitable example of the use of biologic markers to learn more about predilection, incidence, diagnosis, treatment and outcome. One of these markers is cathepsin D. It is an aspartatic lysosomal protease that plays a main role in the multiple tumor progression stages. The purpose of this study is to determine the frequency of expression of cathepsin D in primary breast cancer and its correlation with axillary lymph nodes involvement, grade and stage of tumor. This study is a cross-sectional study, that was performed on 75 tissue samples of patients with breast cancer admitted in Mostafa Khomeiny hospital (2000-2005). After preparing the samples, a tissue section from each sample was obtained. One section of each of the tumoral and lymph nodes were stained by H&E. We determined the type of tumor, the number of lymph nodes, the stage and the grade of the tumor. Another section was stained by a immunohistochemical technique using a monoclonal antibody for cathepsin D. Our study showed that 68% of samples were positive for cathepsin D. 70.7% of patients had lymph nodes involvement. The most of patients were in stage II (57.3%) and 42.7% were in grade II. In this study, we didn’t find any correlation between cathepsin D expression and the factors that we studied. According to the results of previous studies and our study, it seems that more complete studies with more specimens and long-term follow up are necessary to understand exact role of cathepsin D in breast cancer prognosis.

P062. Activation and migration of endothelial cells following CD44 signalling

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CD44 is an extra cellular matrix (ECM) molecule that has Hyaluronic acid (HA) as one of its principal ligands. The CD44-HA interaction plays a major role in stimulation of endothelial cells in neovascularisation and angiogenesis during cancer metastasis. This project was based on the fact that low molecular weight Hyaluronic acid fragments (HAF) brought about increased migration of bovine endothelial cells than HA. This fact was extrapolated to study the effect by addition of concentrations of HA and HAF to Human Umbilical Vein Endothelial Cells (HUVECs). Primary HUVECs, from human umbilical cords were cultured in M199 medium to passage 2 (P2). 1000 cells/ml (P2) were seeded onto colloidal gold-coated matrix. Concentrations of HA and HAF ranging from 10µg/ml to 500µg/ml were added in assays to observe HUVEC migrations. Heparin’s effect on CD44-HA/HAF interaction was also studied. CD44 was visualized using SDS-PAGE and Western blot analysis. Activating antibodies to CD44, D2.1 and IL-1α were added to increase CD44 expression. Expression analysis was done using FACS in HUVEC cell line and SDS-PAGE for primary HUVECs. At a concentration of a 100µg/ml of HA, the HUVEC’s showed much greater migration in cells treated with heparin while in cells treated with HAF at a concentration of 100ug/ml in heparin untreated samples showed greater migration. Expression analysis showed that there were no CD44 expressed in primary HUVECs while CD44 were expressed in the HUVEC cell line using SDS-PAGE and Western Blot. Heparin seems to play a role in the effect of the migration. The lack of expression of CD44 in the primary HUVECs could be the passage number and cells of P2 lack expression. The CD44 receptors could have been internalized during addition of activating antibodies or they could have been destroyed by trypsinisation of the cells.
**P064. Discriminant analysis model of urinaysis for diagnosis of urinary tract infection**

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A simple, rapid and accurate test for definite diagnosis of urinary tract infection (UTI) is necessary. In this research, we studied discriminant analysis model of urinanalysis (U/C) for definite diagnosis of UTI. The cross sectional study had made on 1158 specimens, sent to lab for U/C. For all specimens, routine U/A was done at same time. The specificity, sensitivity, PPV and NPV for different parameters of U/C calculated. Then, we used discriminant analysis model too. Using of discriminant analysis model is able to increasing specificity, sensitivity, PPV and NPV of U/C on diagnosis of UTI. Discriminant analysis model is a new method for increasing diagnostic value of screening tests such as U/C.

**P065. Validation of rapid tests for the identification of *Escherichia coli* in microbiology diagnostic unit**

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Rapid methods for the identification of *E.coli* have been shown to be specific and inexpensive. The Microbiology Laboratory of Selayang Hospital isolates a substantial number of *E. coli* and the introduction of such rapid methods would be considered appropriate. The study was carried out to determine the accuracy of the rapid methods and compared them with the current method used. The identification of *E. coli* by the rapid methods consists of two tests i.e. spot indole and L- pyrrolidonyl-beta-naphthlamide (PYR) and these were compared to the existing biochemical identification kit (Enterotube, Becton Dickinson). Evaluation was performed on 106 clinical isolates of gram negative organisms demonstrating typical lactose fermenting colonies on Mac Conkey agar and were oxidase negative. The isolates were further verified for their identification by the automated identification system (Vitek®, bio Merieux Inc). The methods in demonstrating PYR and spot indole reactions in the isolates that met the core criteria were found to be comparable to the Enterotube test. Both identification systems did not yield misidentifications with the Vitek system. The cost per test for the rapid methods was reduced to half from the existing method used. Our result suggest that the use of the rapid methods in the identification of *E. coli* yields comparable results with the Enterotube test ,saves laboratory resources and provides timely identification.

**P066. Carcinoma ex pleomorphic adenoma of the salivary gland: diagnostic ancillary biomarkers in carcinomatous transformation**

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Carcinoma ex pleomorphic adenoma (CPA) is the 3rd common malignancy of the salivary gland, developing in a long-standing primary or a recurrent pleomorphic adenoma (PA). Little about pathogenesis or specific indicators of malignant transformation of PA has been known. To investigate possible modifications associated with acquisition of malignant phenotype, we examined smooth muscle actin (SMA), p53, p63, bcl-2, EGFR, VEGF, c-erbB-2, c-kit and Glut-1 in 23 CPAs by
immunohistochemistry, and compared with expressions of residual PA. Histologic features including invasiveneness, grade and subtype were additionally analyzed. CPAs were classified into two groups according to their predominant cellular component: carcinomas with luminal differentiation (69.6%, adenocarcinoma, NOS 10; mucoepidermoid carcinoma 4; salivary duct carcinoma 2, high grade 9; low grade 7, invasive 4; minimally/non-invasive 12), and carcinomas with myoepithelial differentiation detected by positive reaction for p63 or SMA (30.4%, myoepithelial carcinoma 5; epithelial-myoepithelial carcinoma 2, low grade 7, invasive 3; minimally/non-invasive 4). In CPA with luminal differentiation, immunohistochemical reactions for p53, c-erbB-2, VEGF and Glut-1 were more common in malignant epithelial cells than in benign components (p=0.012, 0.002, 0.001 and 0.032, respectively) and c-erbB-2 expression was associated with high histologic grade (p=0.041). In CPA with non-luminal differentiation, only p53 expression was more common in carcinomatous elements (p=0.021). In both groups, no biomarker showed association with invasiveness of tumors. Four each cases showed expression of EGFR and c-kit. Different immunoprofiles between carcinomas with luminal and non-luminal differentiations suggest that mechanisms participating in carcinogenesis of CPA are variable, but overexpression of p53 protein in both groups implies that p53 mutation is a critical event in malignant transformation. Selective acquisition of one or more growth factor/receptor appears to be an additional pathogenetic/progressive mechanism and can be utilized for diagnostic or therapeutic purposes in a minor subset of CPA.

P067. Diagnosing tuberculous lymphadenitis on FNA: experience at Hospital Tengku Ampuan Afzan, Kuantan


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Eight million people contract tuberculosis every year, 95% of them in developing countries and one third of the world’s population is infected with Mycobacterium tuberculosis. Annually, tuberculosis causes three million deaths. Today, 3% of the new cases of tuberculosis are related to infection with HIV, a proportion which is rising rapidly especially in Africa and Asia. This trend is echoed in our hospital. Between the period of 01/01/02 - 31/12/06 (5 years), there was 134 cases referred to our department for FNA with clinical diagnosis of TB. 87 (64.9%) of these cases were confirmed and treated as TB. Granulomatous lymphadenitis (47 cases) and suppurative lymphadenitis (41 cases) were seen in almost equal incidence. Majority of these cases 70/80 (80.4%) were in the 20-40 age group. Males predominated with male to female ratio of 5: 1. 53 cases (60.91%) were anti HIV positive.

P068. Glomerular lesions in eastern region of Arab world

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One hundred and eighty eight consecutive renal biopsies were evaluated over a period of two years. As in a standard practice after receiving the ultrasound-guided renal biopsies. The fresh biopsy was divided to three parts, one frozen for immunofluorescence evaluation, one placed in 4% gluteraldehyde for electron microscopic evaluation, and one placed in 10% buffered formalin for light microscopic evaluation. Primary glomerular diseases in 83 biopsies, GN of systemic diseases in 88, glomerular lesions in vascular diseases in 3, glomerular lesions in metabolic diseases in 7, hereditary nephropathies in 2, end-stage kidney in 2, and glomerular lesions in transplantation in 3 biopsies. Among the primary lesions, focal segmental glomerulosclerosis (28) and mesangial proliferative GN (26) were the most common. Lupus nephritis (67) and Ig A nephropathy (20) were the most common of the GN of
systemic diseases. Diabetic glomerulopathy (3) was not as common might one expects. Most likely this is due to sampling and reluctance on part of nephrologists and patients in sampling the kidney in the diabetic patients.

**P069. Brain primitive neuroectodermal tumour (PNET) subtypes in eastern region of Arab world**

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Two relevant subtypes of primitive neuroectodermal tumors (PNET) of central nervous system, lipidized medulloblastoma (medulloctoma) and atypical teratoid/rhabdoid tumor (ATT/RT), were evaluated as two extremes in terms of prognosis. Labeled Streptavidin-Biotin method was used to demonstrate the presence of epithelium membrane antigen, cytokeratine, vimentin, desmin, smooth-muscle Actin, S-100 protein, neurofilament protein, glial fibrillary acidic protein, synaptophysin, alpha fetoprotein, placental alkaline phosphatase, and human chorionic gonadotropin using antibodies on paraffin embedded tissues. Over a period of sixteen years more than 200 cases of CNS-PNET were identified. Medulloctoma was characterized by areas of “lipomatous differentiation”, low proliferative potential, and manifestation in adults. Atypical teratoid/rhabdoid tumor (ATT/RT) was characterized by the presence of rhabdoid cell differentiation and triad immunohistochemical analysis of epithelial membrane antigen (EMA), vimentin, and smooth muscle actin (SMA). This retrospective study made use of more than 200 cases of CNS-PNET, and enabled us to identify these two subtypes to help predict more accurately their histologic behaviour and their clinical outcome, based on their morphological and immunohistochemical profiles.

**P070. Analysis of insulin secretory response using 2-hours continuous infusion of glucose with model assessment in type 2 diabetic subjects**

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Type 2 diabetes is a progressive disease and this progression is principally due to affecting of b-cell function. The present study was aimed to evaluate insulin secretory capacity by pancreatic b-cells in Type 2 diabetic subjects. Subjects and Methods: Thirty-three newly diagnosed Type 2 diabetic subjects were studied with age-and BMI-matched twenty-two Control subjects. The continuous low dose infusion of glucose was started at time 0 minute, extended to 2-hour and calculated per body surface area (180 mg. min.-1. m-2) with sampling at 110, 115 and 120 minute. Serum glucose was measured by glucose oxidase method and serum insulin by chemiluminescence technique. Insulin to glucose ratio was calculated. b-cell function was assessed by 2-hour continuous infusion of glucose with model assessment (2-h CIGMA) using a computer software (HOMA-CIGMA version 2.00). Results: Analysis of beta-cell secretory capacity of two groups showed that the Diabetic group had higher insulin levels, but did not differ significantly.
P071. Interpretation of ADA and ADA2 isoenzymes in pleural effusion

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Routine laboratory tests for tuberculous pleuritis (TP) are time-consuming and unreliable. Pleural ADA levels have been found to be useful, especially in developing countries. These elevated ADA levels have been attributed to ADA2 isoenzyme; however, there are no comprehensive studies evaluating ADA2 as a diagnostic test. To investigate the diagnostic efficacy of ADA and ADA2 in diagnosing TP, a 3-year prospective study was carried out at Tygerberg Hospital. ADA and ADA2 were determined on all patients. A diagnosis was made according to predetermined criteria. A total of 951 samples were received during this period, including 387 patients with TP. An ADA value ≥ 45 U/L yielded a sensitivity, specificity and positive and negative predictive value of 97.3%, 84.2%, 81.4% and 97.7%, respectively. The diagnostic efficiency of this test was 89.7%. An ADA2 value ≥ 40 U/L yielded a sensitivity, specificity and positive and negative predictive value of 97.5%, 94.0%, 92.0% and 98.2%, respectively. The diagnostic efficiency of this test 95.5%. Pleural ADA2 is superior to pleural ADA in the diagnosis of TP and should be used as a routine test in the diagnostic workup of patients with pleural effusions in areas having a high prevalence of TB.

P073. Expression of CD137 in lymphomas

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CD137 (4-1BB) is a member of the tumor necrosis factor receptor family. CD30, well known to be expressed on Reed-Sternberg (RS) cells in Hodgkin lymphoma (HL) and cells of anaplastic large cell lymphoma (ALCL), is another member of this family, and is a costimulatory molecule that is important in regulation of immune responses. CD137 is believed to have similar costimulatory properties. It is conceivable that tumor cells could be exploiting the CD137 / CD137 ligand system to manipulate immune responses. The aim of this study is to determine whether CD137, like CD30, can be found expressed on various lymphoid malignancies. Paraffin blocks were selected from archival tissues available at Tawam Hospital, Al Ain, UAE and NUH, Singapore. After antigen retrieval by heat treatment, sections were stained overnight with anti-CD137. Detection was by the Dako Envision System. Our findings showed that RS cells in 43 out of 45 cases of HL exhibited strongly positive granular cytoplasmic and Golgi staining. Membrane positivity was sometimes observed. In contrast, positivity was present in only 11 out of 18 cases of ALCL and was much less pronounced, with only either membrane positivity or weak Golgi positivity present. Also, the pattern of staining of CD137-positive follicular dendritic cells in reactive follicles differed from that in follicular lymphoma The difference in staining for CD137 in HL and ALCL may be helpful in distinguishing between these 2 entities. The presence of CD137 in these 2 conditions, in which there is a significant inflammatory infiltrate, suggests that CD137 may be important in eliciting and maintaining that inflammatory background.
**P074. Simultaneous detection and genotyping of human papilloma virus based on oligonucleotide DNA chip**

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Human papilloma virus (HPV) is a group of viruses that can cause warts or papillomas. These viruses are small DNA viruses with a double stranded circular genome of approximately 8 Kb enclosed by a 55nm viral capsid and can be grouped into more than 100 different types. Among them, about 30 types of HPV can cause diseases and are divided into high and low risk group. Recent scientific advances have established a method to find out which type of HPV can cause cervical cancer. In this study, a genotyping method based on oligonucleotide microarray was developed for HPV genotyping, which can differentiate HPV into 22 different genotypes. Synthesized oligonucleotide probes of 22-32 nt in length with modification at 5’ end were spotted in duplicate and covalently linked onto glass slide. The oligonucleotide probes were hybridized with complementary DNA target molecules labeled with fluorescence, and the results were analyzed with DNA chip scanner. This study shows that the results of HPV genotyping by oligonucleotide chip were identical with that of sequencing. According to this study, this HPV genotyping method is very simple and can be used as an alternative method for the diagnosis of HPV infection.

**P075. Effect of pleuran beta-glucan pleurotus ostreatus on pathological changes and antioxidant status in dimethylhidrazine-induced rat colon cancer**

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Pleuran is isolated from the wood rotting oyster mushroom Pleurotus ostreatus, and represents the most significant component of the fibre complex of this fungus. The effect of pleuran on the antioxidant status and on the development of precancerous aberrant crypt foci (ACF) lesions in the colon is studied in the male Wistar rats. Colon ACF was induced by treatment with dimethylhydrazine (20mg/kg,12 doses applied s.c. in one-week interval). Eight weeks after starting diet all animals were killed. Conjugated diene content was estimated in serum, erythrocytes, liver and colon. Superoxide dismutase, catalase, glutation peroxidase activity was determined in erythrocytes, liver and colon. A diet containing either 10% pleuran or 10% cellulose was compared with cellulose-free diet and both were found to significantly reduced conjugated diene content in erythrocytes. Pleuran increased superoxide dismutase and glutation peroxidase activity in liver and glutation reductase activity in in liver. Diets containing cellulose and pleuran reduced glutation peroxidase activity and increased catalase activity in erythrocytes. The incidence of ACF lesions developed in the colon of all animals fed a cellulose free diet, however was reduced to 64 and 60% following cellulose and pleuran diets, respectively. Although ACF lesions were reduced by the cellulose diet, the more significant reduction statistically was achievd with the pleuran diet. The antioxidative activity of pleuran is consistent with the structurally identical beta-glucan obtained from Saccharomyces cerevisiae that was reported to act as an ROS scavenger. The protective effect of dietary pleuran in the prevention of, or therapy for, neoplastic and inflammatory disease of colon are promising, especially with respect to the ten-fold higher pleuran content in Pleurotus ostreatus as compared to beta-glucan content in S.cerevisiae, making the production of this substance more profitable economically.
P076. Role of LC-MS/MS spectrometry in diagnosis of various endocrine disorders.

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Until recently most of the phenotypic information on congenital endocrine disorders have relied on biochemical testing of steroids, biogenic amines and peptides but is now being combined with the molecular testing. In spite of the mutational analysis of endocrine disorders the correlation of the phenotype relies more on biochemical testing than the molecular testing. Immunoassays have been the methodology of choice for the analysis of steroids and amines in making diagnosis of patients affected with Cushing’s, bone disorders, pheochromocytoma and congenital adrenal hyperplasia (CAH). But very often the results from the endocrine laboratory had to be repeated with the HPLC-extraction assays to rule out the possible cross reactivities of the glucocorticoids, steroid metabolites and drugs with the antibody detecting the analyte. In the past the use of gold standard MS technology in the clinical diagnostic labs have been limited because of labor intensive extraction, sample preparations and chromatographic separations. Recently the use of MS/MS (tandem MS) technology in liquid and gas chromatography has revolutionized the application of MS technology in clinical laboratories. This is due to reduction in effort for extraction and chromatography and as a result has a scope for expediting the analysis of steroids, biogenic amines and peptides for the diagnosis of various endocrine disorders. We at Mayo have implemented this technology for the routine analysis of steroids, biogenic amines and peptides. We are performing 1500-2000 25-OH-vitamin-D assays using this technology. These methods not only provide reliable results for endocrine disorders but also can be used as reference methods by other laboratories and accreditation agencies.

P077. Granular cell astrocytic tumor: report of a rare glial neoplasm

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Advances in immunohistochemistry have led to the discovery of newer forms of brain neoplasms with unique prognostic implications, and one such entity, albeit rare, is characterized here. A 43-year-old male presented with a 2-month history of ataxia, facial droop, visual disturbances, and headaches. Over two months, he developed left upper extremity weakness. An MRI scan demonstrated a 5.2 cm right basal ganglia lesion that was heterogeneously hyperintense on T1 with contrast, and hypointense on T2. In addition, there was a 2.4 cm satellite lesion in the right frontal paramedian region that demonstrated more typical characteristics of a high-grade glioma. MR Spectroscopy revealed a high Choline/Creatine ratio (12:1), low NAA, and elevated myoinositol. The resected tumor revealed a heterogeneously cellular, glial neoplasm composed of larger GFAP-immunoreactive gemistocytic cells, and smaller GFAP-negative dark-staining cells. In addition, the tumor had a third population of globoid, GFAP-negative cells that were immunoreactive for EMA and CD68 antigens, and demonstrated positive staining for PAS. These were intimately admixed with the glial cells, and appeared to be integral components of this tumor. The findings were consistent with the recently described Granular Cell Astrocytoma (GCA), a rare variant of astrocytoma with important prognostic implications. In spite of high-grade features such as increased cellularity, several mitoses, and a high Ki-67 proliferation index, the tumor strangely lacked vascular proliferation or necrosis, as seen in glioblastomas. Moreover, the patient has been symptom-free after chemotherapy and radiation for the past 2 years. GCA is rare, and easily overlooked unless carefully examined with special stains. Its natural history is not fully understood. Only few cases have been documented, but none in the basal ganglia with multicentric involvement, and such extended survival. Larger multicenter studies will help to better define the biologic characteristics, and appropriate management of these unique neoplasms.
P078. Langerhans cell histiocytosis of the thyroid diagnosed by fine needle aspiration cytology: a case report.

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Langerhans cell histiocytosis (LCH) is a rare disease that extremely rare involves thyroid gland. Moreover, LCH involving the thyroid has rarely diagnosed and described by fine needle aspiration (FNA). We report langerhans cell histiocytosis of the thyroid associated with chronic thyroiditis in an 18 years old woman who complained from neck swelling. Through FNA cytology, histopathologic evaluation revealed LCH confirmed through immunohistochemical staining. Fine needle aspiration of thyroid gland will add LCH to the differential diagnosis of thyroid enlargement.

P079. Identification of JAK2 V617F gene mutation in chronic myeloproliferative disorders among Malay patients.

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Chronic myeloproliferative disorders (CMPDs) are a group of haematological disorders affecting the multi-potent stem cells that resulting in proliferation of one or more blood cell lineages. It is usually diagnosed based on clinical and laboratory criteria such as proposed by polycythaemia vera study group (PVSG) (1). This mutation is a common marker of molecular clinical entity shared by polycythaemia vera(PV), essential thrombocythaemia (ET) and idiopathic myelofibrosis (IMF). However the detection rate of JAK2 V617F gene mutation varies among few studies ranging from 65% to 97% for PV, 23-57% in ET and 35-95% in IMF (2). This variation is due to different methods used for the detection with difference in the test sensitivity. To our knowledge, no study has yet been done in Malay patients with CMPDs. Thus our aim is to identify the frequency of JAK2 V617F gene mutation in CMPDs among Malay patients. A total of 31 Malay patients known cases of CMPDs who attended Haematology Clinic in Hospital University Sains Malaysia were studied. DNA was extracted and allele-specific oligonucleotide polymerase chain reaction for JAK2 V617 gene was performed. The mean age at presentation was 55.1 years-old. Sixteen (51.6%), 4 (12.9%), 9(29%), and 2 (6%) were diagnosed as PV, idiopathic erythrocytosis, ET and IMF respectively. JAK2 V617F gene mutation was detected in 93.7% of those with PV, 44.4% in ET and 100% in IMF. None was detected in idiopathic erythrocytosis. This is the first reported incidence of JAK2 V617F gene mutation among Malay patients in Malaysia with CMPDs and the occurrence is similar to the previous study in the West.
P080. Diffuse cutaneous leishmaniasis in southwestern part of Iran

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Cutaneous leishmaniasis is still a major health problem in southwestern part of Iran. In 2002-2003 the number of cases raised significantly. In this presentation 3 cases of diffuse cutaneous leishmaniasis are reported. The first case was a 14 years old boy with cutaneous leishmaniasis in face and neck for 8 years. The patients had fistulized lymphnode in neck and had tuberculosis as well. The patient had been under treatment with Glucantime for 4 courses during the infection time but received no cure. Combination treatment with anti-TB and anti-leishmaniasis drugs cured the patient. The second case was a man at the age of 57 years and the lesions were on trunk for 4 years. The third case was a 26 years old with lesions on both hands for 3 years. Direct examination and wright Giemsa staining verified the cutaneous leishmaniasis and nested PCR characterized the species of leishmania as L.major.

P081. Evaluation of cytological alterations in clinically normal oral mucosal epithelia of smokers and nonsmokers via AgNOR counts and nuclear morphometry

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Objectives: Smoking is a known risk factor for oral squamous carcinoma. It was found that, 4.5% of clinically benign lesions have dysplastic or carcinomatous features. Thus, cytological screening of smokers and of patients with other known risk factors can be of extreme importance. Nucleolar organizer regions (NORs) are loops of DNA in which ribosomal RNA is encoded. Their number per nucleus has been shown to be correlated with cell proliferation. NORs can be visualized with the use of AgNOR silver staining technique. Nuclear enlargement is known to be one of the important signs of cellular atypia in squamous epithelium. As proliferative activity is also prominent in atypical cells of the epithelium, nuclear areas of the proliferating epithelial cells can be expected to be larger. We aimed to evaluate proliferation of the clinically normal appearing oral mucosal epithelium of smokers and non-smokers via AgNOR counts and nuclear morphometry. Methods: Smears were collected from the normal appearing mouth floor mucosa of 80 patients, 40 nonsmokers and 40 smokers, between ages of 50 and 70. AgNORs were counted in nuclei of the first 50 non-overlapping, well-fixed, nucleated squamous cells and nuclear areas of 50 cells with similar properties were calculated via computerized image analyzing system. Results: Statistical analyzes showed that, mean ± SD of AgNOR numbers per nucleus in the non-smoking group (3.47± 0.30) was lower than the smoking group (4.22±0.39)(P<0.001) and mean± SD of nuclear areas of the squamous cells of non-smokers (87 ± 9.4) (P=0.003). Mean percentage of nuclei with more than five AgNORS were 14.6% and 36.8% in nonsmokers and smokers respectively. AgNOR counts and nuclear areas showed a significantly positive relationship among smokers + nonsmokers (n=80) (r=0.338; p=0.02). Likewise there was a significantly positive relationship between AgNOR counts and nuclear areas among non-smokers (r=0.422; P=0.007). Conclusion: Our results support that smoking is a severe risk factor for oral mucosal proliferative lesions and exfoliative cytology can be the preferred method for screening of oral mucosal lesions.
P082. A rare case of angiomyxolipoma; differential diagnosis from other vascular and myxoid tumors

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Although lipomatous tumors are very frequent among benign soft tissue neoplasms, angiomyxolipoma is a very rare entity with only six cases reported before. Histopathologically, this tumor is composed of an admixture of mature adipocytes, poorly cellular myxoid spindle cell areas and abundant vascular structures. There are both thin and thick walled vessels, some of which may show prominent hyalinization. We present the 7th case of angiomyxolipoma located in the posterior neck region of a 36-year-old man. Hematoxylin-eosin stained slides of formalin fixed and paraffin embedded specimen revealed described features of angiomyxolipoma. Myxoid spindle cell lipoma, vascular spindle cell lipoma, myxolipoma, angiolipoma and pseudoangiomatous spindle cell lipoma were considered in the differential diagnosis of angiomyxolipoma. Though, most lesions can be diagnosed histopathologically without using any additional methods, immunohistochemistry is known to be a helpful tool in the differential diagnosis of this extremely rare entity. In our case, positive staining for vimentin and sparse positivity for CD34, in the absence of reactivity for SMA, desmin, S-100 protein and HMB45 in the spindle cells, were the most important immunohistochemical features that helped in the differential diagnosis.

P083. Primary rhabdomyosarcoma of the uterus

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Uterine sarcomas are rare tumors that account for approximately 3% of uterine cancers. Pure rhabdomyosarcoma (RMS) of the uterus is an extremely rare. Although RMS is a common soft tissue tumor in children, it is uncommon in adults and accounts for less than 5% of all adult soft tissue sarcomas. We reported a case of RMS of the uterus in a 60-year-old post-menopausal woman. A 60-year-old para 2 presented to National University Hospital with 2-week history of post-menopausal bleed, associated with abdominal and back pain. She also noticed a progressively increasing abdominal girth for the past 10 years. Physical examination revealed a 24-week size abdominal mass. Her CA125 level obtained was elevated at 201 units/mL. Computed tomography of the abdomen and pelvis showed multiple large uterine fibroids with areas of necrosis and calcifications. TAHBSO was performed. The tumour located at the lower uterine segment displayed areas of hemorrhage and measured 7 x 6 x 5 cm. Histologically, the tumour consisted of a sheet-like growth pattern of spindle cells with oval nuclei and eosinophilic cytoplasm, in a myxoid stroma. The diagnosis was further supported by immunohistochemical demonstration of desmin and vimentin in the tumor cells. Post-operatively, she was given 6 cycles of VAC. At 6 months followed up she was alive and well. Rhabdomyosarcoma is a tumor of mesenchymal origin which arises from cells committed to skeletal muscle differentiation. It commonly affects the head and neck region, the genitourinary tract, or the extremities. It is primarily a neoplasm of childhood and adolescence. The most common types of uterine sarcoma are malignant mixed mesodermal tumors, leiomyosarcomas, and endometrial stromal sarcomas, which account for over 95% of all cases and the remaining RMS. According to Horn and Enterline, there are four major histological types of RMS: alveolar, pleomorphic, embryonal, and sarcoma botryoides. The most common histologic variant of RMS is embryonal. As RMS is very rare, a throughout sampling and search for carcinomatous component is mandatory to exclude carcinosarcoma.
P084. **Ki-67 proliferation in serous and mucinous ovarian tumors and its relations with clinical and histopathological parameters**

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The present study aimed to evaluate Ki-67 proliferation in malignant serous and mucinous ovarian tumors by immunohistochemistry and to investigate the relations of this marker with parameters like age, tumor size, grade, stage, and lymph node involvement. The study included 29 serous and 22 mucinous carcinomas, diagnosed at the Pathology Department of our hospital between 2002-2006, all cases being in an age range of 17-82 years old. Following the routine pathological process, paraffin blocks were used in the immunohistochemical assessment based on primary Ki-67 antibodies. Malignant cells with positive nuclear staining being $\geq 5\%$ was considered for evaluations. Statistical analysis was performed by the Chi-Square, Kruskal Wallis, Fisher’s Exact, and Mann Whitney-U tests. The rates of Ki-67 expression in serous and mucinous carcinomas were 49\% and 86.3\% respectively. In serous tumors 20\% of Grade I and 70\% of Grade III were stained positive. In mucinous carcinomas these rates were 80\% and 90\%, respectively. In serous carcinomas, Ki-67 expression was observed at a rate of 58\% and 66\% in early (Stages 1-2) and advanced (Stages 3-4) stages, respectively. In mucinous carcinomas, these rates were 60\% and 71\%, respectively. Ki-67 expression was not significantly related with tumor size, age, and lymph node involvement. In serous and mucinous ovarian tumors, Ki-67 protein expression was significantly related with the type, grade, and stage of tumors, while no significant relation could be detected with the age of patients, tumor size, and lymph node involvement.

P085. **Ciprofloxacin resistance in Escherichia coli: A 12 year experience in a major Canadian health care centre**

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**Objective:** To observe an evolution of a Ciprofloxacin resistance pattern in Escherichia coli isolated from a patient population served by a large Canadian hospital over 12 years. **Method:** This retrospective study used E. coli isolated from clinical specimens collected between 1994 and 2005 as the representative organism. Microbiology laboratory database was reviewed for three sentinel years (1994, 1999 and 2005) regarding patient age, gender, specimen source, and date of collection. Organism identification and resistance to ciprofloxacin was determined by either/or: BioMerrieux Vitek, Becton Dickinson Phoenix, Kirby Bauer disk diffusion, AB Biodisk E-test, Becton Dickinson Pasco and/or Dade Behring MicroScan, using resistance criteria from CLSI (formerly NCCLS). Repeat specimens of the same sample type collected within one month were excluded from analysis. Microsoft Excel was used for data analysis. **Results:** The 6584 pathogenic E.coli strains from 5897 patients were distributed uniformly throughout three sentinel periods of the study. The most common specimen source for all three periods was urine, followed by surface swabs in 1994 and 1999, and blood in 2005. Overall ciprofloxacin resistance rates were 0.39\% for 1994, followed by a sharp increase to 3.14\% for 1999 to level off at 16.92\% for 2005. Resistance rates were highest in blood isolates for 1994 and 1999 and surface swabs in 2005. In every sentinel period, highest resistance rates were seen elderly (over 60 years of age); females with resistant E.coli outnumbered the males, particularly in 2005 (36.1\% vs.62.6\%)

**Conclusion:** Overall ciprofloxacin resistance rates increased over a 12 year surveillance period. The unexpected plateau of resistance rates in 1999-2005 was most likely due to change in prescribing habits caused by new region-wide antibiotic control initiative. Since quinolones are the mainstay of antimicrobial therapy worldwide, we advise caution when prescribing these antibiotics for elderly females, to further limit development of resistance.
P086. Synthesis, characterization Schiff bases of hydrazone derivatives and their metal complexes & a study on ethanol-induced ulcer in Sprague-Dawley rats

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Schiff bases of hydrazone derivatives have been used extensively as ligands in the field of coordination chemistry and they were found to be extensively applicable due to the remarkable biological properties such as antibacterial, antifungal, anti-tumor, antiviral and various other biological effects. The Schiff base ligands were prepared by stirring and refluxing hydrazones and carbonyl compounds in acidic ethanol. The solvent was removed to give precipitates or crystals. These ligands were then underwent the same procedure with metal acetate in order to obtain the metal complexes. All of the compounds were characterized by means of IR, UV-Vis, NMR, GC-MS, XRF, X-ray crystallography, CHN and TLC analysis. Sprague-Dawley rats were pretreated orally with different concentration of ligands and complexes before ethanol administration. The rats were sacrificed and their stomachs were removed to measure gastric lesions for anti-ulcer study purpose while the mucus and gastric juice were collected to determine the possible reaction mechanism. The resulting gastric lesions from high dose (250mg/kg) and low dose (62.5mg/kg) were compared to the negative control (10% Tween 20) and positive control (Cimetidine) by calculating the inhibition percentage.

P087. Hairy cell leukaemia (HCL) is a mature B-cell neoplasm that is rare in incidence.

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Although it rarely provides a diagnostic problem, its unique characteristics and behaviour deserves attention. We described a case of HCL in a 56 year old Malay male who initially presented with constitutional symptoms and hepatosplenomegaly for several weeks in duration. He then developed cutaneous lesions during the disease course. The peripheral blood film showed bicytopaenia and presence of mononuclear cells with multiple, fine cytoplasmic projections typical of hairy cell leukaemia cells. Immunophenotyping results showed presence of monoclonal cells that bear the surface antigen expression consistent with that of HCL. The patient unfortunately succumbed, before any treatment could be instituted. The skin lesion was inconclusive of HCL infiltration. It was most likely due to infective process, which is more common in this type of patient. Diagnosis of HCL involves presence of typical clinical presentation and presence of cells with hair-like cytoplasmic projections in the peripheral blood that bear the surface expression of a mature B-cells with the addition of certain activation markers. Conventional treatments include pentostatin, cladribine and interferon-α, however monoclonal antibodies such anti-CD 20 and anti-CD 22 are still under clinical trial for difficult cases.
P088. Study of MTHFR C677T polymorphism as a risk factor for neural tube defects in Malay ethnic origin in Malaysia: A case control study

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**Background:** Major congenital malformations occur in about 3% of newborn. Several studies have suggested that the homozygosity for the C677T methylenetetrahydrofolate reductase (MTHFR) variant is a potential risk factor for neural tube defects (NTDs), as individuals homozygous for the C677T allele exhibit elevated homocysteine concentrations under the condition of low folic acid intake. It has been hypothesized that the maternal folic acid supplementation prevents NTDs by partially correcting reduced MTHFR activity associated with the variant form of the enzyme. However the association has not been found in some ethnic groups. In this study, we attempted to assess the association between NTDs and the MTHFR C677T in Malaysian Malay population.

**Materials and Methods:** Genomic DNA was extracted from saliva of 22 children with NTDs and blood of 20 healthy controls. The MTHFR C677T genotype was determined by polymerase chain reaction with restriction enzyme Hinf I digestion. We investigated whether MTHFR genotype influenced the risk for the NTDs.

**Results:** Among the 22 NTDs patients and 20 control subjects, we found that the MTHFR 677TT genotype was absent in both patient and control groups. The MTHFR 677CT heterozygous genotype was also absent in the NTDs patients. It is however present in 3 of the control subjects (15%). All of our NTDs patients (100%) and 17 of the controls exhibited MTHFR 677CC genotype (85%). There was no Malaysian Malay individual with homozygous MTHFR C677T (TT) genotype found in this study.

**Discussion:** Folic acid is efficacious in reducing NTDs but the extent of this is unascertained in the Malaysian population. The frequency of the MTHFR C677T mutation differs among ethnic populations. In conclusion, we found that NTDs in the Malaysian Malay population is not associated with homozygosity for the C677T mutation in the MTHFR gene.

P089. Platelet rich plasma enhances isolation and expansion of human mesenchymal stromal cell

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Mesenchymal stromal cells (MSCs) are new alternatives for cell-based therapies. In vitro expansion of MSCs has been achieved in medium containing fetal bovine serum (FBS). However, FBS is a putative source of prion or virus transmission; its biosafety is a major obstacle for clinical application. Several studies have been done using platelet rich plasma (PRP) as a natural source of growth factors for expansion of autologous progenitor cells. PRP can be obtained by twice slow spin of venous blood, subsequently by addition of calcium gluconate (100mg/ml) to have the platelets release their granular contents. Also, platelet lysate (PL) obtained from platelet apheresis were shown to be rich in growth factors that promote MSC expansion. In this study, 10% PRP obtained from slow spin, 3000g for 10 min, of blood sample from venesection of healthy human volunteers (average platelet count is 3.35 x 106 per mL) was added to Dulbecco’s Modified Eagle Medium (DMEM). The resultant mixture was left overnight at 4ºC to gel. The gel was then broken up and filtered with 0.2µm pore filter. The filtrate was used as the culture medium. Media with 10% FBS or platelet poor plasma (PPP) were used as control media. Mononuclear cells (MNC) obtained from bone marrow aspirates of non-leukaemic and non-malignant patients were used as a source for MSC. MSC isolation was performed as previously reported. At passage 5, MSC were phenotypically analysed by flow cytometry and found positively
stained for MSC phenotype: CD10, CD 29, CD 44, CD73 and CD147; negative for CD14, CD34, CD45 and HLA-DR. The growth of MSC was observed to be earlier in 10% PRP added culture medium, followed by that of 10% FBS. There was no growth seen in 10% PPP added culture medium. 10% PRP appears to enhance the isolation and expansion of MSC.

**P090. Primary thyroid lymphoma: a report of two cases**

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Primary thyroid lymphoma is a rare disorder accounting for about 2% of all malignant lymphomas and less than 5% of thyroid malignancies. It is an aggressive disease with poor outcome. The majority of thyroid lymphomas are non-Hodgkin’s lymphomas of B-cell origin. The peak incidence is in the sixth decade with a majority of cases occurring in women. Two cases of primary thyroid lymphoma with similar presentations are reported to compare and highlight the clinical issues and challenges posed by this rare disease. The first case was a 65 year old Chinese gentleman who was referred for a sudden increase in thyroid gland within 4 months associated with hoarseness of voice and breathlessness. He had a large multinodular goiter which showed retrosternal extension and bilateral vocal cord involvement but was clinically euthyroid. The second case, a 68 year old Malay lady, also presented with sudden enlargement of thyroid gland associated with pain and breathlessness. She had a large diffuse goiter with retrosternal extension and intravascular invasion. Biochemically she was hypothyroid. Histopathological examination (thyroid gland) of both cases showed diffuse large B-cell lymphoma. The best management for a primary thyroid lymphoma be it chemotherapy, radiotherapy, surgery or monoclonal antibodies is still debatable. The role for surgery has evolved through the years but its importance in emergency situations should not be overlooked. These two case reports illustrate the difficulties in managing this rare disorder. Surgical intervention for primary thyroid lymphomas is mainly for tumour debulking and to relieve obstructive symptoms as was performed in the first patient. The best treatment results for primary thyroid large-cell lymphoma occur with combined-modality therapy as evident in our first patient who has survived for 12 months since presentation of symptoms. Our second patient unfortunately did not receive any therapy as she succumbed to her progressive disease.

**P091. Amniotic membrane as a carrier for cultured human mesenchymal stromal cells without altering morphology and differentiation properties**

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Amniotic membrane (AM) is a multilayered tissue with anti-angiogenic, anti-tumoural and anti-inflammatory properties. Since it does not posses antigens of histocompatibility, this membrane is never rejected by the receiving tissues. Owing to this advantage, we experimented with AM as a carrier for a coat of mesenchymal stromal cells (MSC) before transplantation. The purpose of this study is to examine whether AM interferes with the growth rate and differentiation properties of MSC. Sterilized AM was denuded by trypsin to remove the epithelium layer. Cryopreserved human MSC from previous culture, expanded and harvested at the third passage were then layered onto AM in culture. Morphology and growth pattern of the resultant MSC on AM was examined by light microscopy. MSC on AM were induced into adipocytes or oesteocytes by incubating with adipogenic or osteogenic medium. MSC on
AM apparently propagated slower when compared to MSC grown on plastic culture flask. However MSC on AM still appeared to be spindle shaped, similar to that of MSC grown adherent to plastic culture flask. Lipid droplets were observed in MSC cytoplasm when MSC on AM were induced into adipocytes. While MSC driven to osteogenic differentiation clearly showed an increased in calcium accumulation, as revealed by Alizarin Red S staining. These results showed that AM could serve as a carrier for MSC transplantation in future applications such as healing of ulcers without altering MSC morphology and differentiation properties.

P092. Analysis of post-donation platelet counts in regular plateletpheresis donors

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Introduction: Plateletpheresis is used to obtain large numbers of platelets from random volunteer donors. The donors are allowed to donate approximately 24 times in a year. One unit plateletapheresis contain at least 3 X 10^11 platelets. Because large numbers of platelet have been collected, transient but significant decreases in donors' platelet counts have been known as outcome of plateletapheresis donation. The aim of this study was to determine the outcome of regular plateletapheresis donations on donor platelet counts. Methods: A retrospective study was performed from 2001 to 2006 at Transfusion Medicine Unit, Hospital Universiti Sains Malaysia. The data of plateletapheresis donors were obtained from a computerized database. Data were analyzed using SPSS software. Results: Seventeen subjects were studied in this study. The age was between 33 and 54 years. The duration of the study was 1.4-5.3 years. Transfusion rate of subjects were 3.8 to 19.9 times per year. Average platelet count dropped from 277.8 x 10^9/L to 262.5 x 10^9/L but this drop was not statistically significant (P=0.174). Transfusion rate and duration of donation was not significantly different between the subjects (P=0.567 and P=0.575 respectively). Our results have shown that the donation rate did not influence the reduction of platelet count between subjects using repeated measure of ANOVA (P=0.272). Conclusions: Long-term and regular plateletapheresis donation does not influence donors' platelet counts. However, a decrease in platelet counts was noted transiently after donation.

P093. Immature reticulocyte fraction (IRF): the value in the detection of haemopoietic engraftment in post peripheral stem cell transplant patients

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Background: Immature Reticulocyte Fraction (IRF) is indicative of the least mature fraction of reticulocytes. The hematology analyzers measure IRF based on the fluorescent measurement of the cytoplasmic RNA. Objectives: This study was carried out to elucidate the IRF as a useful indicator of haemopoietic reconstitution after stem cell transplantation in conjunction with the absolute neutrophil count (ANC) which has been used traditionally. Methodology: Serial measurement of IRF and ANC given by the haematology analyzers (Cell Dyn 4000 and Sysmex XE-2100) from 13 adult patients underwent peripheral blood stem cell transplant (PBSCT) were collected from the day of stem cell infusion until engraftment were obtained. The criteria for engraftment for IRF was defined the first of at least 2 consecutive days with IRF >0.17 (Cell Dyn 4000) or IRF >0.05 (Sysmex XE-2100) whereas for ANC was defined as the first of at least 2 consecutive days with an ANC > 0.5 x 10^9/L. The analysis and
comparison for recovery of both IRF and ANC to indicate engraftment was done using Mann Whitney test. **Results:** The recovery of IRF was obtained after a median of 10 days, 2 days earlier than the recovery of ANC which recovered after a median of 12 days. The difference was statistically significant (p=0.016). 61.5% of the patients had the recovery of IRF as their earliest indicator of engraftment as compared to 7.7% of patients that showed earlier recovery of ANC. The different instrument used in this study did not influence the measurements of IRF (p = 0.62) or ANC (p = 0.28). **Conclusion:** IRF is the earlier sign of haematopoietic reconstitution after PBSCT when compared with the ANC which has been used traditionally. Thus, the automated reticulocytes quantifications particularly the IRF may be integrated into the clinical protocols to evaluate bone marrow reconstitution.

**P094. Bone turnover from birth to the end of life**

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Bone is metabolically active throughout life. Age, gender and various diseases exert very different effects on the biochemical markers (resorption and formation) of bone turnover, and hence it is very important to establish relevant reference ranges. The aim of this study was to investigate certain resorption markers: N-terminal telopeptide in the urine (measured by using the VITROS ECI ISM /Ortho-Clinical Diagnostics, USA/), betaCrossLaps in the serum, and the bone formation markers: osteocalcin, total and bone-specific alkaline phosphatase in the serum (measured by using the Elecsys 2010 /Roche Diagnostics, BRD/, with reagents from Roche). The study involved 5622 healthy males and 2081 male patients (age range: 0 day-99 years) and 8130 healthy females and 5783 female patients (0 day-97 years). In the healthy groups, the bone turnover was highest in the first month after birth (in mature infants the markers were significantly increased compared with adults (puberty the markers increase approximately 2 years earlier in girls). The resorption markers increased significantly (increased significantly (Paget’s disease, metastatic tumour, etc.), and during steroid therapy. In summary, each laboratory must define its own reference ranges, the laboratory medicine thereby promoting the clinical usefulness of the bone turnover.

**P095. The predictive and diagnostic values of procalcitonin and C-reactive protein for bacterial sepsis**

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**Introduction:** Diagnosis of sepsis in critically ill patient is frequently difficult. In spite of much-improved rates of survival, early predictors of the specific causes for these infections and appropriate therapy for sepsis continue to elude clinicians. The lack of specific clinical symptoms in sepsis patients, coupled with the constraints of microbiological cultures, has extended the search for newer biomarkers that can be diagnosed and sharpened early therapeutic strategies leading to shorter hospital stay, reduction in non judicious use of antibiotics and more pronounced patient recovery. One such new biomarker specific for bacterial sepsis is Procalcitonin. **Objective:** To compare the diagnostic value of procalcitonin (PCT) and C-reactive protein (CRP) in bacterial sepsis and correlate them with the “gold standard” bacteriological blood culture results. **Methods:** This is a retrospective study from July 2005. Data of patients with clinical evidence of sepsis whose blood were sent for PCT were retrieved. Only patients with sufficient data of PCT, CRP
and blood culture testing performed prior to initiation of antibiotic treatment were analysed. **Results:** A total of 30 patients' data were analyzed. In clinically septic patients, the median serum PCT and CRP levels were 12.87 ng/ml and 8.93 mg/dl respectively. Spearman’s test demonstrated a significant positive correlation between serum PCT and CRP levels ($r = 0.731, p < 0.01$); the correlation between PCT and blood culture results were statistically significant ($r = 0.426, p < 0.05$) whereas there was no statistically significant correlation between CRP and blood culture results. The specificity and positive predictive value of the serum PCT test for bacterial infection was higher than that of the serum CRP test (47.6% vs. 17.65% and 57.14% vs. 48.15%). However, sensitivity and negative predictive value for CRP were better than PCT (100% vs. 92.31% and 100% vs. 88.89%). **Conclusion:** Although PCT and CRP are positively correlated, PCT was observed to be a more specific biomarker and with better positive predictive value for bacterial sepsis compared to CRP.

**P096. Rapid detection of Hb Constant Spring mutation by allelic discrimination using real time PCR**

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Hb Constant Spring (HbCS) is the most prevalent variety of nondeletional alpha-thalassaemia in Southeast Asian populations. The Constant Spring gene arises from a point mutation TAA --> CAA at the terminal codon of the alpha2-globin gene and results in the production of abnormally long alpha-globin chains. Thus, HbCS has an α-thalassaemia like effect and it is the major cause of HbH disease. We have developed a simple allelic discrimination Real Time PCR method based on the Taqman chemistry for the rapid screening of HbCS. Primers and Taqman probes were designed to specifically amplify the point mutation of the HbCS gene. The method was then used to analyze DNA from 40 samples referred to HUKM for diagnosis of alpha-thalassaemia mutation and possible HbCS. The protocol, standardized by analysis of 40 samples was possible for us to distinguish accurately wild type from mutant alleles and also the heterozygous alleles. Genotypes were determined by allelic discrimination and result performed in scatter graph analysis. We found 3 cases out of 40 were mutant (αα<sup>cs</sup>/*-) and 3 were heterozygous (αα<sup>cs</sup> / αα). We had confirmed the result with DNA sequencing. This allelic discrimination analysis is a simple, rapid and effective method for detection of nondeletional alpha thalassaemia.

**P097. Methicillin-resistant staphylococcus aureus infection not responsive to vancomycin therapy: Characterization of the isolate and electron microscopy of its cell wall.**

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A 64-year-old male was admitted to ICU for intestinal obstruction. On the fourth day of admission his blood culture grew methicillin-resistant Staphylococcus aureus. He was immediately started on vancomycin. However after 7 days of treatment his blood culture was still positive for MRSA. The isolate was sent to IMR for MIC determination and to rule out VISA. At Bacteriology Unit, IMR, antibiotic susceptibility tests were carried out using a panel of antibiotics following the CLSI guidelines. MIC determination of vancomycin was carried out using E-test strip. VISA screening was carried out using BHI vancomycin 6 μg/L agar plate. The isolate was also sent for transmission electron microscopy of its cell wall. A control culture ATCC 25923 was also submitted for comparison
of cell wall thickness. The isolate was found to be resistant to amikacin, gentamicin, erythromycin, ciprofloxacin, tetracycline, co-trimoxazole and rifampicin and sensitive to vancomycin, linezolid, fusidic acid, chloramphenicol and clindamycin. The vancomycin MIC was 2 μg/L and VISA screening test is negative. An increase in the cell wall thickness was noted in this isolate when compared with the control strain. Vancomycin treatment failures have increasingly been reported with infections caused by susceptible MRSA strains with relatively high MICs (2 μg/L). Vancomycin acts by binding to the D-alanyl-D-alanine residues of murein monomer thus inhibiting the peptidoglycan synthesis of S. aureus. A thicker cell wall will cause more vancomycin molecules to be trapped in the peptidoglycan layers before reaching the cytoplasmic membrane where peptidoglycan synthesis occurs. This will also prevent further penetration by other vancomycin molecules. A thickened cell wall is the cardinal feature all vancomycin-resistant Staphylococcus aureus (VRSA) strains. Cell wall thickening was observed in this isolate even though the MIC is only 2 μg/L.

P098. PAX8-PPARγ1 fusion oncogene in follicular thyroid carcinoma

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Distinguishing follicular thyroid carcinoma (FTC) from its benign counterpart represents a major diagnostic surgical cytopathology dilemma. The occurrence of the PAX8-PPARγ1 fusion gene, resulting from a t(2;3)(q13;p25) translocation is thought to be restricted to follicular tumors (adenomas and carcinomas) of the thyroid (FTA and FTC). The demonstration of this fusion gene provides a new and promising starting point in the preoperative diagnosis of follicular thyroid neoplasms. Herein we report a case of follicular thyroid carcinoma with nodular hyperplasia and chronic lymphocytic thyroiditis in a 49 year-old lady who was presented to Hospital Universiti Kebangsaan Malaysia (HUKM). Preoperative diagnosis of the patient on fine-needle aspiration cytology (FNAC) suggests a neoplastic process. Histologically, malignant cells arranged in closely packed follicles surrounded by a fibrous capsule with areas of capsular invasion were seen; no vascular invasion was demonstrated. Immunohistochemistry, reverse-transcriptase polymerase chain reaction (RT-PCR) and fluorescence in-situ hybridisation (FISH) were carried out on the tissue sample of the patient to determine the presence of the PAX8-PPARγ1 fusion gene. Immunohistochemistry performed with wild-type PPARγ1 antibody revealed strong, diffuse nuclear expression of PAX8-PPARγ1 in paraffin-embedded thyroid tumor sections of the patient. RT-PCR revealed in-frame fusion between both PAX8 and PPARγ1 genes. FISH studies showed presence of fusion fluorescence signals confirming the presence of the PAX8-PPARγ1 fusion gene. The presence of the t(2;3)(q13;p25) rearrangement in FTC suggest an important role of PAX8-PPARγ1 rearrangement in the development of follicular thyroid malignancy.
P099. A case of light chain deposition disease (LCDD)

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Light chain deposition disease (LCDD) a rare multi systemic disorder is a subtype of monoclonal immunoglobulin deposition disease (MIDD) characterized by tissue deposition of monoclonal light chain that lack affinity for Congo red and do not have a fibrillar organization. The light chain deposits have prevalence of κ light chain (80%) than λ light chain. The most frequently affected organ in LCDD is the kidney which can present with nephrotic syndrome and varying degree of renal failure. Incidence of renal disease caused by LCDD is 0.1 per million of population (IRRB). We described this rare case in a 43 year-old Malay lady who presented with myeloma kidney. She was first seen in 2003 in regional hospital with easy bruising and lethargy and was managed as having megaloblastic anaemia. In June 2006, she started to have left hip pain and loin pain and treated as having urinary tract infection at various hospitals. She then presented to Hospital UKM in January 2007 with proteinuria and acute renal failure. Bone marrow aspiration and trephine biopsy showed plasmacytosis with abnormal morphology. Protein electrophoresis revealed monoclonal band at gamma region which confirmed lambda light chain by immunofixation with Bence-Jones protein detected. MRI of pelvis showed osteolytic lesion Renal biopsy reported glomerulosclerosis with dilated tubules with some showed pale pink casts with cracks in the lumen which do not show apple-green birefringes under polarized light after staining with Congo red stain. She was treated with Vincristine, Adriamycin and Dexamethasone regime and by 3 weeks post chemotherapy her renal function and symptoms improved.

P100. Atypical lymphoid disorders-clinically mimicking lymphoma

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Lymphoproliferative disorders mimicking lymphoma in their clinical manifestations can cause delay in arriving at definitive diagnosis. Castleman disease (CD) is a rare lymphoproliferative disorder that can be found in both nodal and extranodal sites and in many circumstances it is a diagnosis of exclusion. CD is either reactive lymphoid hyperplasia initiated by chronic antigenic stimulation that is associated with a viral trigger or is due to developmental growth disturbance of the lymphoid tissue. Rosai-Dorfmann disease (RDD) is a rare disorder of the hematopoietic system and is characterized by a nonmalignant proliferation of distinctive histiocytic/phagocytic cells within lymph node sinuses and lymphatics in extranodal sites. RDD occurs worldwide and is a disease of childhood and early adulthood however, it is less common in Asia. We describe three patients with lymphadenopathy and constitutional symptoms suggestive of lymphoma. All were referred to the haematology unit with the provisional diagnosis of lymphoma. From histopathological and immunohistochemistry examinations, these patients were finally diagnosed with Castleman disease, Rosai-Dorfmann disease and metastatic cancer with unknown origin. Apart from the basic morphologic study with H&E stains which form the cornerstone of diagnosis, immunohistochemistry may be necessary in some cases. Various markers which are currently available can be used in order to achieve the definite diagnosis. These three cases are highlighted in our case report in order to create awareness amongst clinicians that there are other unusual causes of generalized lymphadenopathy that can mimic lymphoma.
P101. External performance evaluation of the COBAS C 501 analysis system

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Objective: To test the analytical performance of representative reagents on the clinical chemistry module cobas c 501 of the cobas® 6000 analyzer against Modular Analytics and COBAS INTEGRA® 800 (Roche Diagnostics GmbH). Methods: Testing included clinical chemistry (ammonia, direct/total bilirubin, CK-MB, ceruloplasmin, ferritin, fructosamine, prealbumin), TDM (carbamazepine, gentamicin, mycophenolic acid, phenobarbital, phenytoin, theophylline, vancomycin, valproic acid), and urine DAT (amphetamines, benzodiazepines, methadone, THC). Linearity (clinical chemistry), within-run imprecision (21 replicates), total imprecision (triplicates, 21 days), and method comparisons were analyzed. Results: Linearity was well confirmed (recovery 90–110% of expected values). Within-run CVs using controls and/or human material ranged from 0.4 to 3.9% for clinical chemistry, from 0.6 to 4.1% for TDM, and from 0.9 to 5.6% for DAT. Total CVs were 1.0 to 4.8% for clinical chemistry except ammonia (9.2%), 1.9 to 7.8% for TDM, and 3.2 to 8.6% for DAT except amphetamines (11.3/13.1%). Quantitative method comparisons against Modular Analytics SW A revealed slopes from 0.93 to 1.08, intercepts < 55% of lower detection limits, and correlation coefficients > 0.992 except valproic acid (0.981). Systematic deviations were partly higher versus COBAS INTEGRA® systems (slope deviations up to 17%), which can be explained by differences in assay technologies. Agreement in qualitative method comparisons for DAT was 97 to 100%. Conclusions: Linearity, imprecision and comparability against Modular Analytics SW A and COBAS INTEGRA® 800 are very good on the cobas c 501 module. Thus, the analyzer is well suited for routine use in clinical chemistry, TDM and DAT.


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Introduction: Euphorbia hirta is a wild plant that is commonly found in tropical countries and has been widely used in traditional medicine for many therapeutic treatments. This plant has been evaluated for its analgesic, antipyretic and anti-inflammatory properties in mice and rats. Objective: In this study, the anti-inflammatory properties of aqueous extraction of Euphorbia hirta were investigated using a rat model of adjuvant-induced arthritis. Methodology: Dried, powdered whole plant material was extracted sequentially with each of n-hexane, chloroform, methanol and water using a soxhlet extractor. The water extract was freeze dried and then resuspended in water. To induce arthritis in rats, complete Freund’s Adjuvant containing 0.5 mg Mycobacterium tuberculosis was injected in the sub-plantar tissue of the right posterior paw. The injected paws became inflamed and oedematous. Subsequently, the animals were grouped and each group (n=8) received different concentrations of the herbal extract (placebo, 50mg/kg, 100mg/kg and 500mg/kg) for 62 days. Control animals were injected with incomplete Freund’s Adjuvant. The rats were sacrificed, and the joints were processed, blocked and sectioned and stained with H&E for histopathological examination. The microscopic changes were qualitatively classified under the following categories: oedema, inflammatory infiltration, joint space narrowing, synovial hyperplasia and fibrosis. Results and Discussion: Histopathological sections showed that water extracts of E. hirta were most optimal at 50 mg/kg, showing less abnormalities in joint architecture, and had no effects at concentrations at 500 mg/kg which showed features of inflammation, narrowed joint space, fibrosis and oedema comparable to those receiving placebo. Therefore, water extracts of E hirta at low concentrations have effective anti-inflammatory effects.
P103. Efficacy of a commercial genotyping method in identifying HIV non-B subtypes

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Non-B Subtypes (nBS), including Circulating Recombinant Forms (CRFs) and Unique Recombinant Forms (URFs), previously bordered in African countries, have now been shown to circulate in Europe and North America, where, however, Subtypes B (BS) are still predominant. Not only does this new aspect of HIV epidemiology possess clinical and therapeutic implications, but it also has important influence on diagnostics, especially virus molecular biology. In fact, most tests, though not specifically tailored on BS, have at least been developed in the western countries where BS have long been the only known viruses. Thus, primers which perform well with BS may be not so adequate for nBS, as already noticed in some instances. Aims of this study were to evaluate the prevalence of nBS in a cohort of HIV patients in our region and to verify if they can be routinely genotyped. To this purpose, 585 sequences from patients enrolled in four clinical centres in Liguria, obtained using Trugene HIV-1 Kit (Bayer-HealthCare) and recorded in ARCA (Antiretroviral Resistance Cohort Analysis), a public data base structured for antiretroviral resistance studies, have been analyzed. The ARCA data base is able to identify nBS, CRFs and URFs through the analysis of protease (1-99 codons) and reverse-transcriptase (38-299 codons) genes. It was possible to identify 547/585 (93.5\%) patients infected with BS, 19/547 (3.5\%) with non-B clades: 1 clade A1, 4 clade C, 5 clade F1 and 9 clade G; 18/547 (3.3\%) with CRFs: 1 CRF\textsubscript{14-BG}, 2 CRF\textsubscript{01-AE}, 2 CRF\textsubscript{12-BF}, 4 CRF\textsubscript{15-01B}, 9 CRF02\textsubscript{AG} and finally 1 URF. Overall nBS prevalence observed was 38/547 (6.9\%). The results of the present study show that nBS circulate in our country and that, unlike what has been reported for some routinely used PCR test, the genotyping method commonly used in the laboratory could sequence every virus, including all nBS.

P104. Genotyping test useful in HBV-DNA positive patients failing therapy

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An immunomodulator, interferon alfa (IFN-\textalpha{}), two synthetic nucleoside analogues, lamivudine (3TC) and Entecavir (ETV) and a synthetic nucleotide analogue, adefovir dipivoxil (ADV), are currently approved, in Italy, for the treatment of chronic hepatitis B. Several other antivirals are in clinical development. However, the IFN-\textalpha{} response rate is less then 40\% and IFN-\textalpha{} therapy is associated with a number of adverse effects. On the other hand, long-term 3TC or ADV mono-therapy leads to the emergence of drug-resistant HBV strains, that hampers a final positive outcome of treatment. The aim of our study was to evaluate the prevalence of resistance-associated mutations to antiviral drugs in a cohort of HBV-DNA positive patients failing therapy. Fifty-four patients were enrolled in this study, 49/54 were treated with 3TC and 5/54 with 3TC and ADV. All specimens have been sequenced using Trugene HBV Genotyping kit (Bayer-HealthCare), capable to identify the mutations conferring resistance to all three antivirals and located in the domains B, C (conserved YMDD) and D of the POL/RT region. Among the 54 sequences analyzed, 34 (63\%) resulted resistant to 3TC, of which 15/34 (44\%) had the M204I/V mutation, 15/34 (44\%) had M204V and L180M and finally 4/34 (12\%) had V173L, M204V and L180M. Only one patient of 5 treated with 3TC and ADF had the mutation A181V/T, conferring resistance to ADF, together with V173L, M204V and L180M. The most representative genotype was the D (47/54-87\%), following by A (4/54-7.4\%), E (1/54-1.86\%), G (1/54-1.86\%) and F (1/54-1.86\%). The results show that resistance-associated mutations do occur in HBV patients during treatment with nucleoside analogues, similarly to what have been more thoroughly seen in HIV infection. Thus the availability of several current and future molecules is likely to make the use of virus genotyping as useful as in that infection.
P105. Reflex testing in in vitro allergology diagnostics: the case of Bet v 1 and Bet v 2 recombinant allergens

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Introduction: The aim of this work was to assess the cost-benefit ratio of automated reflex testing for in vitro allergology diagnostics and particularly in the use of Bet v 1 and Bet v 2 recombinant allergens. Materials and Methods: Specific IgE have been measured in our laboratory using automated analyzer ImmunoCap 250 (Phadia, Uppsala, Sweden) and we used allergens panels selected on the basis of the epidemiology and the severity of induced pathology. We introduced the reflex rule according to which Bet v 1 and Bet v 2 recombinant allergens are measured when IgE specific for birch (t3) or apple (f49) are > 0.35 KU/L. Results: The results of the new diagnostic algorithm have been evaluated one year after its implementation. We investigated 290 patients: in 89 t3 was positive and f49 negative; in 20 t3 was positive and f49 negative; in 181 t3 and f49 were positive. The rule based on apple alone was not useful since the patients of the second group (t3- and f49+) were negative for the recombinants. We found more recombinant positive cases in the first group (t3+ and f49-) than in the second group (t3+ and f49+): respectively Bet v 1 was positive in 56.2% vs 44.8% and Bet v 2 in 23.6% vs 17.1% case. We investigated the presence of other foods capable to rise the rate of t3 positive subjects for Bet v2. According to our data, they were tomato (f25) and carrot (f31): 39.7% of the subjects t3, f25 and f31 positive and 17.1% of the t3 and f49 positive subjects were Bet v 2 positive. Conclusions: Reflex testing for recombinant allergens diagnostics is useful to detect allergens common to pollens and foods; moreover, the need to choose very carefully the antigen that releases the reflex is confirmed.

P106. Phenotypes and frequencies of dendritic cells subsets in malignant lymphomas

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Introduction: Dendritic cells (DCs) are bone marrow-derived antigen presenting cells (PCs), specialized in antigen capturing and presentation to T-lymphocytes. There are also candidates for inducing anti-lymphoma immunity. At least two subsets of DCs in the peripheral blood have been identified namely the myeloid dendritic cells (MDC) which involved in CD4+ T-cells Th1 response, induced cytotoxic response and resulted in tumour immunity; and the plasmacytoid dendritic cells (PDC) which involved in CD4+ T-cells Th2 response that may resulted in immune tolerance to foreign antigens. The objective of this study is to investigate the frequency and phenotypes of peripheral blood dendritic cells in malignant lymphoma patients in comparison to healthy individuals. Materials & Methods: From July 2003 to July 2006, a total of 22 healthy donors and 30 newly diagnosed malignant lymphomas patients were recruited. Whole blood in EDTA-anticoagulated specimen tubes were used for full blood counts and dendritic cells subsets analysis. Full blood counts were analysed with Coulter Haematology Analyser and Blood Debdritic Cell Enumeration Kits were used for the flow cytometric whole blood phenotypic analysis of dendritic cells subsets. Results: Our results showed that there was statistically significant reduction in myeloid dendritic cells (MDC) in patients with malignant lymphomas as compared to healthy donors.
P107. A case report: Hb E/ Lepore with diagnostic difficulty

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Hb Lepore is a structurally abnormal haemoglobin (Hb) formed by the fusion of the N-terminal portion of δ and C-terminal portion of β-globin chains. In a heterozygous state the phenotype can be distinguished from thalassaemia trait by Hb electrophoresis and high performance liquid chromatography (HPLC). However, when Hb Lepore interacts with other abnormal Hb in the compound heterozygous state the phenotype is variable and may cause a diagnostic difficulty. We report a 19-year-old boy, who presented with mild jaundice and mild anaemia. There was no splenomegaly and no history of red cell transfusion. Blood count showed Hb 11.1 x 10^9/L, red cell count 5.51 x 10^12/L, MCV 59.6 fl and MCH 20.1 pg. Blood film showed marked anisopoikilocytosis and numerous target cells. There were abnormal band at A2 and F region on the Hb electrophoresis and the HPLC showed high Hb A2 levels (64.6%) and high Hb F level (23.2%). The findings were therefore suggestive of HbE/ δβ thalassaemia. Family study, however, showed that the sister and the father were consistent with heterozygous Hb Lepore while the mother was consistent with Hb E trait which suggested that the patient is a possible case of HbE/ Lepore. Thus his initial laboratory findings mimicked HbE/ δβ thalassaemia and on its own had posed a diagnostic difficulty. In conclusion, although the heterozygous Hb Lepore can be easily diagnosed by Hb electrophoresis and HPLC, difficulty in diagnosis may be encountered where there is interaction of Hb Lepore with other haemoglobinopathies. Family study is therefore very important. Investigations which include common β-globin gene mutations analysis for HbE and molecular study to detect the presence of the fusion gene product of Hb Lepore are essential for a conclusive diagnosis of this patient.

P108. The estimation of fetomaternal haemorrhage by flow cytometry

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Fetomaternal haemorrhage (FMH) may occur following a sensitizing event, during pregnancy or at delivery. In cases of rhesus (Rh) incompatibility between mother and the fetus, it can thus subject to the haemolytic disease of the newborn. The Kleihauer test for quantification of FMH lacks standardization and results are less accurate. Furthermore, it cannot differentiate the fetal cell from the adult HbF. Flowcytometry analysis using monoclonal antibodies, is a new technique for the quantification of FMH and it allows larger number of cells to be analysed. It is also able to differentiate the fetal cell from maternal HbF, and thus is more sensitive and accurate. The objective of our study is to determine the FMH using the flowcytometric analysis of anti-HbF antibody and to correlate the FMH using flow cytometry and the standard Kleihauer test. 98 peripheral blood samples from pregnant women at more than 20 weeks of pregnancy and post delivery were analyzed by both methods. The percentage of the fetal cells were recorded and the FMH were calculated. We found a fair correlation between the two methods with the correlation coefficient r = 0.633
P109. Primary cardiac tumours in Malaysia: Histopathological study

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Cardiac tumours are generally rare. Previously, only a few case reports of cardiac tumours have been published from Malaysia and this is the largest series that has been studied so far. This is a retrospective analysis of pathological features of surgically excised cardiac tumours from the National Heart Institute, Kuala Lumpur between December 2001 to December 2006. In this series, 22 cases of benign myxomas and one case of malignant fibrous histiocytoma were noted. Cardiac myxomas: There were 4 males and 18 females patients and the male to female ratio was 1: 4.5. The ages ranged from 11 -75 years (mean 46 years). 12 patients were Malays, 10 Chinese and 1 Indian. All the myxomas were from the atrium and the most common site was left atrium (20 cases). Grossly the majority (16 cases) of the tumours were single lobulated mass exhibiting glistening surface and areas of haemorrhage. They varied from 3.5 to 10 cm in largest dimension. In 6 cases, the tumour was sent in multiple fragments. Microscopy showed elongated and stellate shaped cells in a richly vascular myxomatous matrix with areas of recent and old haemorrhage. None of the cases showed nuclear atypia or mitosis. One case of malignant fibrous histiocytoma was noted in a 37-year-old Chinese female. The tumour sent for histological examination showed multiple pieces of soft whitish tissue, 8 X 5 cm in aggregate. Histology revealed spindle shaped cells arranged in storiform pattern and multinucleated giant cells exhibiting frequent mitotic figures. In conclusion, this study shows benign myxoma is the most common cardiac tumour and the frequent site of occurrence is left atrium.

P110. Genotyping of NT A388G in the organic anion transporter (OATP2) gene by high resolution melting analysis.

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Introduction: OATP2 gene has been shown to be responsible for the transportation of organic anions into hepatocytes and hence, may be involved in the transportation of unconjugated bilirubin. Severe neonatal unconjugated hyperbilirubinemia is common in Malaysia and these neonates are at risk of developing kernicterus. Studies in Taiwan showed that variation at ntA388G of the OATP gene is a risk factor for unconjugated hyperbilirubinemia. In our effort to determine whether abnormalities in OATP2 gene plays a role in the development of neonatal unconjugated hyperbilirubinemia we evaluated a technique based on HRM analysis of PCR amplicon to detect single nucleotide polymorphism (SNP) for nt A388G in the OATP2 gene. Methods: a 150bp fragment of the OATP2 gene including the variation locus (A388G) was amplified by PCR using RG6000, Corbett Research, in the presence of saturating DNA dye Syto9 on DNA extracted from EDTA cord blood samples of 20 neonates with severe neonatal jaundice. The amplicons were melted at the rate of 0.1C /s to generate the high resolution melting profile. Results: results showed three different melting profiles for GG, AA and AG. With that, out of 20 cases, 12 were homozygous (GG), 4 heterozygous (AG) and 4 wild type (AA) for nucleotide 388 of OATP2. The results of the HRM analysis was in completely concordance with the DNA sequencing result. Conclusion: In conclusion, we have found that the HRM analysis technique is simple, rapid and useful for genotyping of nt A388G OATP2 gene polymorphism. This technique can be extended to study other polymorphisms associated with neonatal jaundice and has a great potential as a frontline technique for molecular diagnosis and identification of known genetic risk factors of severe neonatal jaundice.
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The objectives of this study were to evaluate the performance of HbA1c assay on Primus PDQ (Primus Corporation, USA) analyzer with respect to precision, recovery and comparability with Cobas Integra 800 (Roche Diagnostics, Germany) and Bio-Rad D10 (Bio-Rad Laboratories, USA) automated analyzers. Primus PDQ (PDQ) employs High-Performance Liquid Chromatography (HPLC) boronate affinity chromatography methodology. Bio-Rad D10 (D10) uses HPLC cation-exchange chromatography methodology. Cobas Integra 800 employs an immunoturbidimetric method. All these three methods are according to IFCC reference standardization. Precision studies were performed using two patients’ samples and two levels of commercial control sera. Recovery was assessed using a patient’s sample with low HbA1c level admixed, in various ratios, with another sample containing high HbA1c level. Sixty patient samples analyzed on the PDQ were compared to D10 and Cobas Integra 800. The assay time for PDQ was 2 minutes and the throughput was 30 samples per hour. The intra-run coefficient of variations (CVs) on patients’ samples were 0.5% (mean HbA1c = 6.4%) and 0.6% (mean HbA1c = 11.6%). The inter-run CVs on patients’ samples were 1.0% (mean HbA1c = 6.4%) and 0.8% (mean HbA1c = 11.6%). The intra-run CVs were 1.1% and 0.7% for level 1(normal) and level 2(abnormal) controls respectively. The inter-run CVs were 1.7% and 0.8% for normal and abnormal controls respectively. Mean recovery was 99.7%. The PDQ showed good correlation with D10 (PDQ = 0.96D10 + 0.52; r = 0.99) and Cobas Integra 800[Cobas800] (PDQ = 1.02Cobas800 - 0.37; r = 0.99).

In conclusion, the HbA1c assay on PDQ was precise with CVs.

P112. Evaluation of HbA1c Assay on Bio Rad D10 Analyzer in our Clinical Diagnostic Laboratory
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Objective: To evaluate analytical performance of BioRad D10 HbA1c analyzer (HPLC method) and compare it with Roche’s Cobas Integra HbA1c assay (immunoturbidimetry method). Materials and Methods: Within-run imprecision was done by assaying controls (2 levels) and patient samples [medium (mean=6.3%) and high (mean=10.5%) levels] 20 times within the same run while between-run imprecision was conducted by assaying aliquots of controls (2 levels) and patient samples (medium and high levels) in duplicates twice a day for 5 consecutive days. Sixty patient’s samples were assayed on both analyzers concurrently for comparison study. Recovery was established by obtaining neat low and high values of patients’ haemolysates, mixing them in ratios of 1:3, 1:1 and 3:1 and assaying the mixtures in duplicates. Expected HbA1c levels were calculated to obtain recovery. Ten uraemic and ten thalassaemic venous samples were assayed on the analyzers on the same day to assess interference. Results: The D10’s within-run coefficient of variation (CV) for control 1 and 2 were 0.5% (mean=5.9% and 10.0% respectively). Within-run CV for patients’ samples were 1.1% (mean=6.0%) and 1.4% (mean=10.5%) respectively. The between-run CV for control 1 and 2 were 1.0% (mean=6.4%) and 0.7% (mean=10.0%) respectively while patients’ sample CVs were 0.3% (mean=6.4%) and 1.2% (mean=10.5%) respectively. Both analyzers correlate well (r=0.99) and the D10 showed good recovery (mean=101.7%). Generally, both analyzers had lower HbA1c results with uraemic and thalassaemic samples but D10 has the advantage of displaying HbF peak/value on the chromatogram and alerting on low haemoglobin (Hb) samples enabling lab staffs to confidently verify results based on the acceptance criteria set by the manufacturer. Conclusion: In conclusion, D10 exhibited acceptable imprecision with CV.
P113. BRCA1 Sequence Variants in High Risk Breast Cancer Patients in Malaysia

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Breast cancer is the most common malignancy affecting Malaysian women. While most breast cancer cases are sporadic, 10-15% are ascribable to genetic susceptibility. Inherited mutations of the BRCA1 and BRCA2 genes account for about 70-90% of familial breast cancer in most Caucasian populations. Half of the cases are attributable to BRCA1 mutations alone. Knowledge of the role of BRCA1 mutations in Malaysian breast cancer patients is very limited. This study aims to detect the presence of BRCA1 mutations in high risk breast cancer patients in three hospitals in Malaysia; Hospital Universiti Kebangsaan Malaysia, Hospital Kuala Lumpur and Hospital Putrajaya. Eight fully-informed, consented high risk breast cancer patients. We detected four types of sequence variants; 2731T>C, 3232A>G, 3667A>G and 4427T>C in five patients. Three of these patients were noted to have two types of sequence variants each (2731T>C and 3667A>G in one patient; 3232A>G and 4427 T>C in another and 3667A>G and 4427T>C in the third case). All of them were early-onset cases and have positive family history of breast cancer. We detected 3667 A>G and 4427 T>C sequence variants in two other patients while in the remaining three other patients (male, bilateral disease and early-onset cases), no sequence variant was identified. According to the Breast Cancer Information Core (BIC) Database, these sequence variants were polymorphisms. Our preliminary findings suggest that BRCA1 mutation Malaysian patients is comparable with Caucasians.


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Diamond-Blackfan Anemia (DBA) is a rare disorder which usually presents in early infancy with symptoms of anemia, such as pallor and failure to thrive. The diagnostic criteria for DBA include normochromic, macrocytic anemia developing early in childhood, a normocellular bone marrow with selective deficiency of red cell precursors, reticulocytopaenia, normal or slightly decreased leucocytes counts and normal, increased or reduced platelet counts. The incidence of DBA is 5 per million per live births. Approximately three-quarters of the cases are sporadic but dominant or recessive inheritance has been reported in different families. Some patients have chromosomal abnormalities. Mutations in RSP19 causes DBA in some familial and sporadic cases, but other causes of DBA remain to be identified. Somatic abnormalities are present in about 30% of the patients which include short stature, atrial or ventricular septal defects, urogenital abnormalities, microcephaly, cleft palate, micrognathia, macrognathia and deformed thumb. Majority of the patients respond to prednisolone. Bone marrow allografting can restore normal haematopoiesis. We present a case of 15 months old Malay girl who presented at the age of 7 weeks with pallor and failure to thrive. Full blood count showed macrocytic anemia, reticulocytopaenia with normal white cell and platelet count. Hemoglobin electrophoresis showed increased HbF. When first seen in the clinic, she was active, mildly pale with no dysmorphic features. There was hepatomegaly (3 cm) and splenomegaly (tip was palpable). Bone marrow showed selective depression of erythropoiesis. Renal
DTPA showed bilateral duplex system of the kidney with bilateral hydronephrosis. Considering the clinical presentation and laboratory findings, the diagnosis of DBA was made. She had been receiving monthly blood transfusion since then. Currently she is planned for bone marrow transplant with matched sibling donor due to refractoriness to steroid therapy.

P115. Late diagnosis of paroxysmal nocturnal haemoglobinuria in chronic anaemia: A case report.

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Paroxysmal nocturnal haemoglobinuria (PNH) is a rare form of an acquired haemopoietic stem cell disorder characterized clinically by chronic haemolytic anaemia, thrombosis and bone marrow failure. Immunophenotyping play a key role in diagnosing PNH. Treatment is mainly supportive and definitive management is allogeneic bone marrow transplantation. We describe a 41-year-old Chinese male patient who had history of chronic anaemia since 2004. He was thoroughly investigated by the gastroenterologist team and was diagnosed and treated as iron deficiency anaemia. The anaemia had persisted and lactate dehydrogenase (LDH) was noted to be increased despite management and he was referred to haematologist team HUKM. In re-evaluating the history of the patient, he had noticed the discoloration of his morning urine and when he was unwell. Urine haemosiderin and Ham’s test subsequently performed was positive. Immunophenotyping of peripheral blood showed partial expression of CD55 and CD59 in the granulocytes and partial expression of CD55 in the monocytes and lymphocytes lineages. The patient was started on 30 mg of oral prednisolone every alternate day and was planned for allogeneic peripheral blood stem cell transplantation. In conclusion, when evaluating chronic anemia secondary to iron deficiency anemia and persistently high LDH in a young man, PNH should be kept in mind. Because it is so rare, delay in diagnosis is common in patients with PNH, which has a considerable impact in the management and prognosis. The availability of a diagnostic immunophenotyping will further aid in the rapid definitive diagnosis of PNH.


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Chronic myeloid leukaemia (CML) is a clonal disorder of a pluripotent stem cell. Philadelphia (Ph) chromosome (t(9;22)) is positive in more than 95% of cases. The molecular consequences of this translocation is a novel fusion gene, BCR-ABL which encodes a constitutively active protein, tyrosine kinase. The disease is rare in childhood, representing only less than 4% of all childhood leukaemias. However, the molecular features, clinical presentation and response of childhood CML to standard therapies are comparable with adult CML. We describe a 9 year old Malay girl presented with a 4 months history of progressive pallor associated with lethargic, joint pain, loss of appetite and loss of weight. She had hepatosplenomegaly measuring 5cm and 12cm respectively. The peripheral blood smear examination showed hypergranulocytosis with different stages of maturation seen. Serum lactate dehydrogenase was increased and neutrophil alkaline phosphatase (NAP) score was low. Bone marrow morphology was consistent with CML in chronic phase. Molecular studies revealed presence of BCR-ABL fusion gene. Human leucocyte antigen (HLA) class 1 showed no match donor. She was treated with hydroxyurea and interferon. She developed renal impairment while on interferon.
the treatment was discontinued. Unfortunately, the white cell count continued to increase despite being on hydroxyurea. On September 2006, she was started on Imatinib (Glivec). She responded well to Glivec and achieved morphological and molecular remission with the treatment. Glivec is an important option for facilitating induction of complete remission in children for whom there is no HLA-identical donor.

P117. Paraneoplastic pemphigus in a patient with follicular lymphoma: A diagnostic dilemma

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Paraneoplastic pemphigus (PNP) is a rare autoimmune blistering and erosive mucocutaneous disease associated with neoplasia, most notably of lymphoreticular origin. The clinical and immunopathological findings are polymorphic; however the presence of intractable stomatitis and antiplakin protein autoantibodies are constant features. Clinical manifestations include erythema, bullae, papulosquamous eruptions and erythema multiforme-like lesions and severe mucous membrane involvement. PNP has distinct clinical, histologic and immunologic features that differentiate it from classic pemphigus with underlying neoplasia. The diagnosis is often difficult because of the clinical polymorphism, histological variability and immunological complexity. PNP is associated with high mortality and poor response to treatment. Combinations of immunosuppressive agents are usually required to obtain even partial control of the skin lesions. We describe a 70-year-old Chinese man who presented with one month history of painless abdominal mass, which progressively increase in size, associated with loss of appetite and loss of weight. CT-scan showed multiple enlarged para-aortic and mesenteric lymph nodes. Ultrasound guided mesenteric lymph node biopsy showed low-grade follicular lymphoma. He defaulted follow-up prior to bone marrow assessment and treatment. He presented again 2 years later with nephrotic syndrome and extensive exfoliative dermatitis with erythematous base involving the face, scalp, neck, trunk and upper limb. Blisters were noted on the lower limb with pustules at the edge of the lesions and serous, foul-smelling discharge. There was no oral ulcer and the conjunctiva was not involved. He denied taking any traditional medicine. Skin biopsy was inconclusive. He succumbed after 3 cycles of chemotherapy (R-CVP regimen). In conclusion, although the skin biopsy is inconclusive and the rarity of the disease, in view of the clinical presentation of the pemphigus with underlying lymphoma which responded to prednisolone, the diagnosis of paraneoplastic pemphigus cannot be ruled out.
P118. Real time quantification of BCR/ABL fusion gene in chronic myeloid leukemia

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Philadelphia chromosome (t 9; 22) is found in more than 95% CML cases. The novel fusion gene, BCR/ABL besides conferring a diagnostic value for chronic myeloid leukaemias (CML), is used in monitoring therapeutic response, hence important in prognosis of these cases. The objective of this study was to evaluate a current protocol for quantitation of BCR/ABL gene in CML using real-time RQ-PCR. Ten patients diagnosed as CML from Hospital UKM were enrolled into this study. Total RNA was extracted from blood and bone marrow followed by cDNA synthesis. Two sets of primers and Taqman probes for ABL control gene and M-BCR fusion gene together with serial dilutions of plasmid DNA for BCR/ABL gene (101-105) and ABL gene(103-105) were subject to PCR using ABI Prism SDS 7000. The copy number of both genes in patients were quantify by referring to the copy numbers generated by standard curves. A ratio of BCR/ABL and ABL was calculated at diagnosis and follow-up for each patient. The analysis showed the efficiency of the ABL gene is 90.5% (±0.2158) and 93.4% (±0.1573) for BCR/ABL respectively. Both fusion gene and control gene were correlated well with R^2 = 0.99. We are able to detect the BCR – ABL in all the CML patients and their respective follow-up samples. In conclusion, RQ-PCR is a fast and effective technique. It has a potential role as a first line of molecular diagnosis in patients with CML and also useful in monitoring minimal residual disease and genetic recurrence in patients known to harbour this translocation.

P119. Chromosomal changes in thyroid diseases: Involvement of chromosome 21 an early event of thyroid tumorigenesis?

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Multinodular goiter (MNG) is the most frequent type of thyroid disease. Malignant thyroid lesions include papillary (PTC), follicular (FTC), anaplastic (ATC) and medullary thyroid carcinoma (MTC). Among this, anaplastic thyroid carcinoma (ATC) is undifferentiated and highly aggressive. The objective of the study is to identify chromosomal aberrations and common regions in the thyroid lesions using the comparative genomic hybridization (CGH) method. Fifty thyroid lesions (7 MNG, 1 MNG with occult PTC, 2 FTC, 10 FTA, 27 PTC, 2 ATC and 1 MTC) were analysed for chromosomal aberrations using the CGH technique. Tumor DNA labeled with SpectrumGreen and normal DNA labeled with SpectrumRed were hybridised simultaneously to normal metaphase spreads. For each case, 10 metaphase spreads were studied using the Applied Imaging CytoVision System. Gains and losses occurred in all of the benign and malignant thyroid tumours. Five of 8(62.5%) MNG cases did not show any chromosomal alterations. The remaining 3(37.5%) MNG cases including one MNG with occult PTC showed chromosomal alterations involving gains of chromosomes 2q37, 4p16 and loss at 16q21. Two FTC cases showed mainly gains than losses at chromosomes 5p15.1 (50%) and 7q32-q35 (50%). Four of 10 FTA exhibited recurrent losses of chromosome 21p12-q22 (40%). Fifteen of 27 (56%) PTC also showed recurrent losses involving chromosome 21p and 21q. The single MTC case also showed losses within the same region. Our study shows that alterations of chromosomal regions are more common in neoplastic lesions compared to non-neoplastic lesions. The more undifferentiated the tumour, the higher the number of chromosomal aberrations. Twenty of 38 (53%) neoplasms show chromosomal abnormalities involving chromosome 21, suggesting that it may be an early event in thyroid tumorigenesis.
P120. Analysis of P27 and cyclin D1 expression in breast carcinomas
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P27 and cyclin D1 have recently been reported as important markers for regulation of the cell cycle. Overexpression of Cyclin D1 which is proto-oncogene and p27 as tumor suppressor gene may disrupts normal cell cycle control, possibly promoting the development and progression of cancers, including breast cancer. The aim of this study is to examine the expressions of p27 and cyclin D in breast carcinomas and correlate with estrogen receptor and clinicopathologic parameters. Tissue microarray was constructed with 3-4 core (0.6 mm diameter) from 112 paraffin embedding tissues confirmed for breast carcinomas. Expression was determined by immunohistochemistry staining using antibodies estrogen receptor (ER), p53, p27 and cyclin D1. Overexpression of ER, p27 and cyclin D1 were seen in 59% (56/95), 51% (51/101) and 71% (59/83) of the cases. 67% (34/51) cases of ER positive were p27 positive whereas 84% (37/44) cases were cyclin D1 positive. No significant associations were seen in p27 and cyclin D1 expressions with other clinicopathologic parameters. Our results showed significant positive association of p27 and cyclin D1 with only ER status similar to other studies.

P121. Expression of Cyclin E in breast lesions using immunohistochemistry
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Cyclin E, a regulatory subunit of cyclin dependent kinase 2, is an important regulator of the G1 phase transition and expresses during the late G1 phase of the cell cycle until the end of the S-phase. Overexpression of Cyclin E plays an important role in many different cancers including breast cancer. The aim of the study is to investigate the immunohistochemical expression of cyclin E in breast lesions. A total of 112 samples were retrieved from the Department of Pathology at Hospital Universiti Kebangsaan Malaysia from years 2003 to 2005. We reviewed the histopathological slides and performed immunohistochemistry for Cyclin E on paraffin-embedded tissue from 19 cases of fibrocystic changes with adjacent normal breast tissues, 8 cases of usual ductal hyperplasia (UDH), 17 cases of fibroadenomas (FA), 10 cases of ductal carcinoma in situ (DCIS) and 58 cases of infiltrating ductal carcinoma (IDC). Cells labeled by the Cyclin E display a nuclear staining pattern and positivity was recorded when >5% of tumor cell nuclei showed immunoreactivity. A total of 10 cases (17.2%) of IDC (range 0 - 85.1%, mean 4.7%) and 2 cases (20%) of DCIS (range 0 - 48.9%, mean 7.39%) are positive for Cyclin E. There was no reactivity of Cyclin E in all the UDH, FA and fibrocystic changes. Our results showed overexpression of Cyclin E was seen only in malignant breast cases and these findings are within the range of other studies.
P122. Extramedullary relapse in a patient with acute lymphoblastic leukemia following allogeneic peripheral blood stem cell transplantation

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Extramedullary relapses of acute lymphoblastic leukemia (ALL) in children and young adults are rare especially after allogeneic peripheral blood stem cell transplantation (allo-PBSCT) and continues to be a therapeutic problem. The main reason of failure of allo-PBSCT is resistance of the leukaemic cells and fatal side effects of treatment, including infections and graft-versus-host disease (GVHD). We describe a rare case of extramedullary relapse in the breast of a 19-year-old girl who was diagnosed as B-ALL. The extramedullary relapsed occurred 6 months after her allo-PBSCT. She was diagnosed as B-ALL two years ago (2005) when she initially presented with lost of weight and hyperleucocytosis. She did not respond to the standard risk protocol and the high risk protocol treatments. Subsequently due to the resistance of treatment, five cycles of B-ALL protocol (BFM 90) were given and finally she achieved remission. An HLA-identical allo-PBSCT from her 14 year old younger sister was performed in March 2006. There was bone marrow engraftment with development of GVHD (mucositis grade 1). Post-transplantation analysis of haematopoietic chimaerism was performed by a semiquantitative PCR assay for the amplification of STR markers and complete chimaerism was documented. Bone marrow biopsy showed no residual blasts. In September 2006, six months after transplantation, a mass in the left breast was detected and on subsequent follow-ups the lump had increased in size and number involving both sides, ranging 2 to 6 cm, hard and non tender. The biopsy of the breast lumps revealed recurrent extramedullary leukaemia, however bone marrow smear showed normal haemopoiesis, without excess of blasts. She was treated with radiotherapy and the masses completely disappeared. She is currently on myeloablative regime and doing well. In conclusion, although extramedullary relapse especially in the breast is rare, awareness and early diagnosis is important for therapeutic and prognostic implication.

P123. Nonsecretory multiple myeloma - A case report

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Nonsecretory multiple myeloma (NSMM) is a rare variant of the classic form of multiple myeloma (MM) and accounts for 1% to 5% of all cases of MM. The clinical presentation and radiographic findings of both types are the same, except for the absence of renal function impairment in NSMM. The diagnosis of MM requires the detection of a monoclonal gammopathy in the serum or urine. In NSMM, however, no such gammopathy can be demonstrated, making the diagnosis more difficult. The choice of therapy and survival length of patients with NSMM appeared to be similar to those of patients with measurable M-protein since the nonsecretory variant was closely associated with high tumor cell mass, as determined by severe anemia and/or multiple areas of bone destruction. We describe a 71-year-old Malay man who initially had pain over the right rib for few months and was diagnosed as granulocytic sarcoma based on biopsy of the paraspinal mass. Radiotherapy was given and patient was well until nine months later when he came back with back pain and also pain at the left side of the body. No monoclonal gammopathy was found in the serum or urine, but skeletal survey revealed diffuse osteolytic lesions with multiple pathological fracture. The bone marrow biopsy of the lytic lesion on the upper third of the femur showed infiltration of abnormal plasma cells. The immunophenotyping was
positive for HLA-DR, CD 38, cytoplasmic immunoglobulin with lambda restriction. The chromosomal analysis was of normal karyotyping. There was also depression of normal immunoglobulins and the absence of both renal failure and hypercalcemia. The diagnosis of multiple myeloma was finally made based on the radiological and bone biopsy findings. He was treated with radiotherapy and two cycles of chemotherapy (dexamethasone, zometa and thalidomide). Unfortunately he did not respond to the treatment and thus palliative treatment was given to the patient.

P124. Molecular characterization of alpha thalassaemia patients diagnosed in HUKM

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Alpha thalassaemia is the most common inherited disorder in Malaysia. The clinical severity is dependence on the number of alpha gene involved. Full blood count and Hb analysis using either electrophoresis or HPLC are unable to detect alpha thalassaemia carriers. They can only be confirmed by molecular analysis. The objective of this study is to determine the prevalence of different types of gene abnormalities and their haematologic feature in cases of alpha thalassaemia diagnosed in HUKM. Retrospective analyses of all cases diagnosed as alpha thalassaemia using multiplex PCR and real time PCR in HUKM were carried out. From October 2001 to February 2007, there were 228 cases of alpha thalassaemia diagnosed in HUKM. Male to female ratio was 3:4. Majority of the cases were Chinese (57%) followed by Malays (36.8%), and Indian (6.1%). There were 200 cases of alpha thalassaemia trait and the commonest gene abnormality was /-SEA (71.4%) followed by /3.7(24.1%), /CS (2%), -4.2/-3.7 (1%), /-4.2 (0.5%), -3.7/-3.7 (0.5%) and CS/CS (0.5%). From 21 cases of Hb H disease, 19 cases (90.5%) were -3.7/-SEA while only 2 cases (9.5 %) were SEA/CS. There were 4 cases of Hb Barts (-SEA/-SEA) diagnosed. In conclusion, the commonest alpha gene abnormality in our series is the /-SEA deletion followed by -3.7 single gene deletion with the non-deletional Hb CS mutation constituting only 1.8% of the alpha gene abnormalities. Molecular analysis is required for definitive diagnosis of the alpha thalassaemic syndromes and is an important prerequisite for genetic counseling.

P125. A study of angiogenic markers in gastric biopsies of precancerous and cancerous lesions

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Angiogenesis plays a vital role in the growth and metastases of tumours. Recent studies indicate that vascular endothelial growth factor (VEGF) and tumour suppressor gene p53 play an important role in the angiogenic process of many tumours like the colon, breast and stomach. The objective of the present preliminary study was to determine the expression of angiogenic markers VEGF and p53 in gastric biopsies of precancerous and cancerous lesions. 30 cases of precancerous and cancerous gastric lesions and 10 normal gastric biopsies were randomly selected. All samples were collected from Hospital Kuala Lumpur. These were samples of 19 gastric carcinoma lesions, 11 precancerous lesions and 10 normal biopsies. Tissue samples from each patient were initially analyzed with routine H&E. Immunohistochemistry was performed on the gastric specimens to determine the over-expression of VEGF and the positive accumulation of mutant p53. Both positive and negative controls were included in the study. It was observed that 6 out of 19 cases of gastric carcinoma have given the positive result
for p53 (32%). 7 out of 19 gastric carcinomas have given a positive expression of VEGF (37%). 2 out of 11 precancerous lesions showed positivity for VEGF (18%). A similar result was observed for p53. The expression of p53 and VEGF in gastric cancer lesions (32% and 37% respectively) indicate that they do play a major role in angiogenic progression of cancer. However, a larger sample study is required to correlate the simultaneous role of VEGF and p53 in angiogenesis in gastric carcinoma patients and in patients progressing to gastric carcinoma. This will form the basis for further research.

P126. Correlation between DNA flow cytometric analysis findings and histologic grade in transitional cell carcinoma of urinary bladder

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Transitional cell carcinoma of urinary bladder is the fifth most common cancer in IRAN, that the prognosis and choice of treatment is related to several parameters such as histologic grade. In this study, correlation between DNA analysis findings and histologic grade were studied. In a cross sectional study, 75 paraffin embedded blocks of patients suffered TCC of urinary bladder between 2000-2002 from Pathology department of Mostafa Khomeini hospital in Tehran were studied. Histologic grade was determined on basis of WHO/ISUP grading system. Aneuploidy and percent of tumoral cells in S phase in each specimen were determined by flow cytometric analysis. 21.4% of LMP grade tumors, 74.3% of low grade tumors and 73% of high grade tumors showed aneuploidy .Aneuploid cells percent was 1.64+3.33, 4.30+7.71 and 17.57+12.53 in LMP grade, low grade and high grade tumors respectively (p=0.000).Tumoral cells in S phase percent was 8.14+3.66, 12+ 4.75 and 17.3+5.42 in LMP grade, low grade and high grade tumors respectively (p=0.000). High grade tumors possessed high aneuploidy and high S phase fraction. But for determination of role of these findings in prognosis, follow up studies are recommended.

P127. Oral squamous cell cell carcinoma: Analysis of Claudins in a tissue microarray of 105 cases

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Introduction: Claudins of which 24 types have been identified are integral transmembrane proteins of the tight juctions that are critical for maintaining cell adhesion and polarity. They also act as selective barriers. Cells and tissues are characterized by individual claudin patterns and express differently in embryogenesis, normal and neoplastic tissues. Alterations in the expression of individual claudins have been detected in several carcinomas and shown to be related to progression invasion; however the role in carcinogenesis is controversial. Methods: Using a panel of anti-claudin antibodies (anti-claudin 1, 2, 3, 4, 5 and 7) we have mapped claudin expression using immunohistochemistry in 105 specimens of oral squamous cell carcinoma (SCC) organized in a tissue array. The study was performed in quadruplicates and the results analyzed morphologically and semi-quantitavely. Results: All claudins included in the study were present surrounding the cell membrane in the positive controls (normal oral mucosa). In the arrays containing the samples of SCC, claudins were strongly present in well-differentiated SCC, presented mild and low expression on moderated differentiated SCC, and was negative in poorly differentiated SCC. Claudin-7 was
mostly negative or presented weak expression in all cases studied. Expression of all claudins was decreased or absent in areas of invasion front. **Conclusions:** Claudins expression patterns showed a strong correlation with histological type of SCC, being decreased in areas of invasion and negative in poorly differentiated tumors. This expression pattern may be related to evolution and prognosis of these tumors.

**P128. Histo-morphometric study of duodenal biopsies in patients with giardiasis**

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Giardiasis is common in most of the developing countries, including India. It mainly affects the lower duodenum and upper jejunum. The aim of this study was to view the various histological changes associated with giardiasis. 350 duodenal biopsies from patients who underwent upper gastrointestinal endoscopy were evaluated by histo – morphometric techniques. Of these 13.42 (47/350) biopsies showed trophozoites of Giardia lamblia, 36.17% (17/47) exhibited inflammation, 31.91% (15/47) contained increased number of intra-epithelial lymphocytes, 17.02% (8/47) had abnormal villous architecture, and 14.89% (7/47) had congested and dilated blood vessels. By morphometry mean intra-epithelial lymphocytes were 34.92 and mean villous: crypt ratio (V/C) was 3.18. This study highlights the importance of identification of Giardia trrophozoites in duodenal biopsies so as to avoid misdiagnosis of celiac disease or tropical sprue, which may have similar histological features but different clinical management.

**P129. Audit of procalcitonin requesting in a large University Hospital in the years 2001-2006**

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Procalcitonin (PCT) has been measured in our laboratory since 2001 and the requests increased 10 fold (from 1662 to 16093) in the years 2001-2006. The aim of this study was to audit the pattern of PCT requests. We audited the 45966 PCT requests made by the units of a large University Hospital [including a 1100 bed hospital (A) and a 700 bed hospital (B)] and smaller hospitals in the area. All the assays have been carried out on stat basis in heparinized plasma samples using automated chemiluminescence analyzer (Liaison, Diasorin, Italy) and reagents from Brahms (Germany). PCT assay became available in 2001 and in that year 92.8% (1543 out of 1662) of the requests came from Anesthesiology of Hospital A. In the following years the percentage decreased down to 33.2% (5351/16093) in 2006. The Neurosurgery Anesthesiology of Hospital A started to request PCT in 2004 (295/7153= 4.12%); in 2006 the requests have been 5555 (34.5%) while the Anesthesiology Unit of Hospital B started in 2001 (8= 0.5%); its requests peaked in 2004 (23.2%=1660) and slowly decreased to 17.6% (2510) in 2005 and 15.4% (2479) in 2006. Also the requests from pediatric Intensive Care Unit started in 2001 (0.9%), peaked in 2003 (16.2%) and then fell down to 2.8% in 2006. Among the others units notable are the cases of Anesthesiology of an external hospital that increased from 15 (0.3%) requests in 2003 to 192 (1.2%) in 2006 and that of Cystic Fibrosis Unit that fell from 31 (1.9%) in 2001 to 2 (0.01%) in 2006. The pattern of the diffusion of a new test is influenced not only by the mix of the pathologies of inpatients but also by the opinion of physicians of the different units on the basis of rather subjective assumptions.
P130. Alterations in iron and copper metabolism in Alzheimer’s disease and related dementias

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Roles for altered metals have been speculated in the pathogenesis of neurodegenerative disorders such as Alzheimer’s disease (AD). Redox active iron and copper, capable of generating reactive oxygen species, have been documented in association with amyloid plaques and neurofibrillary tangles (NFT), and alterations in iron regulatory proteins (IRPs) have been reported in AD. However, little is known about the relative distribution of iron, copper, and IRP activity at different stages of these diseases. To address this, we measured the levels of copper, and loosely bound, non-heme and total iron in the frontal cortex and hippocampus of patients with mild, moderate and severe AD, as well as dementia with Lewy bodies (DLB,) using graphite furnace atomic absorption spectrometry. Additionally, a novel monoclonal antibody to iron regulatory protein 2 (IRP2) was generated, and its expression examined in relation to pathological hallmarks of AD and DLB by immunohistochemistry. Strong neuronal expression of IRP2 was observed in aging brains, but our preliminary studies showed no significant differences between groups, or in relation to plaques, NFTs, and Lewy Bodies. However, we found significantly decreased loosely bound iron in the hippocampus of mild-moderate and severe AD patients (3.7 ± 0.4 and 3.7 ± 0.3, respectively) compared to controls (5.2 ± 0.6 µg Fe/g wet weight), and decreased total copper in severe AD and DLB frontal cortex (6.9 ± 1.2 vs. 3.9 ± 0.3 and 3.6 ± 0.6 µg Cu/g wet weight, respectively, compared to controls, with a trend toward lower levels in the white matter. These findings indicate imbalances in brain metal levels in both AD and DLB, while shedding light on the temporal sequences of such alterations in their pathogenesis. Further studies will focus on forms of dementias associated with frontotemporal degeneration, and cerebral amyloid angiopathy.

P131. An investigation into the application of a technical labour productivity measurement tool in a tertiary Chemical Pathology hospital laboratory

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The ongoing quest to ensure optimal efficiency and cost effectiveness in diagnostic clinical laboratories led to many changes in the way most public health and private sector laboratories are operating, nationally and internationally. Central to any such operational changes to enable more cost effective service delivery is the ability of the laboratory manager to obtain the relevant information needed to direct these changes and to assess the outcome of affected changes, whether positive or negative. Ratio analysis, productivity measurement and ultimately the process of benchmarking with or without peer review are all tools used by the laboratory manager. In this retrospective study a universal technical labour productivity tool (= Billable tests / Technical FTE’s)* was applied to the Chemical Pathology Laboratory for February 2006. Data was obtained from retrospective staff rosters and the laboratory’s information system. The tool was applied to the laboratory as a whole with a sub-analysis performed on the semi-automated and manual sections of the laboratory. The total technical labour productivity ratio of the laboratory was 2244 (= 95092 billable tests/39 FTE’s) vs. the semi-automated and manual sections of the laboratory with a productivity ratio of 46 (= 530 billable tests/11 FTE’s). 29% (11/39 FTE’s x 100%) of the total technical labour was used to produce just 0.56% (530/95092 billable tests x 100%) of the total laboratory output in the semi-automated and manual sections. It is clear that when this tool is applied judiciously, keeping in mind its limitations, it is a very powerful management tool that can influence staff allocation, labour costs, productivity and substantiate laboratory performance claims to administrators in a meaningful, reproducible and quantifiable way

*FTE’s = Full Time Equivalents*
P132. Pleural effusion with crystals, an unusual feature of multiple myeloma

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We showcase a 54-year-old female patient who presented with weight loss and sudden onset of weakness and paraesthesia of the lower limbs. The patient was chronically ill, anaemic, had bilateral pleural effusions and neurological deficit at T5 level. Special investigations showed bicytopenia, normal renal function and hypocalcaemia. CXR confirmed bilateral pleural effusions. The pleural effusion cytopsin was hypercellular with numerous plasma cells and macrophages, some filled with crystals and globules. Free crystals and globules were also present. Trephine biopsy revealed >80% plasmacytic infiltration and monoclonal peaks were present in both serum and urine. Bone marrow immuno-histochemistry revealed IgA Kappa monoclonality. MRI of the spine showed multiple plasmacytomas with spinal chord compression. The patient passed away within one week of admission. Pleural effusions in Multiple Myeloma occur in approximately 6% of cases. The aetiology is multifactorial. Effusions due to myelomatous pleural involvement are rare.

P133. Verification of the reference interval of procalcitonin using indirect methods in 30334 inpatients results

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There is no agreement about the reference limits of procalcitonin (PCT) and very different Upper Reference Limits (URL) have been proposed (0.5-2 f g/L). According to IFCC recommendations, reference values must be calculated from reference subjects collected with utmost care. Since 1960, the production of ‘normal’ intervals using routine laboratory data has been advocated but it has been hampered mainly by the difficulty of manually manipulating many thousands of results. This requirement is now met by the availability of large number of results stored in the LIS. The aim of this study was to assess PCT Health Related Limits (HRL). We downloaded from LIS the 30334 PCT results obtained in 2005 and 2006 in our laboratory (Liaison automated analyzer, Diasorin, Italy) and we displayed, using the indirect method proposed by Kairisto and Poola and the GraphROC software, the distribution of all the PCT results and of the patients admitted to Intensive Care Unit (ICU) and to Neurosurgery Intensive Care Unit (NSICU) both in 2005 and in 2006. Notwithstanding the bimodal distribution of the results, some comments should be pointed out: 1) the mean PCT value (n=30334) is 0.57 ug/L; 0.5, 1, and 2 ug/L represent respectively 65th, 82.4th and 95th percentiles; 2) the mean PCT value (n=4914) of ICU patients in 2005 is 1.05 ug/L; 0.5, 1, and 2 ug/L represent respectively 46th, 67th and 84th percentiles; 3) the mean PCT value (n=5567) of NSICU patients in 2006 is 0.24 ug/L; 0.25, 0.5, 1, and 2 ug/L represent respectively 67th, 88th and 99.8th percentiles. In our opinion, the indirect method appears a very convenient way for producing and/or validating the HRL/reference interval also of PCT; our data convincingly confirm that 0.5 ug/L is consistent with URL and 2.0 ug/L with decision limit.
P134.  **Helicobacter pylori in areas of gastric metaplasia in gallbladder and isolation of H pylori DNA from gallstones**  
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**Objectives**: To assess if the areas of gastric metaplasia in gallbladder are colonized by Helicobacter pylori and to do a molecular study of gallstones for presence of H. pylori DNA.  

**Methods**: Sections from 111 gallbladders with evidence of gastric metaplasia on haematoxylin and eosin and AB-PAS (pH 2.5) stain were further stained with Loeffler’s methylene blue and Warthin Starry stain for demonstration of H. pylori. Confirmation of H. pylori was done by immunohistochemistry. Formalin fixed mucosal tissues and gallstones from 11 cases showing heavy colonization by H. pylori were subjected to molecular analysis.  

**Results**: Helicobacter pylori were present in 50/111 (45%) sections with gastric metaplasia. Areas adjacent to gastric metaplasia in gallbladder showed acute inflammation (6%) and lymphoid follicle formation in 58% cases with H. pylori that was significantly higher than those seen in sections without H. pylori. In molecular study 8/11 gallstones showed 16S rDNA. Amplification of material from one stone showed positivity for atpA, efp, mut y, ppa, trp C, Ure1 and Vac A genes. Phylogenetic affiliation study of the isolates indicated that H. pylori sequence from the gallstones clustered with Indian strains of H. pylori. No considerable difference was observed in phylogenetic affiliations of 8 stones studied.  

**Conclusion**: H. pylori colonizes areas of gastric metaplasia in gallbladder producing histological changes similar to those seen in gastric mucosa. Isolation of H. pylori DNA from gallstones further support its presence in gallbladder.

P135.  **Hormone receptor status in breast carcinoma: Comparison of immunocytochemistry and immunohistochemistry**  
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The objective was to study the estrogen receptor (ER) and progesterone receptor (PR) profile of breast carcinoma and to compare the findings on immunocytochemistry (ICC) on fine needle aspiration cytology (FNAC) with immunohistochemistry (IHC). A total of 69 cases of breast cancer selected over a two-year period, that had both ICC and IHC were analysed in this study. Cellularity of smears, staining intensity, background staining and percentage of tumor cells showing staining were tabulated and compared with percentage positivity on histopathology. ICC staining for ER and PR was available in 69 and 38 cases respectively. ER and PR status was available in all cases on histopathology. On cytology, ER was positive in 39 cases and negative in 28; it was confirmed on histopathology in 36 and 22 cases respectively (sensitivity - 85.7%; specificity - 88%; PPV - 92.3%; NPV - 78.5%). Two smears were unsatisfactory for evaluation due to poor cellularity. Moderate to strong intensity of staining for ER was noted in 35 smears. None of the cases with more than 80% cells positive on cytology were negative in histopathology; however, 4 cases that were positive in fewer cells showed no reaction in histopathology. PR was positive in 16/38 cases on cytology, all of which were confirmed in histopathology and negative in 21. Of the latter, there were five false negatives (sensitivity - 76%; specificity - 100%; PPV - 100%; NPV - 76%). One of the smears was unsatisfactory due to poor cellularity. Among the PR positive cases, moderate to intense staining was noted in 14/16 cases and mild in 2 cases. Cytologic assessment of hormonal status is comparable to histopathology in relation to staining intensity and proportion of stained cells. Adequate cellularity of smears and prompt fixation are two factors that may influence ICC.

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Chronic lymphocytic leukaemia (CLL) result from accumulation of small, mature monoclonal B lymphocytes expressing CD5 and CD23 markers. Prognosis of B-CLL is based on clinical staging which the limitation is the failure to assess whether the disease will progress or remain stable. Soluble CD23 (sCD23) was proposed as a marker of disease activity and can provide additional prognostic information.

Objectives: 1) To determine the prognostic significance of sCD23 in CLL patients. 2) To correlate sCD23 level with the value of known parameters like clinical staging, lymphocyte doubling time, CD38, ZAP-70 and bone marrow morphology.

Methods: In this prospective study, serum of 11 B-CLL patients were collected. Serum samples taken were stored at -80°C. Serum sCD23 levels of patients and healthy control were measured with enzyme linked immunosorbent assay.

Results: The range of serum sCD23 level in B-CLL patients was 5-650 U/ml while the cut off point was defined as the median level of 23.4 U/ml. One patient in Rai stage 0 (50%) had higher than the median level of sCD23. Eight patients in Rai stage II group (72.73%) had high sCD23 and three patients had low level. One of the patients had the highest sCD23 level had disease progression from Rai stage II to Rai stage IV with lymphocyte doubling time (LDT) less than six months. Seven patients with LDT more than six months (70%) had high sCD23 level. Three patients with diffuse bone marrow involvement had high sCD23 (75%) while all patients (100%) with focal bone marrow involvement had low sCD23 level.

Conclusion: Serum sCD23 were higher in B-CLL patients with LDT less than six months, diffuse bone marrow involvement and higher clinical staging. It could therefore be used as another prognostic indicator in B-CLL patients.

P137. Mucosal malignant melanoma of the maxillary sinus: A case report

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Mucosal malignant melanomas (MMM) are rare accounting for less than 1% of all melanomas and < 5% of all sinonasal tract neoplasm. As for (noncutaneous) upper respiratory tract region, nasal cavity is most frequently affected, followed by a combination of the nasal cavity and paranasal sinuses. We report a case of MMM occurring in a 53-years-old Chinese man. He presented to Hospital Kuala Lumpur with 1-month history of left nasomaxillary sinus swelling. Examination revealed a palatal swelling measuring 20 x 10 mm. Wide excision of the tumour was done. Macroscopically, there is an irregular polypoidal growth occupying the left maxillary sinus measuring 55 x 50 x 40 mm. Cut sections show a brownish mass extending from the floor of the maxillary sinus infiltrating into adjacent structures. Microscopically, the mass is composed of hypercellular tumour interspersed with tumour cell necrosis. These tumours are comprised of prominent spindled cells admixed with epitheloid cells, arranged in solid to fascicular patterns. The tumour cells exhibit medium to large sized cells with high nuclear to cytoplasmic ratio, pleomorphic vesicular nuclei with coarse chromatin pattern and some with prominent nucleoli. Mitoses including atypical forms are easily identifiable (20/10 HPF). Occasional tumour cells contain intracytoplasmic melanin pigment that is confirmed by Masson Fontana stain. Vascular invasion and neurotropism are identified. Immunohistochemically, the malignant cells are immunopositive for vimentin, S100 (focal) and HMB45 (focal). They are negative for cytokeratin, EMA, leucocyte common antigen, smooth muscle actin, synaptophysin, chromogranin and desmin. Based on
the morphology and immunohistochemistry findings, the diagnosis of mucosal malignant melanoma was made. Primary mucosal melanoma has a more aggressive clinical course than its cutaneous counterpart. The histology of these lesions varies, with differing degrees of melanin production and growth pattern. Metastatic malignant melanoma to the sinonasal tract, although highly uncommon, should be excluded as the prognosis is even poorer.

P138. Trisomy X and primary myelodysplastic syndrome (MDS) with eosinophilia

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Primary myelodysplastic syndrome (MDS) is a clinical syndrome whereby a genetically injured haematopoietic stem cells populated a bone marrow and lead to ineffective production of haemopoietic cells. Not only that, the quality of the cells produced are also compromised. We report a case of a young lady who presented with symptoms of anaemia and investigations revealed bicytopenia and eosinophilia. Bone marrow aspiration revealed myelodysplastic syndrome with eosinophilia and cytogenetic findings revealed the presence of trisomy X and a marker chromosome at chromosome 11. She was planned for allogeneic peripheral blood stem cell transplantation (alloPBSCT). Conventional cytogenetic G-banding revealed the presence of trisomy X but multicoloured fluorescence in situ hyridisation (M-FISH) added new information to the cytogenetic abnormalities that this patient had. The cytogenetic findings help in prognosis & further management of this patient. The rationale of using M-FISH will be discussed further. In conclusion, the presence of eosinophilic MDS with underlying complex cytogenetic abnormalities (trisomy X and marker chromosome 11) is a rare entity. The underlying mechanism of these chromosomal abnormalities causing myelodysplastic syndrome is yet to be known.

P139. Specific prenatal protocols for Thalassaemia and HbE in Malaysian ethnic groups – Easy and cost-effective implementation in government hospitals

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Alpha and β-thalassaemia and the haemoglobin variant HbE are recognised as public health problems in the Malays, Chinese and Orang Asli populations in Malaysia. About 4.5% of Malaysian Chinese are carriers of α⁺-thalassaemia (α⁺⁻SEA/αα) and about 3.5−4.5% of the population are heterozygous carriers for β-thalassaemia. Cost-effective prenatal diagnostic protocols for these disorders have been developed for easy implementation in government hospitals. Using a Duplex-PCR, the Southeast Asian deletion (α⁺⁻SEA) responsible for the fatal condition, Hb Bart’s hydrops foetalis in Malaysian Chinese, can be confirmed. The Duplex-PCR specifically amplifies both the 20.5kb SEA deletion and the normal sequence between the ψα-α2 globin genes, thus, differentiating between normal, carrier and Hb Bart’s affected foetuses in a single PCR. Combine-ARMS confirms two to three -globin gene mutations in a single reaction. Beta-thalassaemia in 72% of the Malaysian Chinese and 37% of the Malays can be identified using C-ARMS for CD41-42/IVS2-654 and C-ARMS for CD41-42/CD17/IVS1-5 respectively. HbE, commonly present in about 30.7% of Malaysian Malays can be rapidly detected using an in-house developed ARMS protocol. The 45 kb Filipino deletion is the most common defect responsible for thalassaemia in the Orang Asli. Using gap-PCR, 99% of β-thalassaemia in these indigenous groups can be detected in one reaction. Highly trained technical staff is not required for
performing these protocols as the target sequences are amplified as discrete distinct bands. The high specificity and sensitivity of these protocols also allows easy implementation in government hospitals with basic laboratory set-up and limited resources.

**P140. Oxidative DNA damage in leukocytes and its association with metabolic control in diabetic patients with and without microalbuminuria**

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Oxidative damage was suggested in the development of diabetic microangiopathic and macroangiopathic complications in these patients. Since the long term complications are the main cause of morbidity and mortality in diabetic patients, a quantitative determination of the level of oxidative stress is a valuable indicator of the degree of the severity of the disease and of the effectiveness of the treatment. The main aim of this study is to evaluate the extent of oxidative DNA damage with different severity of microalbuminuria in diabetic patients and its associations with other metabolic controls. The comet assay was adopted to measure the level of oxidative DNA damage in leukocytes of 127 Type II diabetic patients with and without microalbuminuria. Patients were grouped into four groups based on the findings in the urine test. Fasting blood samples were collected for the determination of fasting blood glucose (FBG), glycated haemoglobin (HbA1c), lipoprotein (a) and lipid profile. Low DNA Damage (type 0 and 1) was seen in normal subjects and patients with Normoalbuminuria (0mg/dL and 20mg/dL urine microalbumin tests). High and complete DNA damage (type 2, 3 and 4) were detected at microalbuminuria marked 50mg/dL and more. The microalbuminuria groups had shown a significant elevation of DNA damage compared to Normoalbuminuria groups ($18.54\pm3.17\%$ Vs $12.00\pm1.37\%$, $p<0.01$). There were a significant correlation ($p<0.01$) between FBG and HbA1c in both Normo- and Microalbuminuria groups. But no significant different was detected in the mean values of FBG and HbA1c between all diabetic groups. The level of oxidative DNA damage was increased significantly after the concentration of 20mg/dL microalbuminuria. Total cholesterol, triglyceride, LDL-chol and concentration of Lp(a) did not show any association with the level of oxidative DNA damage. This study suggests that oxidative stress occurred in the early stage of diabetic complication and even before overt microalbuminuria was detected.

**P141. Huge upper limb mass in a 13 year old boy: A case of small cell variant of anaplastic large cell lymphoma with positive immunoreactivity for CD99**

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Anaplastic large cell lymphoma (ALCL) is a high-grade lymphoma characteristically showing a pleomorphic CD30 (Ki-1) positive large cell infiltrate, with a predominantly T-cell immunophenotype and co-expression of epithelial membrane antigen (EMA). However, variants of ALCL have been described, including lymphohistiocytic, sarcomatoid and small cell. A 13 year old boy presented with a huge 18x20cm mass on his right arm of 6 months duration. MRI showed an enhancing soft tissue tumour involving the whole triceps with no bony involvement. Histopathological examination revealed sheets of malignant small round blue cells with marked apoptosis, numerous mitoses and necrosis. Immunohistochemistry demonstrated positivity for LCA, CD43, CD45R0, CD30, EMA, ALK-1 and CD99, with negativity for CD20, TdT, myogenin, myoD1, NSE, bcl-6, bcl-2 and CD10.
FISH testing also excluded the diagnosis of Ewing’s sarcoma/PNET. ALCL occurs in all age groups, with approximately 20% of cases being under 20 years. The small cell variant (SCV) exhibits similar immunohistochemical properties as ALCL, and has been seen to transform into the classic anaplastic large cell form. CD99 (MIC2) was originally described as a diagnostically useful marker for Ewing’s sarcoma/PNET, but has also been documented in a variety of other tumours, notably hematologic neoplasms such as T and B lymphoblastic lymphomas/leukemias, acute myelogenous leukemia and chronic myelogenous leukemia in blastic crisis. A recent study by Chang et al revealed unexpectedly high expression of CD99 (70%) in ALK-positive ALCLs (compared to 20% in ALK-negative ALCLs), but negativity in other mature T/NK cell neoplasms. The reason is unexplained, but the role of ALK is highly suggestive. Pathologists need to be aware of the diagnosis of a small cell variant of ALCL, as well as of the fact that CD99 expression commonly occurs in cases of ALK-positive ALCL, in order to distinguish this entity from Ewing’s sarcoma/PNET.

P142. A late presentation of germ cell tumour: A case report

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A 2 years old boy presented with one week history of abdominal pain and distension associated with difficulty breathing. No symptoms of alteration bowel habit documented. Physical examination revealed the child was dyspnoeic with gross distension of the abdomen. There were palpable mass at the right hypochondrium and suprapubic. Urgent ultrasound performed revealed hepatomegaly with multiple echogenic mass and also suprapubic mass which in favoured of malignancy. Urgent CT Scan abdomen did the next day, findings showed large pelvic mass with metastasis to lungs and liver. However the origin cannot be determined. The differential diagnoses were Rhabdomyosarcoma and Neuroblastoma. During admission, the child developed acute respiratory distress with drastically increased abdominal distension. Urgent ultrasound did reveal increasing ascites with possibility of intra peritoneal bled. Emergency laparotomy and debulking of tumour were done. Intra operative findings of ruptured liver mass with big suprapubic mass noted during the operation. Post operative management in ICU noted the abdominal distension recurred, and the process of weaning off ventilator unsuccessful. The HPE result was Germ Cell Tumour and he was planned for chemotherapy. However, the condition worsening and the child passed away in ICU on day 13 post operative. We present this case because of the need for early detection so that the proper treatment can be instituted.


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Maternal floor infarction of the placenta is a relatively rare disorder that on gross examination is characterized by a thickened gray-yellow maternal floor of the placenta with histologic evidence of massive fibrin deposition (Gitterinfarkt or Netzinfarkt) involving the decidua basalis and the contiguous villi. Entering and wrapping of the villi cause atrophy of the villi. The lesion is often associated with the x cell proliferation, and the forming of placental cysts. Three cases of maternal floor infarction of the placenta were reported. Their ages were thirty, twenty three, and thirty respectively. The findings of gross examination include a thickened, yellow maternal floor with large deposits of fibrin in the
placenta as were seen in lamellar cutting of 0.4 cm of thickness in the first, 0.3 cm in the second, and 0.4 in the third case. All of the cases had hematoma in some parts of the villi and had been associated with fetal death.

**P144. Detection of Mycobacterium tuberculosis from sputum and lymph node aspiration by microscopic and rapid ICT test compared with PCR test on suspected TB patients**

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The objective was to analyze the ability of tests for detecting *Mycobacterium tuberculosis* by microscopic (acid fast bacilli or AFB smear) and rapid ICT (Mycotec TB) compared with polymerase chain reaction (PCR) on suspected pulmonary and lymphadenitis tuberculosis (TB). Serum samples were obtained from 109 suspected TB patients for ICT test. Sputum samples were obtained from 74 suspected pulmonary TB and lymphnode aspiration were collected from 35 suspected lymphadenitis TB. for microscopic tests in the Clinical Pathology Laboratory of Wahidin Sudirohusodo Hospital and PCR tests using IS6110 primers in the Molecular Laboratory, Medical Faculty of Hasanuddin University. Positive-test on suspected pulmonary TB were: ICT (58,11%), PCR (50%), culture (33,78%), AFB smear with decontamination (29,73%) and AFB smear (28,38%) respectively, on suspected lymphadenitis TB were cytology (80%), ICT (68,5%), PCR (62,8%), AFB smear (31,5%) respectively. Sensitivity and specificity tests to culture test on suspected pulmonary TB were: AFB smear (52% and 83,67%), AFB smear with decontamination (56% and 83,67%), ICT (60% and 42,86%), and to PCR test were: AFB smear (29,73% and 72,97%); AFB smear with decontamination (29,73% and 70,27%); ICT (67,57% and 51,35%). Sensitivity of combination tests (if one of the test was positive) to culture were: AFB smear plus ICT (72%) and AFB smear with decontamination plus ICT (76%), and sensitivity of the two combination to PCR were 67,57%. Sensitivity and specificity tests to PCR on suspected lymphadenitis TB were cytology (72,3% and 7,69%), ICT (63,64% and 23,08%), and AFB smear (22,73% and 58,85%), and sensitivity of combination tests (if one of the test was positive) were: AFB smear plus ICT (63,64%), Cytology plus ICT (86,36%) and Cytology plus AFB smear (72,73%). On suspected pulmonary TB, ICT had the highest sensitivity to culture and to PCR, but on lymphadenitis TB, cytology test had highest sensitivity. Sensitivity value can be increased by combinating 2 tests (if one of the test was positive) showed the highest value was AFB smear plus ICT on suspected pulmonary TB, and cytology plus ICT on suspected lymphadenitis TB.
P145. Guidelines for the implementation of an ethical code for the Indonesian clinical pathologists.

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All medical doctors in Indonesia, including clinical pathologists are obliged to become members of the Indonesian Medical Association. In doing their medical professions, all medical doctors, clinical pathologists and other medical specialists must based on the Indonesian Ethical Code of Medicine, professional standards and other ethical conditions in Indonesia. The objectives are to achieve welfare and healthy people, to prioritize the patient and community’s healthy and safety and to prevent malpractice, dissatisfaction and conflicts of clinical pathologists services to the community, non ethics and non legal aspects. Ethical conditions and standards as guidelines for clinical pathologists are: 1) the ethical code of medicine; 2) the professional and medical service standards and the ethical code of clinical pathologists; 3) the regulation of the Indonesian Association of Clinical Pathologists; 4) the Indonesian constitution and philosophy; 5) the norm of law in Indonesia; 6) the development of medical science and technology; 7) the value of the community development; 8) the competence certificate; 9) the registration certificate; and 10) the licence of medical practice in Indonesia. Beside the above ethical conditions or standards, guidelines for implementations of ethical code of clinical pathologists in doing their professions are obliged to follow three obligations e.g. general obligations; obligations to the other clinical pathologists and other medical professions and obligations to clinical laboratory, patients and specimens. As conclusions, clinical pathologists in doing their medical professions in Indonesia are obliged to follow guidelines of the ethical code of the Indonesian clinical pathologists and other ethical conditions and standards as legal aspects in Indonesia.

P146. Diagnostic usefulness of a 7-feature, 15-point scoring system in the interpretation of liver biopsy in neonatal cholestasis

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**Background:** Accurate interpretation of liver biopsy in neonatal cholestasis requires considerable experience. We aimed to assess the usefulness of a scoring system devised to assist interpretation of liver biopsy in neonatal cholestasis. **Methods:** Materials obtained from infants with neonatal cholestasis referred to the Department of Paediatrics, University of Malaya Medical Centre, Kuala Lumpur were reviewed by the authors, initially blinded to the final diagnosis. Author’s histological diagnosis, based on a 7-feature (portal ductal proliferation, bile plugs in portal ductules, porto-portal bridging, lymphocytic infiltration in portal region, multinucleated hepatocytes, neutrophils in the infiltrate, hepatocellular swelling), 15-point (minimum 0, maximum 15) and without the scoring system, was compared with the final diagnosis. **Results:** 84 liver biopsy materials from 78 patients were reviewed. Without the scoring system, biliary atresia (BA) was correctly diagnosed histologically in 30 cases, labelled as neonatal hepatitis (NH) in 3. NH was identified correctly in 33 cases, labelled as BA in 2 cases. Of the remaining 15 patients with other causes intrahepatic cholestasis, the author’s diagnosis was BA (n=5) and NH (n=9). Overall sensitivity for BA was 91%, sensitivity 86% and accuracy 88%. With the scoring system, a score of 7 has the best diagnostic utility to differentiate BA from other intrahepatic cholestasis histologically (sensitivity 88%, specificity 94%, accuracy 92%). Four patients with a score < 7 had BA, and 3 patients with a score 7 had NH. **Conclusion:** A 7-feature histological scoring system has good diagnostic accuracy in the interpretation of liver biopsy in neonatal cholestasis.
P147. α1-antitrypsin deficiency is not an important cause of childhood liver diseases in a multi-ethnic Southeast Asian population

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Aim: We conducted a prospective study to determine the role of α1-antitrypsin (α1AT) deficiency in the pathogenesis of neonatal cholestasis and other childhood liver diseases in a multi-ethnic Southeast Asian population. **Patients and methodology:** Prospective patients with neonatal cholestasis (group 1), other liver diseases (group 2), and children with other medical conditions (group 3) referred to the Paediatric Unit, University of Malaya Medical Centre, Malaysia, from May 2002 to June 2005, were screened for α1AT level and phenotype. α1AT level below 80 mg/dL was considered as low. **Results:** Of the 114 patients (group 1, n=53; group 2, n=42; group 3, n=19) screened, 7 patients (6% of total; group 1, n=1; group 2, n=4; group 3, n=2) had a α1AT level below 80 mg/dL. All had marginally low level (range 57 – 79 mg/dL), but none had a clinical diagnosis of α1AT deficiency. One patient had PiZ- heterozygous phenotype (α1AT level 217 mg/dL) while another patient had PiMS heterozygous. **Conclusions:** α1AT deficiency is not an important cause of neonatal cholestasis and childhood liver diseases in Malaysian children. In Malaysian children with neonatal cholestasis or other liver diseases, routine assay for α1AT phenotype is not recommended if there is no family history of neonatal cholestasis of uncertain aetiology, or if α1AT level is above 80 mg/dL.

P148. Patterns of chromosomal alterations in breast cancer and deletion on chromosome 22 are associated with lymph node-negative

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Breast tumour development and progression are thought to be driven by an accumulation of chromosomal aberrations. The pattern of chromosomal aberrations was reported to be associated with disease recurrence or survival in lymph node-negative women. The objectives of this study are to identify and map regions of the genome with DNA sequence copy number changes in primary breast cancer and to determine specific chromosomal regions associated with clinical outcome in lymph node-negative tumours. Chromosomal aberrations were examined by comparative genomic hybridization in 32 primary breast cancer specimens. Tumour DNA was labeled with SpectrumGreen and normal DNA was labeled with SpectrumRed, subsequently hybridized on the normal metaphase. The most frequent chromosomal losses mapped to chromosome 19 (88%). Other chromosomal losses were found at 1p36.2-p36.3 (72%), 17 (72%), 22q11.2-q13 (69%), 16p12-p13.3 (56%), 16q22-q24 (50%), 9q34 (47%) and 12q24.1-q24.3 (38%). The common sites of chromosomal gains were found at 3p12-q12 (75%), 6p11.2-q16 (63%), 4p13-q24 (59%), 5p13-q11.2 (53%), 13q21-q31 (50%), 1p3 (44%), 5q15-q21 (41%) and 8q21.1-q21.3 (34%). Deletions of chromosome 17 (72%; P = 0.027) and 22q11.2-q13 (69%; P = 0.033) were significantly higher in those with HER-2/erbB2 receptor negative. Loss of chromosome 22q11.2-q13 (P = 0.020) occurred in the vast majority of lymph node-negative cases (13 of 32). There is considerable scientific and clinical interest in locating lymph node status-dependent chromosomal 22q11.2-q13 regions to find genes that may be responsible for metastasis formation. Our results indicate that certain genomic lesions, especially loss of chromosome 19 and gain of region at chromosome 3p12-q12 may play a role in breast tumourigenesis.
P149. Detection and characterisation of the BRCA2 gene in Malaysian breast cancer patients
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Mutations in the BRCA2 (OMIM: #600185) gene have been linked to an elevated risk of breast cancer which has been demonstrated to be due to the inheritance of dominant susceptibility genes conferring a high risk of the disease. The contribution of BRCA2 mutations to breast cancer in Malaysian women remains to be elucidated. The purposes of this study are, first, to investigate the presence of sequence variants in the BRCA2 gene and, second, to evaluate the sequence variants between simple sequence polymorphisms or pathogenic mutations. Germline BRCA2 mutations were screened in 17 Malaysian high-risk breast cancer patients composed of familial, early-onset and male cancer groups. Clinical information and peripheral blood were obtained from fully-informed and consenting individuals. Genomic DNA was extracted from the peripheral blood and amplified by polymerase chain reaction (PCR). Direct DNA sequencing was subsequently performed on the PCR product to detect sequence variants. In the coding (10,257 bp) sequences of BRCA2, seven sequence variants were identified. Sequence variants were detected in 17 of 17 (100%) unrelated patients. One novel sequence variant, (2024T>C) was identified in 12 (71%) patients resulting in the amino acid change from Phenylalanine to Serine. Seven patients had a missense mutation in exon 10, (1342C>A) which has been previously detected in individuals from Central and Western Europe. Another sequence variant in exon 10, (1593A>G) was detected in one patient. Two sequence variants (3624A>G and 4035T>C) were detected in exon 11. Another sequence variant, (203G>A) was detected in the 5’ untranslated region of seven patients. In exon 14, one sequence variant, (7470A>G) was detected in three patients. These findings were consistent with the result formerly reported in Breast Cancer Information Core (BIC). DNA sequencing is still on going using a larger sample size with more interesting findings anticipated.

P150. Paraganglioma: A report of three cases in unusual sites
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Extra-adrenal paragangliomas are uncommon entities. They can be classified into four basic groups according to their anatomic sites, i.e. branchiomeric, intravagal, aorticosympathetic and visceral autonomic. Similar tumour may arise in sites away from usual distribution of the sympathetic and parasympathetic ganglia, e.g. orbit, nose, small intestine and even the pancreas. We report three cases of paraganglioma which were found in unusual sites such as urinary bladder, thyroid gland and on the wall of the inferior vena cava. Case 1 was a 40-year-old Chinese woman with multinodular goiter and a separate nodule in the right upper thyroid pole. This separate fleshy and vascular nodule measured 2.5cm across in largest dimension and was composed of an intrathyroidal tumour made up of clusters of uniform bland tumour cells segregated by haemorrhagic fibrovascular tissue. Case 2 was a 43-year-old Indian man who had a 6cm-diameter tumour on the inferior vena cave, just below the junction of the renal vein. The tumour was vascular and composed of focally pleomorphic tumour cells with hardly any mitoses. Both tumours in Cases 1 and 2 were partially encapsulated and expressed chromogranin as well as synaptophysin. Case 3 was 25-year-old Chinese man who presented with
painless haematuria. Examination showed a solid tumour in the lateral wall of the urinary bladder. The tumour was removed piecemeal and measured 3cm in aggregate. Histology revealed a tumour composed of nests of relatively uniform cells, with moderate nuclear pleomorphism and occasional mitosis, invading into the submucosa and muscle wall. There was no capsule seen. Tumour cells expressed vimentin and synaptophysin.

P151. **Histopathology appearance from blighted ovum diagnosed by ultrasonography**

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Anembryonic pregnancy caused by blighted ovum is a pregnancy in which a visible embryo never develops within the gestational sac. The frequency has been assessed as 49% – 90% of all spontaneous abortions. Diagnosis of the pregnancy can be based on ultrasonography methods when the pregnancy has reached 8-10 weeks or more and histopathology examination of the uterine curettage. This study is aimed to evaluate the histopathology findings in the abortive tissues in whom blighted ovum has previously been diagnosed by careful ultrasonography examination. This study consists of 43 samples of abortive tissues from 8 – 16 weeks gestation sent by obstetricians after blighted ovum was diagnosed by ultrasonography methods. Samples were examined macroscopically then the tissues were processed with paraffin blocks and the histopathology appearance of the chorionic villi were examined under the microscope. There was no fetal tissue observed in all of the cases, either macroscopically or microscopically. The histopathology findings were hydropic degeneration in 43 cases (100%), villous hypoplasia in 33 cases (77%), fibrosis villi in 41 cases (95%), cavitation in 24 cases (56%), invagination in 28 cases (65%) and inclusion in 21 cases (49%). Histopathologically blighted ovum gave the villi choriales changes as hydropic degeneration, villous hypoplasia, fibrosis villi, cavitation, invagination and inclusion. By observing the changes it is suggested that further examination should be done, to know wheather the changes related to chromosomal abnormality, by chromosome karyotyping and immunohistochemistry.

P152. **Detection of BRCA2 gene mutation in breast cancer patients using denaturing high performance liquid chromatography (DHPLC)**

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With the increasing number of sequence variants identified in the BRCA2 gene among high risk breast cancer patients, there is a need for high throughput methods for mutation and/or polymorphism detection. Denaturing high-performance liquid chromatography (DHPLC) is a recently developed method for detecting DNA sequence variants. It has high sensitivity and successfully detects single-nucleotide substitutions, small deletions and insertions. This study aims to assess the reliability of this method as a mutation detection tool for BRCA2 gene in high risk breast cancer patients. Twelve fully-informed, consented breast cancer patients who fulfilled our inclusion criteria which included positive family history of breast cancer, early-onset (< 40 years) and male breast cancer patients from Hospital Universiti Kebangsaan Malaysia (HUKM), Hospital Kuala Lumpur (HKL) and Hospital Putrajaya (HPJ) were selected. DNA material was extracted from their peripheral blood followed by polymerase
chain reaction (PCR) amplification and subsequently analysed using DHPLC technique. Direct DNA sequencing was performed on fragments which showed elution profiles that were distinct from those of the wild-type samples to determine the sequence variants. Our preliminary results showed DHPLC successfully detected two sequence variants in nine of the 12 patients. Sequence variants 1342C>A in exon 10 was found in four patients (33.33%) and 4035T>C in exon 11 in five patients (41.67%), which was confirmed by direct DNA sequencing. Two of the nine patients showed presence of both sequence variants. These two sequence variants have been previously reported as polymorphism in other studies. Our early findings suggest there is a potential role of DHPLC as a mutation detection method for BRCA2 gene in breast cancer patients.

P153.  Inhibitory activity of vitamin E on the susceptibility of LDL to oxidation reaction

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There is increasing evidence that the oxidation of LDL plays an important role in atherogenesis. Our understanding of the mechanism of LDL oxidation and factors that determine its susceptibility to oxidation is still incomplete. Copper is a candidate for oxidizing LDL in atherosclerotic lesions. Antioxidants that can prevent LDL oxidation may act as antiatherogens. Therefore aim of this study is to determine the effect vitamin E on LDL oxidation by copper as in vitro. In this study LDL was isolated from plasma by ultracentrifugation using a single-step discontinuous gradient. Then various concentrations of vitamin E was added to LDL and incubated for 1 hour at 37°C. The oxidizability of LDL was estimated by conjugated dienes method after CuSO4 addition. Our results were showed that vitamin E by increasing of maximum time for conjugated dienes formation significantly. We find that α-tocopherol with inhibition of oxidation of LDL may have a role in ameliorating atherosclerosis.

P154.  Metaplastic papillary tumor of fallopian tube: A case report with ultrastructural study

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Metaplastic papillary tumor of the fallopian tube is a rare lesion distinctive for pregnancy and postpartum period, which morphologically may mimic malignancy, but the tumor shows completely benign behavior clinically. Only seven cases have been reported in the literature by now and this is the 8th patient with unique ultrastructural findings. A 27 years woman G2 P1 A0 L1 a case of elective cesarean section. During the operation there was a firm solid mass attached to the left fallopian tube measuring (4 cm in diameter).pathologic and electron microscopic study was performed. Electron microscopy study showed multiple membrane-bound vesicles with different size in the cytoplasm of the tumor cells and sometimes a single large membrane-bound vesicle in the cytoplasm. The inner layers of the cytoplasmic vesicles are covered by membrane arranged in microvillous pattern with different length. The cytoplasm revealed polar aggregation of mitochondria. This unique ultrastructural feature by electron microscopy has not been reported in the literature for this tumor by now.
P156. Multiparameters flow cytometry immunophenotyping of multiple myeloma and monoclonal gammopathies of uncertain significance

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Monoclonal gammopathy of undetermined significance (MGUS) and multiple myeloma (MM) are the most frequent forms of monoclonal gammopathies. Flow cytometric parameters may help us to understand plasma cells biology and pathology. Normal PCs from healthy donors express a typical phenotype: CD19+ and CD56 negative whereas malignant plasma cells do not express CD19 and generally express CD56. We have applied flow cytometric analysis to evaluate the bone marrow plasma cells in 58 patients with MGUS and 65 patients with multiple myeloma, of which 45 pts have been evaluated at diagnosis and after therapy, 10 after peripheral stem cell transplantation and 10 in progression disease. A flow cytometric technique based on 7-color multidimensional flow cytometry using k/l/CD38/CD19/CD56/CD138/CD45/CD20 and two-step acquisition procedure was performed on cytometer ADPCyan TM (Dako, Ft Collins, CO, USA). In the first step 50,000 events corresponding to the total of BM MNC were acquired. In the second step only those events included in the “live-gate” drawn on SSC/CD38 bright fraction (where plasma cells are located) were acquired and studied for the relative expression of CD56, CD19 and CD20. In MGUS we found that the CD38 bright cells percentage was <5.5% of bone marrow nucleated cells. In 22 patients with MM at diagnosis we found a percentage of CD38 bright of 10%-60% of bone marrow nucleated cells with CD56+/CD19- cells=67%±30%, normal PCs with CD56-/CD19+ phenotype were <2%±0.4% and CD56+/CD19+ cells=1%. We found both the populations CD56+/CD19- and CD56-/CD19+ in CD38 bright cells’ fraction in MGUS patients and the exclusive presence of CD56+/CD19+population in MM patients at diagnosis. Moreover our study gives original data about CD56+/CD19+ population which seems to spread in disease control phases. Our results, even if preliminary, show that 7-color multidimensional flow cytometric analysis can be useful in qualitative and quantitative bone marrow plasma cells assay.

P158. Evaluation of the correlation between MDM2 protein and histologic grading of cerebral astrocytoma

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MDM2 is a protein factor that plays an important role in inhibition of P53 and P73 related apoptosis. The main aim of this study was to observe MDM2 expression in different histologic gradings of cerebral astrocytomas. 104 cases of astrocytic tumors including 4 different grades were selected randomly. 4 micro meter sections of paraffin blocks of tumors were prepared, then the slides were stained by mdm2 antibody. The results were analysed by SPSS software. The intensity of nuclear staining for MDM2 marker has a statistically significant correlation with histologic grading. There was no significant correlation between percentage of nuclear staining and histologic grading. The positivity of mdm2 marker and also the multiplying of intensity and percentage of nuclear staining in s.score had a significant correlation with histologic grade. The s.score correlation with grading of astrocytic tumors was analysed by Roc cure analysis meted and showed that the s.score can be useful for differentiation of glioblastom multiform from other grades with 95.3% specificity. Our results show the role of MDM2 protein as an oncogenic factor in progression of astrocytic tumors. The MDM2 expression in astrocytic cerebral tumors can be specifically useful only for distinction of glioblastoma multiform from other grades of glioma.
P159. Diagnostic value of bone marrow aspiration in comparison to bone marrow biopsy in metastatic tumors

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Introduction and Purpose: For detection of the metastatic tumors to the bone marrow usually bone marrow biopsy is done. Sometimes it is difficult to do biopsy and one must rely on aspiration alone. The purpose of this study is to determine whether bone marrow aspiration is a valuable method for detection of metastatic malignant tumors to bone marrow with comparison to biopsy or not. Materials and Methods: This is a 10 year retrospective study of bone marrow samples, present in the file of bone marrow center of Shiraz University Medical Sciences which is located in Faghihi Hospital. All patients with clinical impression of R/O (ruled out) metastatic tumor to bone marrow and pathologic proved malignancy of other organs were included in this study. The bone marrow reports with positive metastatic tumors were 167. Patients that had adequate biopsy and aspirate smear in the file were 150 as follow, Hodgkin’s disease (29), Non- Hodgkin’s lymphoma, NHL (64), small cell malignant tumor including neuroblastoma, retinoblastoma, Ewing’s sarcoma (19), Metastatic carcinoma of known origine (34), metastatic carcinoma, unclassified (4). Patients with positive biopsy and positive aspiration were 105 out of 150 (70%), and those with negative biopsy and positive aspirate were 8 (5%) which were small cell malignant tumor (6) and NHL (2). With simultaneous bone marrow aspiration and biopsy in patients with NHL and small cell malignant tumor involved bone marrow, 100% showed metastatic cells in aspiration. In metastatic carcinoma, 65% aspirate of patients with involvement of bone marrow biopsy showed metastatic cells. Conclusion: The results of our study indicates that even the aspirate is more accurate in detection of involvement of bone marrow by NHL as well as metastatic small cell malignant tumors such as neuroblastoma, retinoblastoma and Ewing’s sarcoma with better visualization of cell morphology.

P160. Haemoglobin H disease due to co-inheritance of two alpha gene deletion and Hb Constant Spring: A case report

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Haemoglobin H disease, alpha thalassaemia intermedia is usually caused by three alpha gene deletion, as a result of coinheritance of the common deletional forms of alpha and alpha + alleles. However, alpha thalassaemia can also be caused by the non-deletional gene abnormality, Hb Constant Spring (the most prevalent non-deletional alpha thalassaemia in Southeast Asian populations). It is difficult to detect Hb CS on haemoglobin electrophoresis in the heterozygous state because of its low level and unstable nature. We described a 3 year 9 month old Malay boy, referred for moderate anaemia with upper respiratory symptoms. On physical examination, patient was febrile and pale with hepatosplenomegaly. Blood investigation showed hypochromic microcytic anaemia with haemoglobin level of 7.0 g/dl, (MCV; 58.3 fl, MCH; 17.2pg). Haemoglobin analysis showed normal A2 level and mild raised Hb F (3.4%) level and presence of a fast H band, consistent with the diagnosis of Hb H disease. Both parents showed normal haemoglobin level with low MCV (father: 75.8fl; mother: 64.0 fl) and MCH (father : 25.6 pg; mother: 20.3 pg) and haemoglobin analysis revealed father’s Hb A2 and Hb F levels to be normal and the mother had findings consistent with Hb E trait. DNA was analysed for alpha gene deletions by PCR using allele-specific primers and results revealed two alpha gene deletions in patient and his mother. However, the father’s DNA analysis showed four intact alpha genes. DNA was analysed for the common non-deletional Hb Constant Spring mutation by Q-PCR. The presence of Hb Constant Spring mutation was confirmed in both patient and the father (heterozygous) by DNA sequencing. This case illustrates that although Hb H can be easily diagnosed by routine methods, DNA analysis provides the individual’s exact genotype, important for definitive diagnosis and genetic counseling.
P161. Autopsy and laboratory study of a hydrops fetus with anti-Ro and anti-La antibodies
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Autoantibodies to intracellular soluble ribonucleoproteins 48 kD SSB/La, 52 kD SSA/Ro, and 60 kD SSA/Ro are closely associated to the patients with Sjogrene syndrome in adult and neonatal lupus erythematosus syndrome with congenital heart block. We present an autopsy case with hydrops fetalis and anti-Ro & anti-La antibodies in serum. **Materials:** The fetus was elective terminated due to severe hydrops at the gestational age of 24 weeks. Cytogenetic study showed normal karyotype (46, XY). His mother, G1P0, had Sjogrene syndrome found 4 years ago and was under steroid therapy irregularly. Antinuclear antibody (ANA), speckle type, and anti-SSA/Ro antibody were noted at the gestational age of 23 weeks. Her blood type was O+ without RBC irregular antibodies. Autopsy and postmortem survey from blood were performed for genetic counseling and clarifying the cause of hydrops fetalis. **Results:** The stillbirth presented generalized subcutaneous edema, massive ascites, pleural & pericardial effusion. The heart showed myocarditis, myocardial necrosis, fibrosis, dystrophic calcification and perineural lymphocyte infiltration. No sialoadenitis was identified. Anemia with erythroblastosis in peripheral blood and extra-medullary hemotopoiesis in multiple solid organs were noted. Anti-SSA/Ro & anti-SSB/La antibodies, antinuclear antibody (ANA), speckle type, and anti-SSA/Ro antibody were noted at the gestational age of 23 weeks. Her blood type was O+ without RBC irregular antibodies. Autopsy and postmortem survey from blood were performed for genetic counseling and clarifying the cause of hydrops fetalis. **Conclusions:** The fetus with hydrops, erythroblastosis, cardiac fibrosis and calcification might relate to trans-placental anti-Ro/SSA & anti-La/SSB antibodies.

P162. Uterine leiomyoma with eosinophils: A report of a rare case

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Although leiomyoma of the uterus is common, hematopoietic components within this tumor is not. Lymphoid and other hematopoietic elements have been previously recognized, but eosinophilic infiltrates in leiomyoma has received little attention in the literature. We report a case of smooth muscle tumor of uncertain malignant potential (STUMP) with infiltration of numerous eosinophils. The patient was a 31-yr-old virgin that presented with abdominal pain for 2-3 yrs duration. Sonography revealed ecogenic uterine mass with necrotic center, measuring 101.91mm. WBC count was 9730 with no peripheral blood eosinophilia. No clinical evidence of allergy or parasitic infection was present. Myomectomy was done. Microscopic examination revealed highly cellular smooth muscle tumor with hyaline and coagulative necrosis, mild cellular atypia and rare mitosis, as well as infiltration of large numbers of eosinophils in some area. This was a rare case because only two cases reported in literature.
P163. Keratoacanthoma centrifugum marginatum: A report of a rare variant of keratoacanthoma

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Keratoacanthoma centrifugum marginatum is a rare clinical variant of solitary keratoacanthoma characterized by progressive peripheral growth with coincident central healing. Such lesions may be 20 cm or more in diameter. There is no tendency toward spontaneous involution. The most common locations are the dorsa of the hands and the legs. We report one case, 49 years old male, with large skin lesion of dorsa of hand with raised, rolled border since 1/5 year ago.

P164. Clear cell adenocarcinoma of cervix in a pregnant female (not associated with diethylstilbestrol): A case report

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Although cervical carcinoma is the most common gynecologic malignancy associated with pregnancy, its occurrence is rare with an incidence of approximately 1 per 1200 to 10000 pregnancies. We report the clinicopathologic features of a pregnant young female with clear cell adenocarcinoma of cervix. This patient is the first reported pregnant female with primary clear cell adenocarcinoma of cervix not associated with DES, in Iran.


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Gestational choriocarcinoma is an epithelial malignant neoplasm of trophoblastic cells derived from any form of previously normal or abnormal pregnancy. Choriocarcinoma is a rapidly invasive widely metastasizing malignant neoplasm. Favored sites of involvement are the lungs (50%) and vagina (30% to 40%), followed in descending order of frequency by the brain, liver and kidney. Cutaneous metastasis is very rare. We report a case of choriocarcinoma with one cutaneous metastasis in finger of hand.

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Fungal infection of female genital tract is common, with Candida spp. being the most frequent type seen on cervico-vaginal (Pap smear) test. There are rare reports of other fungal types, such as Paracoccidioides and Aspergillus fumigatus, in a Pap test. In one study in Egypt only 7.4% of reproductive tract infections was Aspergillus. We report an unusual case of Aspergillus spp. In a cervico-vaginal (Pap) test. A 43-yr-old female with medical history of spotting underwent Pap test and cervical biopsy. Cytologic examination revealed “fruiting body” of Aspergillus, some candida and malignant squamous cells. Microscopic diagnosis of cervix biopsy was “invasive squamous cell carcinoma”.

P167.  A case of transient myelopoiesis disorder in a Down’s syndrome infant

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Infants with Down’s syndrome have an increased incidence of unusual clonal megakaryocytosis syndrome called transient abnormal myelopoesis (TAM). The disorder is usually transient and self-limiting. About 20% of these patients will develop full blown acute megakaryoblastic leukaemia (AMKL) during their first 4 years of life. Here, we present a case of 2 month old Down’s syndrome infant with trisomy 21 who presented with prolonged jaundice and hepatosplenomegaly since birth. The diagnosis of TAM of Down’s syndrome was made from the peripheral blood film, bone marrow aspirate and immunophenotyping findings. She was managed conservatively and was advised for close haematological follow up, including weekly full blood picture. 3 months after the diagnosis, the blood count had return to normal with no blast cell seen in the peripheral smear. The hepatomegaly regressed. The baby thrived and has so far remained free of haematological problems.
P168. Association between fibrinogen and features of the metabolic syndrome in a rural population of Malaysia

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A number of studies have shown that plasma fibrinogen level is linked with different features of metabolic syndrome (metS) like hyperglycemia, hypertriglyceridemia, hypertension, obesity and hyperinsulinemia. However, there is no such data in a Malaysian population. The purpose of this study was to assess whether elevated fibrinogen levels were associated with the features of metS in subjects residing in a rural community in Malaysia. In this cross sectional study, data was collected from 503 men and 675 women. Data on blood pressure, BMI and waist were determined by standard method. Blood specimens were taken for glucose, insulin, and lipid profile, CRP, and apoB. Fibrinogen was measured by the Clauss method using the ACL 9000 instrument. Insulin resistance was assessed by homeostasis model assessment (HOMA-IR) and metS was defined according to the revised 2004 NCEP definition. Subjects with metS had significantly higher fibrinogen, hsCRP, insulin and HOMA-IR than those without metS. There was statistically significant positive crude correlation between fibrinogen and measures of BMI, HOMA-IR, fasting insulin, fasting glucose, (r range from 0.1 to 0.4, p<0.01). A negative correlation was found between fibrinogen and HDL cholesterol (R =0.07, P<0.05). There was a linear increase in fibrinogen levels with an increase in the number of components of the metS (fibrinogen: 3.45, 3.52, 3.62, 3.72g/L for those with 0, 1, 2, 3 features of the metS, respectively; P trend < 0.001). This finding suggests that increasing number of features of the metS is associated with increasing hemostatic factor that may contribute to adverse cardiovascular outcomes. Thus, measurement of fibrinogen adds clinically important information to metS in our population.

P169. Comparison between serum apolipoprotein B and non-HDL cholesterol in the dyslipidemic classification of Type 2 diabetes subjects

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The Adult Treatment Panel III has proposed that non-HDL cholesterol may be used for clinical decision making in hypertriglyceridemic patients. Non-HDLc also has been suggested as a surrogate marker for apoB which measures total atherogenic particle numbers. Objective: To compare non-HDL cholesterol and apoB in the identification of dyslipidemic phenotypes in type 2 diabetes subjects. Methods: A total of 317 diabetic subjects who were followed up in an on-going prospective study of prevalence of non-communicable disease were included in this study. Fasting blood specimens were taken for the measurement of total cholesterol, HDLc, triglyceride and apoB. LDLc was calculated by the Friedewald’s formula and non-HDLc obtained by subtracting HDLc from total cholesterol. They were classified as hypertriglyceridemic if TG >1.7 mmol/L; as hyper-non-HDLc if non-HDLc > 4.13mmol/L and as hyper-apoB if apoB concentration > 94 mg/L. Both classifications was compared by pearson correlation, concordance and kappa statistics. Results: 192 subjects were hypertriglyceridemic and 125 were normotriglyceridemic. The Pearson correlation showed that non-HDLc was strongly correlated to apoB in both groups (r=0.84 for normotriglyceridemic and r=0.79 for hypertriglyceridemic; p<0.001). In the hypertriglyceridemic group, 157 had increased apoB and 153 had increased non-HDLc, and 17 out of the 39 subjects with normal non-HDLc had increase apoB. The kappa index was 0.498- this
showed moderate agreement between non-HDLc and apoB in this group. In the normotriglyceridemic group, 80 had increased apoB and 66 had increased non-HDLc, and 22 out of the 59 subjects with normal non-HDLc had increase apoB. The kappa index was 0.512 - which showed moderate agreement between non-HDLc and apoB in this group. **Conclusions**: Although non-HDLc was found to be strongly correlated with apoB, apoB identifies subjects at risk better than non-HDLc in our studied population. Hence, non-HDLc might not be suitable as a surrogate marker for apoB in our population.

**P170. Apoptosis-induced chromosome breaks within a common chromosome deletion region in nasopharyngeal carcinoma**

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**Introduction**: Nasopharyngeal carcinoma (NPC) is well-associated with Epstein-Barr virus (EBV) infection and chromosome rearrangements such as additions and deletions. Chromosome 11q23 which contains the Mixed Lineage Leukaemia (MLL) gene appears to be a common region of deletion. Till date, the mechanism of chromosome rearrangements is unknown. However, apoptosis-induced chromosome break within the MLL gene have been reported for the leukaemic cells. This project aims to elucidate the role of apoptosis/EBV-induced apoptosis in chromosome rearrangements in NPC. **Methods**: NPC cell line was grown to high cell density to induce apoptosis. Subsequently, chromosome breaks within the MLL breakpoint cluster region (bcr) was analysed by non-isotopic Southern Hybridisation and nested Inverse Polymerase Chain Reaction (IPCR). In addition, NPC cells were also transfected with the EBV Latent Membrane Protein 1 (LMP1) gene. Expression of LMP1 gene (together with the V5 epitope) was confirmed by Western blotting using anti-V5 antibody as well as anti-LMP1 antibody. LMP1 transfection-induced chromosome breaks within the MLL bcr were analysed by nested IPCR. **Results**: The Southern Hybridization results showed that apoptosis induced chromosomal break within the MLL bcr in NPC cells at a position similar to the one reported in leukaemic cell. This break was significantly inhibited by caspase inhibitor, suggested the involvement of Caspase-Activated DNase (CAD). In addition, LMP1 transfection induced apoptosis in NPC cells and resulted in chromosome breaks within the MLL bcr. **Conclusion**: It is very likely that apoptosis or EBV infection-induced apoptosis results in chromosome breaks, possibly within the MLL bcr at 11q23 and that may eventually lead to chromosome rearrangements observed in NPC.

**P171. The role of histone deacetylase inhibitor in growth inhibition of nasopharyngeal carcinoma**

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**Introduction**: Nasopharyngeal carcinoma (NPC) is a common malignancy in southern China and Southeast Asia. Interestingly, a recent study reported that native people of Bidayuh tribe have a high incidence of NPC in Sarawak. Although chromosomal rearrangement and deletions have been reported in NPC, the molecular pathogenesis of NPC remains unclear. Current research findings reveal that alteration of histone acetylation status by histone acetyltransferases (HATs) and histone deacetylases (HDACs) induce global chromatin structural changes and thus gene expression. Disruption of the equilibrium of histone acetylation levels may thus lead to deregulation of the transcription of genes responsible in maintaining normal cellular processes, resulting in development of cancer. Therefore,
this study aims to determine the role of HDAC inhibitor, Trichostatin A (TSA) in the growth of NPC cell lines. The hypothesis of this study is that TSA inhibits the growth of NPC cell lines. 

**Methods & Results:** Two poorly differentiated NPC cell lines, HONE-1 and SUNE-1 were treated with increasing doses of TSA for 24 hours and the percentage of cell death was assessed by Trypan Blue exclusion assay. Our data showed that HONE-1 and SUNE-1 cells treated with high doses of TSA had 35-40% cell death. Both cells treated with low dose of TSA showed no significant cell death. The effect of TSA on the growth of NPC cells was assessed by crystal violet clonogenic assay. Our results showed that the survival of both cells decreased with increasing doses of TSA treatment. Furthermore, TSA-induced growth inhibition was shown to be time-dependent. However, HONE-1 cells were more sensitive to inhibition by TSA. 

**Conclusion:** TSA inhibits the growth of NPC cells in a dose- and time-dependent manner, and this may be achieved by arresting the cell cycle. Future studies on TSA regulation of the expression of genes responsible in apoptosis and growth arrest are warranted. A better understanding of the molecular mechanisms of growth inhibition by TSA may lead to new insights into potential therapeutic agents of NPC.

P172. **Distribution pattern of haemoglobin variants detected in Singapore General Hospital**

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**Aims:** To study the distribution pattern of alpha and beta haemoglobin variants detected in Thalassaemia Lab of Singapore General Hospital for a period of 6 months (June to December 2006). 

**Methods:** The standard protocol for thalassaemia and abnormal haemoglobin screening consisted of red blood cells indices obtained from cell counters (LH Coulter), electrophoresis on cellulose acetate at pH 8.4, HPLC electrophoresis on Bio-Rad Variant Plus for the quantitation of Hb-A2 and Hb-F and detection of Hb-H inclusion bodies. Sickling tests were done for samples suspected to have Hb-S variant. Samples displaying abnormal bands on the alkaline gel or abnormal variant peaks on the Bio-Rad chromatograms were then run on Beckman’s acid gel electrophoresis at pH 6.3 followed by direct DNA sequencing for definitive diagnosis. 

**Results:** Out of a total of 4593 samples received, 251 were of variants, 40 with variants and 3 with variant (Hb Lepore). For the variants, heterozygous Hb-E (195), homozygous Hb-E (10), Hb-E thalassaemia (3), Hb-E (2), Hb-E/Hb-NewYork (2) and Hb-E/Hb Neopolis (1), homozygous Hb-D (3), heterozygous Hb-D Punjab (10), Hb-D Iran (2), Hb-S (7), Hb-C (1), Hb- New York (7), Hb-J Bangkok (2), Hb-K Ibadan (1), Hb-J Rajappen (1) and Hb-Queens (1), Hb San Diego (1), Hb-Kenitra (1) and Hb-K Ibadan (1) were reported. For α thalassaemia, heterozygous Hb-Constant Spring (17), homozygous Hb-Constant Spring (2), heterozygous Hb-Q Thailand (10), Hb-Q India (6), Hb-G Singapore (3) and Hb-Siam (2) were reported. 73% of the Hb-E trait carriers were Malays, 10% Chinese, 2% Indians and 15% of other races. 42% of Constant Spring traits were Chinese, 35% Malays and 26% Others. 

**Conclusions:** There were more variants detected, the most common being Hb-E. As more foreign talents join the country’s workforce and subsequently the population, the distribution pattern of variants will change over time.
P173. **Changes of contractile wall of the epididymis in men with obstructive azoospermia.**

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The time taken for spermatozoa to appear in the ejaculate is very high (6-12 months) after the microsurgical repair of obstructive azoospermia (Matthews et al., 1995). The cause of this fact is structural changes in the contractile wall with alteration of transport and storage function of spermatozoa (Pelliccione et al., 2004). The objective is study of histological and ultrastructural changes of contractile wall of the caput epididymis in men with obstructive azoospermia. The contractile wall in the tubules of the caput epididymis was analyzed by light microscopy and transmission electron microscopy in 8 patients with a bilateral postinflammatory congestive obstruction of the epididymis. Six specimens from the caput epididymis, obtained from fertile men who had undergone an orchidectomy because of testicular cancer, served as control. The contractile wall was strongly thickened when compared with controls (74.5±6.2 µ vs 18.23±3.2 µ), the flat myoid cells were partially replaced by large smooth muscle cells with features of contractile activity (a large number of myofilaments coalescing into dense bodies) coexisting with secretory activity (a developed Golgi complex and endoplasmic reticulum, scattered lipid inclusions and a thickened continuous basement membrane-like material). The increased mechanical forces on the epididymal wall, proximal to the obstruction, can activate the differentiation of myoid cells into smooth muscle cells. After the microsurgical repair of the obstruction for restore an altered transport and storage of spermatozoa require some time to realign phenotype of smooth muscle cells.

P174. **Purification and characterization of alkaline phosphatase from human hydatidiform mole**

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**Objective:** Purification of alkaline phosphatase from human hydatidiform mole. **Design:** Study in period from third of July 2005 till end of July 2006. **Setting:** Department of Biochemistry, Ahwaz Jondishapur University of Medical Sciences, Ahwaz, Iran. **Patients and Methods:** Five human hydatidiform mole subjects from Taeghani hospital, Tehran, Iran were included in this study. An alkaline phosphatase from human hydatidiform mole was purified by a protocol involving solubilization using precipitation by butanol, acetone, ammonium sulphate, Sephadex G200, ion exchange chromatography and preparative electrophoresis. **Results:** The enzyme was purified 800-fold to apparent homogeneity. This enzyme has 5.2% carbohydrate content. The optimum temperature and pH are 40 centigrade degree and 10.4 respectively. **Conclusion:** Human hydatidiform mole alkaline phosphatase is a novel alkaline phosphatase and it is different from the others.
P175.  Relationship between levels of homocysteine in diabetic & nondiabetic patients with coronary artery disease
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Background: Atherosclerosis manifest earlier and more extensive in severity among diabetic patients. It seems homocysteine is one of the accelerating factors in development of atherosclerosis and serum levels homocysteine increase in patients with coronary artery involvement. The aim of this investigation was to compare homocysteine levels between diabetic and non-diabetic patients with coronary artery disease (CAD).

Methods: As a case-control study via non-randomized sampling method we collected 33 diabetic CAD patient and 34 CAD patient with normal GTT test. All participants were matched age, sex, family history of atherosclerosis, blood pressure, smoking, the level of triglyceride, total cholesterol, LDL-C and body mass index (BMI). Fasting serum homocysteine was measured via ELISA technique and other selected variables also were assessed via conventional laboratory methods. The quantitative variables between two groups analyzed by the Student’s t-test, while the qualitative variables examined by x 2. We used Pearson-Spearman test for correlation analysis between two groups.

Results: 33 diabetic and non-diabetic CAD/MI cases were participated. Among diabetic and non-diabetic subjects, mean age was 59±10.5 and 62.5±9 respectively. The mean duration of diabetes was 5.4±4.1 years. Comparison of the mean homocysteine levels between two groups revealed a significant difference, 16.2±4.8 mmol/L in diabetics vs 10.6±4.8 mmol/l in non-diabetics (P>0.5).

Conclusion: Fasting serum homocysteine in diabetic CAD subjects were higher than non-diabetic CAD. Our findings may suggest that the increased level of homocysteine is correlated to high prevalence and severity of atherosclerosis among diabetic individuals.

P176.  Blood-borne infections
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Although the blood supply is already extremely safe, there are still concern regarding the potential transmission of blood-borne infections. Data from studies indicate that the risk of receiving unit of infectious blood is less than 1:500,000 for HIV, 1:100,000 for hepatitis C and 1:70,000 for hepatitis B. The donor history provides the first major level. Using a closed System for collecting blood that cannot be tampered with or re-used is also one more step in ensuring the safety of the blood collected. Viral serology and NAT testing for the various transfusion transmitted disease including HIV 1/2, HTLV 1/2, hepatitis B and C provides the second major level of protection. Factors that can contribute to viral transmission through blood transfusion include the window period, antibody negative chronic carries, mutant infection and laboratory error. Nonetheless, blood units are not routinely screened for serologic markers of HHV-6, HHV-8 or SEN-V infection. A third major tier of safety currently being developed is pathogen inactivation technology by addition of various additives to blood products to inactivate viruses, bacteria, fungi, protozoa and other transfusion transmitted pathogens. None of the currently available systems are to inactivated prions such as variant Creutzfeld-Jakob disease or viruses such as HAV or HEV infection. It has been reported that pre-storage leukodepletion of blood components may be as seronegative blood components for viruses or parasites such as CMV, EBV, Toxoplasmosis or Chagas disease.
P177. Detection of *Pseudomonas aeruginosa* producing metallo-beta-lactamase in burns patients.

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The emergence of metallo-beta-lactamase (MBL) producing pathogens is an increasing therapeutic problem. These enzymes have a broad substrate spectrum, they hydrolyse all beta-lactams except for the monobactam aztreonam. The aim of this study was to determine MBL presence in clinical isolates of *Pseudomonas aeruginosa*. During the period from October 2005 to July 2006, 100 *P. aeruginosa* nonduplicate isolates were obtained from burn patients. Forty-one strains were selected because of resistance to imipenem. We studied the sensitivity to different antibiotics in these strains. Meropenem and Piperacillin were the most active antibiotics. To determine the presence of MBL, we used the Etest with imipenem/imipenem plus EDTA. Among imipenem resistant *P. aeruginosa* strains, 8 were found to be metallo-beta-lactamase producers. These eight strains were positive for the blaVIM gene as determined by a polymerase chain reaction method.

P178. Correlation between peripheral blood CD34 count by flowcytometry and immature reticulocytes fraction measurement by automated haematology analyser

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**Background:** Autologous PBSC transplant is now increasingly being used as an alternative to autologous bone marrow transplantation because of rapid hematologic recovery, reduced requirement of antibiotic and blood transfusion and shorter hospitalization. To collect a sufficient number of progenitor/stem cells for rapid engraftment, it is critical to optimize the harvesting procedure, especially the timing of stem cell collection. Serial enumeration of CD34⁺ cells in peripheral blood (PB) before apheresis is currently being used to time stem cell collection. But flow cytometric techniques used are complex and expensive. We evaluate a low cost, less complex method of measuring Immature Reticulocytes Fraction (IRF) in PB as an alternative approach. **Methodology:** Thirty chemotherapy-induced mobilizations from 21 patients with hematological malignancies who were scheduled for autologous PBSC transplant were assessed. IRF and peripheral blood (PB) CD34⁺ cells were measured daily from day 10 to 17 post chemotherapy. The correlations between PB CD34⁺ cell and IRF on 2 days before or on the day of stem cell harvesting as well as correlation between PB CD34⁺ cells or IRF 2 days before or on the day of stem cell harvesting and stem cell yields were evaluated. **Results:** IRF showed high variability post chemotherapy. IRF did not correlate with circulating PB CD34⁺ cells or the yield on the first day of stem cell harvesting. PB CD34⁺ cells show a rise and fall pattern post chemotherapy. PB CD34⁺ cells were correlated significantly with the number of CD34⁺ cells per kg of patient’s body weight in the apheresis product. (Spearman’s rho r² = 0.77, p < 0.021). **Conclusion:** IRF measurement post chemotherapy is not useful to predict stem cell harvest. PB CD34 quantification remains useful to predict stem cell harvest.
P179.  Cytology of neuroendocrine carcinoma of the breast: A case report

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Neuroendocrine carcinomas of the breast are uncommon tumors known to occur in the elderly. While focal neuroendocrine differentiation may be noted in many ductal and lobular carcinomas, the term neuroendocrine carcinoma is to be applied when more than 50% of the tumor shows such differentiation. This case report details the cytological features of neuroendocrine carcinoma that was encountered in our hospital. The fine needle aspiration (FNA) smears showed discohesive polygonal cells with abundant cytoplasm, many of which contained eosinophilic granules located at one pole. The background showed several cells with stripped nuclei (bare nuclei). Histology of the mastectomy and axillary lymph nodes specimen from this patient showed features of neuroendocrine carcinoma - solid type, with metastasis, confirmed with immunohistochemistry. The patient is disease free seven months after surgery. This case highlights the need to observe cytological details to identify this rare tumor that may otherwise appear to be infiltrating duct carcinoma: not specified type, on FNA.

P180.  Serum hepcidin in rheumatoid arthritis

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Introduction: Hepcidin is considered the central regulator of iron metabolism. It has been proposed as a factor regulating the uptake of dietary iron and its mobilization from macrophages and hepatic stores. Hepcidin is considered as a mediator of anaemia of inflammation. Aim: To assess whether serum hepcidin concentration is able to distinguish iron deficiency from anaemia of inflammation in patients with rheumatoid arthritis (RA). Methods: Blood samples were obtained from 20 healthy donors, thirty RA patients who presented with anaemia and thirty patients who had pure iron deficiency anaemia (IDA). The samples were analysed for full blood count, iron, ferritin, transferrin, soluble transferrin receptor and prohepcidin. Results: The mean hepcidin prohormone level in the control subjects was 255.73 ng/ml. Hepcidin level was significantly lower in IDA patients (99.7 ng/ml) (p<0.05). Higher sTfR levels were observed in IDA (p<0.05) RA patients were divided into iron depleted and iron repleted subgroups based on the ferritin level. Hepcidin in the iron depleted group was significantly lower than the iron repleted group and the control. sTfR levels in iron depleted group were significantly higher than the control and the iron repleted patients (p<0.05). Conclusion: It is clear from our study that reduced serum hepcidin and increased sTfR indicates iron deficiency. However, a few patients in the iron repleted RA subgroup who had raised sTfR, did not have reduced hepcidin. Further evaluation with a larger cohort of patients is required to assess the usefulness of hepcidin in differentiating iron deficiency from anaemia of inflammation.
P181. Complexed prostate-specific antigen for the detection of prostate cancer

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Introduction: Prostate cancer is the most common malignancy in men. Prostate specific antigen (PSA) is useful marker for the screening, diagnosis and follow-up of patients with prostatic carcinoma. PSA exists in two forms in the blood: free prostate specific antigen (fPSA) and complexed prostate specific antigen (cPSA), which is bound to proteins. Total prostate specific antigen (tPSA) is the measurement of free and complexed PSA. Studies have shown that cPSA has higher specificity in detecting prostate cancer than the commonly-used total prostate specific antigen (PSA) test.

Aim: To evaluate the diagnostic use of total, free and complexed prostate specific antigen, and their ratios in detecting prostate cancer.

Methods: Serum samples were collected from 81 men with prostate disease. Only patients who had tPSA <20ng/ml were included for the study. All these patients had diagnosis confirmed by prostate biopsy or prostate chips from transurethral resection. 19 patients had carcinoma and 62 patients had no malignancy. Total PSA and cPSA were measured using ADVIA Centaur® Immunoassay System; fPSA levels were calculated from tPSA and cPSA.

Results: The mean levels of cPSA (3.6 ± 4.3 ng/ml) in malignant patients were not statistically different from those with no malignancy (4.77 ± 3.14ng/ml) (p>0.05). However, significantly lower fPSA levels (p<0.05) and fPSA/tPSA ratio (p<0.001) were observed in prostatic adenocarcinoma.

Conclusion: The measurements of fPSA and fPSA/tPSA ratio are more useful compared to that of tPSA and cPSA levels in differentiating prostatic cancer from non-neoplastic prostatic lesions.

P182. Bcl-2 expression in invasive ductal carcinoma of the breast in the north-east of Malaysia

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Bcl-2 is an anti-apoptotic protein belonging to a family of proteins that act as regulators of apoptosis in mammalian cells. Bcl-2 expression has previously been reported in normal breast ductal cells and its involvement in the hormonal regulation of hyperplasia and involution was further suggested, and it was thought to be expressed through hormone-dependent pathways. Bcl-2 is a cytoplasmic oncoprotein which is highly expressed in human solid tumours. In breast cancer cells, however, bcl-2 expression is down regulated, the exact mechanism and the effects of which are not clearly defined, as bcl-2 expression appears to be inversely correlated with the presence of p53 mutations. This work aimed at investigating the expression of bcl-2 in invasive ductal carcinoma of the breast utilizing an immunohistochemistry assay as well as studying the clinical correlations of bcl-2. Survivin was detected in 43.7% of 382 IDC study cases. Its expression correlated positively, with lower age of patients, higher histological grades, large tumour sizes, estrogen receptor positivity and progesterone receptor negativity. However, the statistical correlations were rather weak. With the data obtained, bcl-2 detection alone may not be very helpful in the clinical diagnosis. However, it may be useful in the clinical judgment regarding the treatment protocol, and the prognosis.
P183. Localisation of carcinoembryonic antigen, vimentin and estrogen receptors in discriminating primary cervical adenocarcinoma from endometrial adenocarcinoma.

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The aim of the study is to find out whether immunohistochemical (IHC) localization of carcinoembryonic antigen (CEA), vimentin (VN) and estrogen receptor (ER) can distinguish endocervical adenocarcinoma (ECA) from endometrial adenocarcinoma (EMA) in our materials. 46 cases each of histologically diagnosed cases of ECA and EMA from the files of Pathology Departments of Hospital USM and Hospital Kota Bharu during January 1994 to September 2006 were included. IHC stain employing primary mouse monoclonal anti-human IgG antibodies, DAKO: carcinoembryonic antigen (clone II-7), antivimentin antibody (clone Vim 3B4) and estrogen receptor (clone ID54), were performed using DAKO Envision+ system –HRP (DAB) and slides examined separately by one pathologies and then together by all 3 pathologists. Cytoplasmic and/or apical cell membrane staining for CEA was taken as positive. VN positive was defined as crisp perinuclear staining. For ER, distinct nuclear staining was taken as positive. The staining was graded as - , +, ++ & ++++. Histological types of ECA included 18 mucinous, 15 endometrioid, 11 villoglandular, 1 adenosquamous and 1 clear cell carcinoma respectively. Materials consisted of 35 cervical biopsies and 11 hysterectomies. IHC stain for CEA was positive in 42 cases of ECA, 30 of them strongly. VN was negative in 41 and estrogen receptor in 44. Of 46 cases of EMA, 31 were abdominal hysterectomies with bilateral salpingo-oophorectomy and 15 endometrial curettage. The histological types included 43 endometrioid and 3 villoglandular variant. Immunostain showed negative CEA in 38, strong positive in 1 and weak positive in the rest. Vimentin was positive in 41 and ER in 36. Statistical analysis of the result of the 3 stains in the 2 conditions were significant. This study confirms that monoclonal immunoglobulin IgG, CEA, VN and ER are valuable in the distinction of endocervical adenocarcinoma from primary endometrial adenocarcinomas.

P184. A clinicopathological study of nine cases of gallbladder carcinoma in 1122 cholecystectomies in Johor, Malaysia

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Nine cases of primary carcinoma of gallbladder were analysed in an audit of 1122 cholecystectomies for a 6-year period from 2000 to 2005. There were 6 females and 3 males between ages 27 to 81 years. Only 2 out of 9 cases were clinically suspected of carcinoma pre-operatively while 3 cases were misdiagnosed as acute or chronic cholecystitis, two as cholelithiasis, one as intestinal obstruction and the other as ovarian cyst. Another four more cases were suspected to be gallbladder carcinoma intraoperatively. The remaining 3 cases which were not suspected of carcinoma had multiple gall stones. Altogether only 5 out of 9 cases (55.56%) of gallbladder carcinoma were associated with gallstones. Six cases of gallbladder carcinoma (66.67%) had abnormal macroscopic lesions noted; either as with papillary lesions or polypoid mass. The remaining 3 cases had thickening of the wall, consistent with chronic cholecystitis. Seven cases were found histologically to be adenocarcinoma, two of which were papillary carcinoma and one of signet ring cell type. One case of squamous cell carcinoma and one case of adenosquamous carcinoma were noted. This study highlights the importance of careful macroscopic and microscopic evaluation of a routine pathological examination of gallbladder removed for cholecystitis or cholelithiasis. It also shows the incidence of gallbladder carcinoma in patients who have undergone cholecystectomies in a large government hospital in Johor.
P185. The role of 38 kDa adhesin protein Mycobacterium tuberculosis in induction of secretory Immunoglobulin A secretion of bronchiolar and intestinal mucus BALB/c Mice

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Background: While BCG vaccination has remained a mainstay of control programs for more than half a century, no vaccination campaigns have prevented the recent resurgence of tuberculosis (TB). One of the initial and crucial events in any infectious process is the adherence of the microorganism to its target tissues. This attachment is due to virulence structures, called adhesins, that are produced on the surfaces of pathogenic bacteria. The mucosal immune system is a complex and redundant system that generates large amounts S-IgA. The best defense against these predominantly mucosal pathogens would be vaccines, preferably mucosal vaccines capable of inducing both systemic and mucosal immunity. The aim of this study was to determine the role of adhesin of M. tuberculosis in induction of S-IgA secretion into intestinal and bronchiolar mucus.

Methods: In first study, we can isolated haemagglutinin protein of M. tuberculosis B665BT, and then prove that haemagglutinin protein M. tuberculosis B665BT is adhesin protein. We could isolated and determined molecular weight of adhesin protein of Mycobacterium tuberculosis, that was 38 kDa. So in study II, we used this protein in our in vivo experiment. We have challenged BALB/c mice with this adhesin per-oral with/without CTB adjuvant. After 2 weeks, S-IgA in intestinal and bronchiolar mucous of mice that had been vaccinated with adhesin and/or CTB ajuvant and control group, were measured by ELISA and immunohistochemistry.

Results: There was significantly difference between the absorbances of S-IgA intestine of control (mice without adhesin per-oral) and mice with adhesin with or without CTB. The same result was provided in bronchiolar mucous. Immunohistochemistry showed increased level of S-IgA in mice mucosal intestine.

Conclusion: The 38 kDa adhesin protein of M. tuberculosis B665BT could induce S-IgA from mucosal intestine and bronchiolus peroral.

P186. The prevalence of nutritional anaemia in pregnancy at Hospital Universiti Sains Malaysia

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Introduction: Anaemia in pregnancy continues to be a common clinical problem in many developing countries and significantly contributes to maternal mortality and both maternal mortality and fetal morbidity. Objectives: The objectives of this study is to determine the prevalence of anaemia in pregnancy and to study specifically the prevalence of iron, folate and vitamin B12 deficiency during pregnancy in Hospital Universiti Sains Malaysia. Methodology: A cross sectional study of pregnant women attending the antenatal clinic at HUSM was conducted. A systemic random sampling method was applied and 278 patients were included in the study. Full blood count, serum ferritin, serum folate, red cell folate and vitamin B12 levels were determined by automated haematology analyzer and access immunosystem. The results were analysed using SPSS version 11.5. Results: The means of age, period of amenorrhoea and gravida were 31.1 years, 20.67 weeks, and 3 pregnancies respectively. Sixty five percent of the study population were in the second trimester and 62.2% were multigravida. The mean of haemoglobin level was 11.2g/dl. The prevalence of anaemia was 37.1%. The means for serum ferritin, serum folate, vitamin B12 and red cell folate were 17.7 ug/L, 22.9 nmol/L, 282.8 pmol /L and 944.7 nmol/L respectively. Pregnant ladies having a serum ferritin less than 12 ug/L constituted 37.4%, while those with a serum ferritin between 12-50 ug/L were 47.6%. Conclusion: The prevalence of anaemia in pregnancy is similar to previous studies despite the improvement of the standard of living. Iron deficiency anaemia is still the commonest compared to folate and vitamin B12 deficiency.
P187. **Fibrinolytic markers in acute stroke patients: HUSM experience**

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**Introduction:** The fibrinolytic system plays an important role in preventing intra-vascular thrombosis. Previous researchers associate the stroke occurrence with the abnormality in fibrinolytic system. This study was done to compare the levels of three fibrinolytic markers i.e. plasminogen (plg), tissue-plasminogen activator (t-PA) and plasminogen activator inhibitor type-1 (PAI-1) between acute stroke patients and stable non-stroke individuals and to investigate the clinical significance of these markers. **Methods:** This study was conducted in HUSM for one-year period from March 2005 to February 2006. Stroke patients were selected from adult wards whereas control individuals were chosen from various clinics. One hundred and six individuals and 51 acute stroke patients were selected. Both groups have similar risk factors. Their blood were tested for the level of t-PA and PAI-1 using ELISA technique (Biopool TintElize) whereas plasminogen level was tested with colorimetric assay using Hemosil Tm. They were follow-up over a period of 3 months to detect their survival and recovery. **Results:** We found only t-PA level was significantly higher in acute stroke patients compared to control group even after adjusting the cofounders using ANCOVA test. There were no significant statistical association between the three fibrinolytic markers and age, gender, number of risk factors, disease severity, survival and neurological recovery. We observed all the eight patients who died during hospitalization or at the time of follow-up possessed high level of t-PA although statistically not significant. **Conclusion:** High t-PA level indicates abnormal intravascular fibrinolysis which is probably an initiator of the cerebrovascular event or indicating of underlying endothelial damage. This finding supports the hypothesis that disturbances in fibrinolysis occur in stroke patients during cerebrovascular event. An association between high t-PA antigen level and stroke with a 4.6-fold odd ratio was found. t-PA antigen could be a marker to predict high-risk patients for stroke development. A bigger study including baseline t-PA level is needed to confirm this finding.

P188. **A novel human serum α-mannosidase: determination of molecular weight, chemical modification and effect of metal ions**

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We purified a novel α-mannosidase activity from human serum by initial ammonium sulphate precipitation, preparative gel filtration on Biogel P-100 and affinity chromatography on Con A-Seralose. These steps resulted in a purification of 13846-fold, a yield of about 35.1%, and a specific activity of 2160 U mg−1 protein. The purified enzyme (apparent pH optimum 4.2; temperature optimum 40 degrees C). α-mannosidase was purified to electrophoretic homogeneity; analysis of the protein by SDS–PAGE revealed three subunits with molecular masses of 186.2, 125.3 and 112.2 kDa. The proteins contain α-mannosidase were eluted from the column using concentration gradient of glucose / methyl -D-glucopyranoside / methyl -D-glucopyranoside in ethylene glycol (40%, v/v). Homogeneity of the enzymes was confirmed by gel filtration and polyacrylamide gel electrophoresis [PAGE]. Chemical modification studies revealed the involvement of tryptophan for enzyme activity. The enzyme was found to depend on the presence of Zn +2 and Mn +2.
P190. Diagnosis of Helicobacter pylori infection in noninvasive stool samples by PCR-ELISA method

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Introduction: Helicobacter pylori are a major bacterial pathogen in human, which is associated with peptic ulcer disease, gastritis, duodenitis, and non ulcer dyspepsia. A reliable test to detect etiological agent is crucial. None of the test available is suitable for all situations, because each having its own drawbacks and pitfalls. Several attempts have been made to culture H. pylori from stool, but there is very few report of its isolation from culture. Recently, PCR-ELISA technique was tested for diagnosis of some bacterial pathogens. Objective: The aim of this study was developed specific and Sensitive PCR-ELISA assay for detection of H. pylori in stool samples. Materials and Methods: 67 samples were collected from patients with gastrointestinal endoscopies referred to Hazrat Rasoul Akram Hospital, IUMS, Tehran, Iran. The patients Stool and biopsy were used for extraction of DNA followed by amplification, ureC gene of H. pylori by using PCR technique. Simultaneously the PCR-ELISA was carried out for all the samples. Results: Of 67 patients stool samples and biopsies specimens, 31 (46.1%), 34 (50.7%), and 42 (62.6%), 47(70.1%) were showed positive H. pylori by PCR and PCR-ELISA respectively. Conclusion: The result of this study revealed that detection of H. pylori in stool sample by PCR-ELISA having high specificity and it can be used as a primary detection of organism.

P191. The antigen expressions of BCL-2, BCL-6, CD10, CD138 and MUM-1 in diffuse large B-Cell lymphomas: A cohort study

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Diffuse large B-cell lymphoma (DLBCL) is the most common type of malignant non-Hodgkin’s lymphoma. It can be further divided into two subgroups: germinal centre B-cells (GCB) derived DLBCL and non-GCB derived DLBCL based on cDNA microarray studies and immunohistochemical staining profiles. The aim of this study is to investigate the correlation of DLBCL immunophenotype patterns among Malaysian patients towards the survival rate. A total of 84 DLBCL cases were studied retrospectively for expression of BCL-2, BCL-6, CD10, CD138 and MUM-1 by immunohistochemistry and statistically analysed by SPSS programme. Of these cases, 33 (39.3%) were found to be GCB type and the others (60.7%) non-GCB type. Among these cases, 45 were made available for survival rate analysis. Patients with low and low intermediate International Prognostic Index (IPI) score showed better overall survival (OS) (p = 0.009), but not for disease free survival (DFS) (p = 0.137). The expression of BCL-2 protein did not influence the OS (p = 0.949) and DFS (p = 0.851). In contrast, expression of BCL-6 showed lower OS (p = 0.027) but not DFS (p = 0.118). Our study showed that there is no significant difference in the OS and DFS in the two groups of patients with GCB and non-GCB histological type, which differ from cDNA microarray data.
P192. The distribution frequency of hMLH1, hMSH2 and hMSH6 loss in colorectal cancers from a cohort of multiethnic patients

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The mismatch repair (MMR) protein is one of the frequent inactivated events occurring in colorectal carcinogenesis. This study was undertaken to evaluate the loss of MMR protein expression in a cohort of multiethnic patients. A total of 163 colorectal cancer cases were analysed for hMLH1, hMSH2 and hMSH6 protein expressions by immunohistochemistry and findings were correlated with patients’ demographic data and pathological stage. The majority of our cases were male (88/163, 54.0%), Chinese (103/163, 63.2%), tumour located on left-sided colon (128/163, 78.5%), presenting in stage Duke’s B (67/163, 41.1%) and with Grade 2 (moderate differentiation) histomorphology (135/163, 82.8%). As expected, poor differentiation was significantly associated with mucinous histology (p = 0.000). Young age group (<55 years) was significantly associated with poorly differentiated (p = 0.005) and mucinous tumours (p = 0.000). The individual hMSH6 protein loss was the highest with 34 cases (20.9%) followed by hMSH2 (16/163, 9.8%) and hMSH1 (15/163, 9.2%). We found that 27.0% (44/163) of the cases had at least one MMR protein loss. Meanwhile, hMSH2 and hMSH6 double defects were significantly associated with mucinous adenocarcinoma (p = 0.032) and non-Malay (p = 0.043). There were only four cases with total MMR proteins loss. Analysis of MMR protein expressions did not reveal major difference from this cohort of multiethnic patients. We suggest that colorectal tumours of the mutator pathway may be heterogeneous and uniformly distributed in both gender, age and ethnic groups.

P193. The Papanicolaou smear: Concurrence and inter-observer variability using the Bethesda System among pathologists in the Philippines

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Objective: The study aims to determine the cytologic and cytohistologic concurrence and inter-observer variability among eleven general pathologists and one cytopathologist. Methodology: Thirty nine conventional cervicovaginal smears with subsequent cervical biopsy were randomly reviewed by eleven general pathologists and one cytopathologist of a tertiary government hospital. Cytologic and cytohistologic concurrence were determined. Kappa index was computed. Inter-observer variability was calculated as 100 % minus inter- observer agreement. Results: There was concurrence among pathologists in eight smears. Of 31 remaining smears, 3 were miscategorized as epithelial cell abnormalities, the rest showed variability in assessment of non- neoplastic findings with 15 cases overdiagnosed as having inflammation, 5 as shift in vaginal flora, 5 as atrophy and 3 as unsatisfactory for evaluation. Cytohistologic concurrence was 75% ( 29 of 39 ) for general pathologists and 82 % ( 32 of 39 ) for the cytopathologist with a kappa index of 0.280 ( poor concordance ) and 0.425 ( moderate concordance ), respectively. Inter- observer variability was highest in assessment of specimen adequacy ( 96 % ) followed by diagnosis of reactive cellular changes due to inflammation ( 80 % ), NILM ( 58 % ), shift in vaginal flora ( 42 % ), ASCUS ( 42 % ), LSIL ( 42 % ), HSIL ( 8 % ), and atrophy ( 0 % ). Eighty five percent of discrepant diagnosis was one category away from the correct cytodiagnosis. Conclusion: The low concurrence and significant inter- observer variability among general pathologists and cytopathologist could be attributed to differences in application and interpretation of criteria in three categories of The Bethesda System including assessment of specimen adequacy. A review of these criteria and strict adherence to objective qualifiers and details in subcategories should be implemented to improve cytodiagnosis.
P194. Is there any relation between grade in transitional cell carcinoma (TCC) and expression of Epidermal Growth Factor Receptor (EGFR)?

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Background: The present study was undertaken to investigate the correlation of Epidermal Growth Factor Receptor (EGFR) expression with grade of Transitional Cell Carcinoma (TCC). Methods: Tumor samples of 75 patients from Mostafa Khomaini Hospital with Transitional Cell Carcinoma of the bladder were analyzed by immunohistochemistry for expression of EGFR. In this context, we assigned the bladder tumors a grade according WHO classification. Results analyzed for possible correlation with the expression status of the Epidermal Growth Factor Receptor (EGFR). Results: This cross-sectional study showed that all grades of Transitional Cell Carcinoma expressed EGFR, and 14 cases were LMP (18.9%) which 10 cases among them had negative cells according EGFR point of view (71.4%) and 4 cases had reported positive (28.6%). Thirty five cases were low grade (46.7%) which 18 cases among them had reported negative cells (51.4%) and 17 cases had positive cells (48.6%). Twenty six cases were high grade (34.7%) that 9 cases among them had reported negative cells (34.6%). Seventeen cases had positive cells (65.4%). Mann-Witney test showed relation between grade and expression of EGFR (P<0.05). Conclusions: Our study showed that expression of EGFR is correlated with grade of tumor.

P195. Male breast cancer in Iran

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Carcinoma of the male breast is a rare disease and it is reported as, less than 1% of all breast cancers. A high incidence, in a lower average age, with disease presentation in more advanced stage is reported in native Africans and Indians. Predisposing factors include family history (in first degree female and male relatives), hormones (particularly high estrogen and prolactin levels), radiation exposure, hereditary factors and diseases associated with hyperestrogenemia like cirrhosis of the liver or genetic syndromes, such as Klinefelter disease. Mostly such patients are in their late sixties and a palpable lump is the main presentation. Infiltrating ductal carcinoma is the main histological type reported in world literature. Latest data from the National Cancer Registry showed an age-standardized incidence rate (ASR) of 0.48 for male breast cancer in Iran. Meanwhile, of 125 male breast cancer cases in Iranian men during 2004, the most common cases were Infiltrating Ductul Carcinoma, NOS (%88). Studies showed the seventh decade of male’s life is the most prevalent age group for male breast cancer in Iran. This paper will compare epidemiologic finding of breast cancer in Iran as compare as other countries.

P196. In vivo and in vitro study of Iranian garlic on Salmonella typhimurium

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Introduction: Salmonellosis is considered as a major hazard to the health and food poisoning is one of the most commonly related by Salmonella typhimurium. In this study, we studied in vivo and in vitro effects of aqueous extract of Iranian garlic on Salmonella typhimurium. Methods: The aqueous extract of garlic had prepared with the concentration of 20gr/lit. After prescribing a concentration of $10^9$ of Salmonella typhimurium per one milliliter on culture media to 60 rabbits, the symptoms such as
fever and diarrhea were developed. Treatment of involved rabbits by garlic is done three times in the
day for duration of six days. After sampling from animal’s stool in times 0, 24,48,72,96,120 and 144
hour, bacterial colonies were counted in culture media. In other part of this study the zone inhibition
of garlic were measured. Results: The statistical analysis by ANOVA stated that aqueous extract of
garlic could decrease bacterial colonies in culture media.

P197. Epidemiology of breast cancer in Iran
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Breast cancer is the commonest cancer in women in most parts of the world. In Asia, there has been a rapid
increase in the incidence of breast cancer in recent years, and the disease may occur at a relatively young
age. As well as the large variation in the incidence, there is also a wide variation in the mortality and survival
between different countries and regions and also within specific populations. Many complex factors
underlie these variations, including population structure (age, race, and ethnicity), lifestyle, environment,
socioeconomic status, risk factor prevalence, mammography use, disease stage at diagnosis, and access to
high-quality care. Further research is needed to fully understand the reasons for variation in breast cancer
outcomes and to aid in the development of tailored strategies to improve outcomes in general as well
as the standard of care for underserved populations and reduce the burden of breast cancer worldwide.
Data from the National Cancer Registry of Iran showed that breast cancer is the commonest cancer in the Iranian women. Data showed an age-standardized incidence rate (ASR) of 18.2 for breast cancer in Iran. Meanwhile, of 4557 breast cancer cases in Iranian women, the most common cases were Infiltrating Ductal Carcinoma, NOS. In all provinces, breast cancer is the most prevalent cancer in women. Moreover, distribution of breast cancer in all provinces is almost the same. Other studies showed the forth decade of women’s life is the most prevalent age group for breast cancer in Iran. This paper will compare epidemiologic finding of breast cancer in Iran as compare as other countries.

P199. Gene expression profiles of Malaysian breast carcinoma with distinct tumour grades
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Breast cancer is the third most common form of cancer worldwide, after lung and stomach cancer, and
is the most common cause of cancer death in women. In Malaysia, breast cancer is the commonest
cancer in women amongst all races from the age of 20 years and it formed 31% of newly diagnosed
cancer cases in women in 2003. The current hypothesis of tumourigenesis suggests that most invasive
breast carcinoma appear to develop gradually from defined precursor lesions. Thus, the gene expression
profiles of the distinct pathological stages of breast carcinoma, such as atypical ductal hyperplasia
(ADH), ductal carcinoma in situ (DCIS) and invasive ductal carcinoma (IDC) are highly similar to
each other at the level of the transcriptome. In other words, the genetic changes in primary tumours
that favor metastasis occur early in tumour progression. Regardless of pathological stages, many have
reported that tumour grades also play a crucial role in predicting survival and prognostic significance
of breast cancer patients although the molecular events underlying distinct histological grades in breast
cancer is still unclear. In our present study, we are interested to determine gene expression profiles in
different histological defined tumour grades. We used Affymetrix HG-U133A Genechip, to monitor
gene expression profiling of 22,283 probe sets in 40 differently defined breast tumour grades and 40
patient-matched normal controls. We identify a subset of genes that could distinguish between different breast tumour grades. Perhaps, these genes could be used as molecular grading system for Malaysian breast cancer patients, which allowed for greater precision in classifying breast cancer if compared to the currently available conventional grading method.

P200. Insulin-like potential of Annona reticulata leave aqueous extract

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Diabetes mellitus (DM) is a chronic metabolic disorder primarily characterized by hyperglycemia. DM usually occurs due to defects in insulin secretion, insulin action or both. In this study, the adipogenic, lipolytic and glucose uptake activities of Annona reticulate (custard apple) leaf aqueous extract in primary rat adipocytes were assessed using Oil Red O quantitative assay, glycerol quantitative assay, and 2-deoxy-D-[2,6-3H]glucose uptake assay respectively. Traditionally, the leaves of A. reticulata have been used for the treatment of diabetes. A. reticulata exerted a biphasic effect on adipogenesis in the concentration range of 0.1 µg/ml to 1000 µg/ml. At 0.1 µg/ml, A. reticulata exerted the highest adipogenic activity (195.5 %), whereas insulin (1 µM) only caused 119.5 % adipogenesis compared to untreated cells. When A. reticulata (0.1 µg/ml) was co-incubated with insulin (1 µM), additive adipogenesis was observed. Similar concentration range of A. reticulata also exerted a biphasic effect on epinephrine (1 µM) induced lipolysis. At a concentration of 100 µg/ml, A. reticulata suppressed 80.7 % whereas insulin (1 µM) suppressed 38.7% of epinephrine (1 µM) induced lipolysis. A. reticulata (0.1 µg/ml) caused 500.8 % increase in glucose uptake and in the presence of insulin (100 µM), additive increase in glucose uptake was observed. Insulin and several common oral antidiabetic drugs for example rosiglitazone and pioglitazone are also known to have similar activities in promoting adipogenesis, inhibit epinephrine induce lipolysis as well as stimulate glucose uptake in primary rat adipocytes. The present study shows that A. reticulata is a potential ‘insulin-mimicking’ agent since it significantly induced adipogenesis and glucose uptake in primary rat adipocytes but inhibits epinephrine induced lipolytic activity.

P201. Non-invasive assessment of oxidative stress status in breast and colorectal cancer patients

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Oxidative stress which occurs when excessive production of free radicals overwhelms the antioxidant defense system has been implicated in the etiology of cancer. In Malaysia, breast cancer is the most prevalent cancer among women whereas colorectal cancer is the commonest cancer among men. This study was conducted to investigate the levels of oxidative indices and the levels of non-enzymatic antioxidants in breast and colorectal cancer patients. Various studies have reported on lipid peroxidation (LP), hydrogen peroxide (H2O2) and ferric-reducing antioxidant power (FRAP) levels in the serum of cancer patients. To the best of our knowledge, we are the first to highlight on the significance of urinary advanced oxidative protein product (AOPP) in cancer patients. In this study, the levels of AOPP, H2O2, LP and FRAP were measured in urine samples from breast and colorectal cancer patients (n=101 and 49 respectively) attending the Oncology Clinic, University Malaya Medical Centre and compared to that of 95 age-matched normal healthy individuals (volunteers from the Klang Valley). The levels of AOPP, H2O2 and LP were significantly higher.
P202. Comparison of the validity of three biomarkers, carcinoembryonic antigen, pepsinogens, and high sensitive c-reactive protein for gastric cancer screening

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To investigate the desirable screening serum marker for gastric cancer, we evaluated the validity of three biomarkers, carcinoembryonic antigen (CEA), pepsinogens (PGs), and high sensitive C-reactive protein (hsCRP) in asymptomatic and symptomatic people in Korea. We estimated the mean serum levels of CEA, PGs, and hsCRP and compared the sensitivity and specificity of these three biomarkers in total 378 subjects who were classified into seven groups; normal, chronic atrophic gastritis (CAG), intestinal metaplasia (IM), adenoma, early gastric cancer (EGC), advanced gastric cancer (AGC) without metastasis, and AGC with metastasis (M1). In CEA and hsCRP, there was no significant difference among normal, high-risk groups (CAG, IM, adenoma), and EGC. However, CEA was relatively higher in AGC with intestinal type and far-advanced cancer (M1). Combination of serum hsCRP with PG I/II ratio would be helpful as a screening tool for gastric cancer in high incidence area and pave a way to select high-risk subjects that need further specific invasive screening tool such as endoscopy.

P203. Analysis of peripheral blood markers in diabetic retinopathy

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To evaluate the clinical usefulness of peripheral blood biomarkers for prediction of diabetic retinopathy (DR), peripheral blood level of various biomarkers were measured in diabetes mellitus (DM) patients with or without DR. Samples of peripheral blood were analyzed for biochemical markers in 98 DM patients and 41 healthy subjects. The biochemical profiles of blood were analyzed such as glucose, BUN, creatinine, lactate dehydrogenase, total cholesterol, HDL-C, LDL-C, LDL diameter, triglyceride and various cytokines such as IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, EGF (epidermal growth factor), VEGF (vascular endothelial growth factor), INF-β, TNF-α, MCP-1 (monocyte chemoattractant protein-1). The results suggested the DM duration was an important risk factor of DR prediction. Serum glucose, total cholesterol, and LDL-cholesterol level would be helpful to evaluate DR in DM patients. In addition, IL-6, TNF-α, and small LDL diameter would be useful for prediction of PDR in DM patients with DR.

P204. Changes in liver contents of lipid fractions following titanium treatment

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The potential to cause tissue damage by metal ions is the subject of widespread investigation. Various studies have demonstrated the role of reactive oxygen species in the toxification of trace elements in exposed animals. One of these elements with oxidation potential is titanium (Ti). Ti salts are widely used in industry for ceramic painting, in pharmacy for tablet coating and making chemical sunscreens and in medicine as photocatalysts with bacteriocidal activity. This may address the idea that the exposure to these salts could play a role in metabolic disorders. In this study the effect of Ti on liver contents of lipid fractions were investigated. Male wistar rats (200-250g) were used for the experiments. Groups of animals were injected for 10 days with 2.5 mg/kg of titanium.
chloride, as acute dose and for 30 and 60 days with 0.75 mg/kg as chronic doses. At the end of the experimental period animals were anaesthetized, the abdomens were opened and the livers were perfused with appropriate buffer. Livers were then removed immediately and used fresh or kept frozen until analysis. They were homogenized and their contents of triglycerides and phospholipids were determined. Blood samples were also collected before killing to measure the lipid parameters. Titanium led to a significant increase in phospholipid content of the liver (about 66%) whereas triglyceride decreased by about 26 to 30 percent in all treated animals. Titanium also reduced plasma free fatty acids and triglycerides significantly but cholesterol and LDL levels were increased. Lipoprotein lipase activity was also inhibited in titanium treated animals. This study is significant because it shows that chronic inhalation or exposure to titanium at workplaces is associated with changes in liver lipid metabolism. Plasma lipid related parameters were also affected. Although less information is available concerning the mechanism of toxicity, the induction of reactive oxygen species production may be responsible for this effect.

P205. Human mesenchymal stromal cells maintain their proliferative, immunophenotype and differentiation properties following electroporation

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Mesenchymal stromal cells (MSC) are pluripotent progenitor cells that can be found in human bone marrow, umbilical cord blood and adult peripheral blood. These cells have low immunogenicity and could suppress alloreactive T cell responses. In our previous study, we have successfully transfected human MSC with erythropoietin (EPO) gene in vitro by means of electroporation using a Nucleofector and Human Mesenchymal Stem Cells Nucleofection Solution (Amaxa GmBH, Germany). The transfected MSC samples were able to express high EPO and the therapeutic protein could induce hematopoietic stem cells into erythroid colonies. In this study, we compared the transfection efficiency and cytotoxicity of two transfection methods, i.e. lipofection (Lipofectamine 2000) and electroporation (Nucleofection). We also investigated the capacity of the MSC to proliferate, maintain the phenotype of MSC and differentiate into adipocytes and osteocytes following electroporation of a reporter plasmid (pmaxGFP). Our results showed that the nucleofection at a dose of 2 µg of plasmid resulted in higher transfection efficiency (54.07%) and less cytotoxicity (16.27%) than nucleofection at a dose of 4 µg of plasmid (47.15%) and higher cytotoxicity (27.92%). Meanwhile lipofection seemed to be less efficient (approx. 8.13% transfection efficiency at a dose of 4 µg of plasmid) and more harmful to MSC (35.39% cytotoxicity). The nucleofected MSC could also be isolated in single cell and cloned by serial dilution. The cloned cells showed positive staining for CD90 and CD105. They were also capable to differentiate into adipocytes and osteocytes. Thus, we conclude that electroporation does not alter the proliferation, immunophenotye and differentiation properties of MSC.
P206. A novel minimal-size vector (MIDGE) improves erythropoietin expression in human bone marrow mesenchymal stromal cells in vitro

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Mesenchymal stromal cells (MSC) are pluripotent progenitor cells that can be found in human bone marrow, umbilical cord blood and adult peripheral blood. These cells have low immunogenicity and could suppress alloreactive T cell responses. In this study, we compared the nucleofection efficiencies of Sleeping Beauty (SB) transposon system, corresponding plasmid and Minimal-Size Vector (MIDGE) encoding erythropoietin gene in human bone marrow MSC. The nucleofection was performed by using a Nucleofector and Human Mesenchymal Stem Cells Nucleofection Solution (AMAXA GmBH; Germany). Our previous results showed that the nucleofection at a dose of 2 µg of control plasmid encoding green fluorescent protein (GFP) resulted in highest transfection efficiency and least cytotoxicity. However the Sleeping Beauty on a trans delivery system (pT2/HB-EPO and pCMVSB11) failed to deliver the transgene into MSC at a dose of 2 µg as measured by Human Erythropoietin ELISA kit (StemCell Technologies Inc.; Vancouver, BC, Canada). The EPO expression of 2 X 10^5 cells transfected by 10 µg pT2/HB-EPO was only detected on Day 5 post-transfection and the level was low (7.21 mU/mL). Co-transfection with pCMVSB11 to improve transgene expression was helpless as higher DNA concentration was found to be harmful to the cells. In another study, the expression rose to 4779.4 mU/mL EPO per 1.0 µg of vector per 1 x 10^5 cells on Day 1 in pMCV1.2-transfected MSC and the yield dropped sharply to 28.53 mU/mL on Day 22. However the cells still maintained the low expression for three months in culture. Meanwhile the MIDGE vector-transfected MSC expressed highest amount of EPO on Day 6, up to 2683.76 mU/mL EPO per 1.0 µg of vector per 1 x 10^5 cells, and the yield dropped to 641.56 mU/mL after three months post-transfection. Thus, we conclude that the MIDGE vector is more superior to plasmid and Sleeping Beauty (SB) transposon system in long term expression involving erythropoietin gene in-vitro.

P207. Evaluation of the Synchron lx20 Pro system in UMMC

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Our goal was to consolidate different methodologies in the clinical chemistry laboratory and replace aging analyzers; therefore, we evaluated the newly available Beckman Coulter LX20 (Brea, CA). Results were obtained for linearity, within- and between-day precision, correlation, interference, and serum-vs-plasma studies. Satisfactory precision results were obtained, with most assays demonstrating within-day coefficients of variation less than 2% and between-day coefficients of variation less than 5%. The linearity for all assays was acceptable over the range tested. Correlation results were adequate with variation for most of the analytes were very small. This evaluation protocol is established based on the guidelines from SYNCHRON Clinical Systems Performance Verification Manual1 and James O. Westgards’s Method Validation Process and Procedures2. A technical and efficiency assessment was done to assess the suitability of this system. Performance was evaluated for linearity, accuracy, analytical precision (using commercial control materials) and functional sensitivity. For precision studies 20 replicates were run by using BIORAD control and the result is compared with target mean. The differences between the mean and the “true” value is calculated and expressed as percentage of deviation. For correlation studies, a minimum of 40 fresh human specimens were selected to cover
the wide analytical range of the method. At least 8 specimens per day were analyzed within 2 hours by the test and comparative methods, over 5 days to eliminate bias. Correlation data of different assays was obtained against the existing Dade RxL analyzer. Data analysis is carried out by using the Analyse-It program whose tool kit includes linear data plotter, SD or CV calculator and paired data calculator. Correlation data was analyzed using MS Excel linear regression. Generally all chemistries correlated well with the Dade RxL (R values: 0.92 to 1.00). The within-run standard deviation ($s_{w \text{-run}}$) for all the analytes are less than the defined allowable total error to be acceptable, $s_{w \text{-run}} < 0.25 \text{ TE}_{a}$. The variation for most of the analytes are very small and within specification except CREm, ALP and LD. The total standard deviation ($s_{w}$) for all analytes are $1/3$ less than the defined TE, $s_{w} < 0.33 \text{ TE}_{a}$, except CREm, ALP and URIC. The variation for most of the analytes were very small. We conclude that the LX20 demonstrates good performance capabilities, making this instrument suitable for a medium- to high-volume laboratory.

P208. Development of dendritic cell-based vaccine for cancer therapy

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Immunotherapy is showing renewed promise as a modality for treating malignancies. An effective cancer immunotherapy approach requires the activation of host T cells capable of recognizing tumour target antigens, and T cells activation needs appropriate antigen presentation by antigen presenting cells (APC). Dendritic cells (DCs) are the most potent professional APC, with exquisite capacity to interact with T cells and initiate immune response. Dendritic cell-based vaccines are currently the most potent method for stimulating antigen-specific cellular immune responses. Many explorations have focused upon finding a feasible and effective DC-based vaccine, including pulsing DCs with tumour antigen peptides, transducing gene encoding tumour antigens into DCs, and fusing tumour cells with DCs. In this study, the effects of DCs expressing MUC-1 and/ or IL-18 on stimulation of naïve T cells were investigated in mixed leukocyte reaction and the cytotoxic activities of these stimulated T cells were evaluated on human breast cancer cell lines. Autologous T cells were stimulated by DCs transfected with plasmids pVAX1/MUC-1 and/ or pVAX1/IL-18 to investigate the capacity of gene-modified DCs to prime naïve T cells in T cell proliferation assay. The most remarkable T cell proliferation was observed after stimulation with DCs expressing MUC-1 and IL-18, with Stimulation Index of 1.94 versus 0.96 for negative control. Enhanced cytotoxic activity of these stimulated T cells as the effector cells was also augmented against the target human breast cancer cell lines, T47D and MDA-MB-231. At Effector: Target (E: T) ratio of 10: 1, 87% of cytotoxicity was observed in T47D and 79% in MDA-MB-231, respectively after T cells stimulated with DCs expressing MUC-1 and IL-18. These results indicate that DCs expressing both tumour antigen MUC-1 and cytokine IL-18 are potential to be used as a vaccine and its application in immunization may be an effective strategy for a successful therapeutic vaccination against human breast cancer.
**P209. Apoptosis-induced chromosome breaks in the AF9 gene**

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Chromosome translocations are commonly associated with leukaemia. The actual mechanism is unclear. However, the apoptotic nuclease has been implicated in the initial step of translocation that is the introduction of chromosome breaks. Subsequently, erroneous repair of chromosome breaks by DNA repair mechanisms may lead to chromosome translocations. The AF9 gene located at 9p22 is frequently translocated with the Mixed Lineage Leukaemia (MLL) gene at 11q23 resulting in t(9;11)(p22;q23). It is hypothesised that the AF9 gene may be cleaved during apoptosis and eventually fuses with the MLL gene during the attempted DNA repair. This study aims to investigate the role of the apoptotic process in chromosome breaks in AF9. CEM and U937 leukaemic cells were treated with etoposide (VP-16) to induce apoptosis. Formation of DNA nucleosomal ladder indicates apoptosis induction. Non-isotopic Southern Hybridisation performed using an AF9 DNA probe detected a 10.2 kb intact and a 600 bp cleaved AF9 fragments. Nested Inverse Polymerase Chain Reaction (IPCR) was also employed and detected numerous AF9 cleavage fragments. The cleavage sites were mapped to a 0.5 kb region of the AF9 gene. This 0.5 kb region falls within the defined breakpoint cluster region 1 (bcr1) that encompasses intron 4 of the AF9 gene. In order to assess the role of apoptotic nuclease, cells were pre-treated with caspase 3-inhibitor II (Z-DEVD-FMK) prior to VP-16 treatment. In these pre-treated cells, no cleavage fragment was detected. These results suggest that chromosome cleavage within the 0.5 kb region of AF9 may be due to the apoptosis process and implies the involvement of apoptotic nuclease.

**P210. Congenital disorders of glycosylation (CDG): characterization by isoelectric focusing (IEF)**

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Congenital disorders of glycosylation (CDG) are a series of autosomal recessive enzyme deficiencies which result in incomplete glycosylation of plasma protein. Most CDG are multisystem disorders with mild to severe neurological disease and variable involvement of many other organs, including dysmorphism and birth defects. It is known that there are at least ten subtypes of CDG Type I and six subtypes of CDG Type II. The diagnosis of CDG is most widely made by characterizing the number of glycosylation of transferrin (Tf) using isoelectric focusing (IEF) technique. In this study, separation of human serum Tf glycoforms was carried out using IEF in a pH gradient 4-6.5, followed by immunoblotting. Prior to IEF, serum sample was saturated with ferric citrate in order to digest sialic acid chains and to obtain the different glycoforms of Tf. Transferrin (Tf) is the most suitable marker for the detection of CDG. Our findings showed, from 175 patients referred to our laboratory in the past two years, eleven patients (6%) were exhibited abnormal Tf IEF pattern. From eleven patients, six of them (55%) were shown to have typical banding pattern of CDG Type I; namely decreased of tetrasialo- band, and increased of disialo- and asialo- bands. Another five (45%) were shown to have banding pattern of CDG Type II characterized with decreased tetrasialo- band and increased disialo-, trisialo- and monosialo- bands. From this study, it has been shown that Tf IEF is a rapid and simple screening test for CDG. However, the diagnosis of CDG should be confirmed by a cascade of tests such as DNA mutational analysis, functional enzyme analysis, lipid linked oligosaccharides (LLO) testing and proteomics analysis. In conclusion, Tf IEF is the best screening method for differentiating CDG Type I and II.
P211. Morphological changes of spinal cord motoneurons following hemisection in rats

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Motoneuron morphometric data may contribute information that could provide a more complete and comprehensive understanding of the functional changes in spinal cord motor function following spinal cord injury. However, there are not many reports on the changes to the motoneurons following spinal cord hemisection in rats. We investigated the morphological changes of spinal motoneurons at the site of hemisection at T12 and those located 1mm rostral and caudal to the injured site. Routine epoxy semithin sections were stained with toluidine blue. Light microscopy analysis revealed no changes in terms of size of motoneurons, size of nucleus, position of nucleus and density of Nissl Bodies in motoneurons rostral to T12 at 7 and 9 days post operation (p.o.). However, at 14 days p.o. the size of the motoneurons and the density of Nissl Bodies decreased compared to those on the opposite side of the cord and compared to those in normal rats. The nuclei of the motoneurons were eccentric and slightly enlarged. On the other hand, motoneurons at the site of hemisection and those caudal to the site were much more affected. There was a decrease in the size of motoneurons and nuclei and in the density of Nissl Bodies in all rats at 7, 9 and 14 days p.o. In addition, collagenous scar formation was present at these sites in 9 and 14 days p.o. sections. Thus, hemisection decreased the size of motoneurons and size of nuclei, decreased the density of Nissl Bodies of motoneurons at the hemisected site and those caudal to the site but the effects were less remarkable in motoneurons rostral to the site of hemisection.

P212. Serum C-reactive protein levels in relation to insulin resistance and beta cell function in Iranian women with subclinical hypothyroidism.

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Subclinical hypothyroidism (SCH), defined as an asymptomatic state characterized by normal serum concentration of free thyroxine and elevated serum concentration of TSH. The association between coronary heart disease and SCH is unclear. The aim of this study is to investigate the complex interplay between hyperinsulinism, insulin resistance, beta cell function and low-grade chronic inflammation in Iranian women with SCH. Eighty women with SCH (age 33.2±5.0 years) and 80 healthy women (age35.1±4.0 years)as controls matched to the patient group for sex, age and body mass index(BMI), were enrolled in this prospective cross sectional study. TSH, free T3,free T4,highly sensitive C-reactive protein(hs-CRP),fasting insulin, fasting glucose, total HDL and LDL-cholesterol, Triglyceride, HOMA-IR index and HOMA-B index(beta cell function) were determined. hs-CRP was not statistically different between the SCH patients and control group (3.7±3.2 Vs 3.6±4.0,P>0.05). Total cholesterol and LDL-cholesterol were significantly higher, however triglyceride and HDL-cholesterol were not statistically different in patients with SCH as compared with control group. Fasting insulin levels and HOMA-IR were significantly higher in SCH women comparable to the control group. Beta cell function (HOMA-B) was not statistically different between these two groups(206.2±171.5 Vs 168.5±76.98 P>0.05). We showed a positive correlation between HOMA-B and TSH levels(r=0.191, P<0.05) We also found a positive correlation between insulin levels and hs-CRP(r=0.22, P<0.05) and also between
insulin levels and TSH\((r=0.312, P<0.05)\) and DL-cholesterol\((r=0.27, P<0.05)\). We think that Iranian women with SCH exhibited elevated atherogenic parameters (hyperinsulinemia, LDL-cholesterol and total cholesterol) and HOMA-IR.

P213. **Laboratory support in the Ministry of Health Malaysia Liver Transplant Centre: The Selayang Hospital experience.**

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To study the preparatory procedures performed in the transplantation work-up and peri-operative period and challenges faced by the various units in the laboratory involved in liver transplantation in our centre. We assessed if there are any differences in the procedures during the early and recent phases of the program. We studied 2 representative cases of liver transplantation and analyzed the laboratory procedures undertaken for each of them. A total of 25 cases were performed from April 2002 till May 2007. One case in the beginning of the program and one of the most recent cases were randomly chosen. There were a few differences noted and these will be highlighted during the presentation. There were a few differences noted between the work processes then and now. The changes reflected the confidence of having managed more liver transplantation cases over the years.

P214. **Amyloidosis of the liver: A case report.**

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Hepatic involvement in the three major forms of amyloidosis has been reported to be common. One autopsy report showed hepatic deposits in 70% of cases. We had a case of a 52 year old Malay male who was admitted with a clinical suspicion of subacute liver failure secondary to drug induced injury. He had no other previous history of other disease. Screening for Hepatitis A, B and C were negative. Features of early cirrhosis was suspected on ultrasound abdomen and magnetic resonance imaging (MRI). A post mortem biopsy of the liver revealed a classical case of amyloidosis with a linear sinusoidal pattern of amyloid deposition. The amyloid was demonstrable by conventional Congo Red stain. This case illustrates that amyloidosis may often be missed and not thought of as a differential diagnosis.

P217. **Blast characterization of acute myeloid leukaemia in Sarawak by 3-colour flow cytometry: A one-year study**

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Multiparameter flow cytometry (FCM) plays an increasingly important role in the laboratory diagnosis at post-treatment monitoring of acute myeloid leukaemia (AML). Three-colour FCM was first introduced in Sarawak at the University Malaysia Sarawak (UNIMAS) in March 2006. This study aimed to characterize the blast populations of all AMLs analyzed at UNIMAS from March 2006 to
March 2007. A total of 30 cases of AML and 4 cases of RAEB (adults to paediatrics ration of 1.3:1 and male to female ration of 1:1.4) were diagnosed. Morphologically, the breakdown according to the FAB classification was as follows: M1-3, M2-8, M3-2, M4-3, M5-4 and unknown -10. For FCM, the bone marrow aspirates were first screened using an acute leukaemia screening panel consisting of four tubes of markers to determine the lineage. Once myeloid lineage was confirmed, further blast characterization was achieved using the AML panel consisting of seven tubes of markers. The blast populations of 97.1% of the cases expressed CD45 dimly, 76.5% and 97.1% were positive for CD34 and cytoplasmic MPO respectively. The expression of the other markers was as follows: CD33-97.1%, CD13-94.1%, HLA-DR-88.2%, CD117-79.4%, CD64-64.7%, CD15-52.9%, CD11b-29.4%, CD14-26.5%, CD10-11.8% and CD71-2.9%. None of these cases expressed Gly-A and CD61. Aberrant expressions of CD56 and CD7 were found in 32.4% and 20.6% of the cases respectively. In short, the application of 3-colour FCM in UNIMAS over the studied period had resulted in rapid and accurate identification as well as comprehensive characterization of AML. This in turn permitted quick commencement of specific treatment strategy. In addition, unique antigen expression pattern in each case provided useful framework for disease monitoring and MRD study.

P218. Immunohistochemical markers, are we having too many choices?

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As more and more antibodies are made available for immunohistochemical studies, pathologists are increasing being challenged to make a rational choice from multiple antibodies all of which are reputedly suited for the same or similar diagnostic application. A recent review was conducted into the practice of a group of histopathologists working in a general teaching hospital in how they had used the three antibodies, namely smooth muscle actin, calponin and p63, each of which could be used as a marker for myoepithelial differentiation. The findings are presented and discussed. Immunohistochemical studies recently carried out in the histopathology laboratory of the Queen Mary Hospital, Hong Kong were recorded in a computerized database. The database was searched for all stains performed for actin, calponin, and p63 from Jan 2005 to the end of April 2007. Parameters selected were the accession number complete with the tissue block number, antibodies stained and the pathologist ordering the stains. A total of 1148 blocks were stained for actin (n=803), calponin (n=127), and p63 (n=574). Pathologists varied widely in their choice of applying these antibodies. While it is uncommon to use all 3 antibodies on a single block (n=38), one pathologist alone was responsible for 76% (n=29) of this usage. Slightly more blocks were stained for p63 alone (n=291) than those stained in combinations with actin and/or calponin (n=283). Significant variation in the use of the 3 antibodies was observed among a group of 24 pathologists working in a single public institution. Stereotypic patterns of applying immunohistochemical stains may result in excessive procedures. A system to monitor and provide timely feedback on the usage of immunohistochemical markers with the adoption of common protocols would effect cost cuts and more efficient running of laboratory services.
P219. Renal pathology after haematopoietic stem cell transplantation: A study of 13 patients

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The ever-growing number and increasing survival of haematopoietic stem cell transplantation (HSCT) allow better recognition of its associated renal injuries. We aimed to characterize renal pathology after HSCT by reviewing percutaneous renal biopsies after HSCT. A retrospective clinicopathologic study of all renal biopsies archived to the Department of Pathology, Queen Mary Hospital during the period January 1999 to December 2006 was performed. Biopsies from patients with HSCT were selected. Clinical data on presentation and follow up were retrieved from hospital records and physicians. In the 8-year period, a total of 2,585 renal biopsies were archived. 14 (0.54%) biopsies were from 13 patients (11 allogeneic, 2 autologous). All but one patient were male. The age at biopsy ranged from 7-63 year (median: 35 year). The median interval of renal biopsy after HSCT was 18 months (range, 1-134 months). Evidence of graft-versus-host disease (GVHD) was recorded in 9 patients. Presentation of renal disease included significant proteinuria (10 cases) and renal impairment (9 cases). Predominant histological changes were membranous glomerulonephritis (MGN) (n=4) and thrombotic microangiopathy (TMA) (n=3). Four patients died at 0-11 months after renal biopsy. Of the remaining 9 patients with a mean follow up of 39.4 months (range, 6-98 months), chronic renal impairment was found in 4 (44.4%) while proteinuria persisted in 5 patients. Renal involvement investigated by biopsy after HSCT primarily affect male. Among the various renal lesions, two types of glomerulopathy, namely MGN and TMA were the most common. Mechanisms of renal injury include GVHD-associated immune complex (IC) deposition and non-IC injury on endothelial cells, glomerular epithelial cells, mesangium, interstitium and tubular epithelium. Recurrence of paraneoplastic syndrome after HSCT potentially modifies the renal pathology. Pathologists and physicians should attend to the histological and temporal heterogeneities of renal injury when managing patients after HSCT.

P220. Genotypic and phenotypic divergence: Lessons from a family of Fabry’s disease

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Fabry disease is an uncommon X-linked disease in which the activity of α-galactosidase A (AGalA), a lysosomal enzyme, is deficient. Clinical manifestation results from occlusive microvascular lesions affecting the heart, kidneys, peripheral nerves and brain. A Chinese kindred with 5 members in two generations were found to be affected by Fabry’s disease. Diagnosis was established by genetic analysis and/or measurement of serum AGalA activity. The index patient was a 24-year-old pregnant woman who was heterozygous for Fabry’s disease seeking genetic counseling. The patient was reassured after a chorionic villus sample showed only wild type AGalA gene in a male fetus. The baby was delivered full term and postnatal serum AGalA was assessed to be normal. Her mother was investigated for asymptomatic proteinuria and a renal biopsy revealed podocytes packed with myelin bodies. Their serum AGalA activities were subnormal. The two maternal uncles of the patient had clinically manifest Fabry’s disease. One of them suffered end stage renal disease when 35 years old and the other had cardiomyopathy since he was 33 years of age. They had low serum AGalA activity. Direct sequencing of the AGalA gene of the uncles identified a missense mutation in exon 1 predicting a leucine to praline substitution (L14P). The patient’s 22-year-old younger sister had a normal serum AGalA activity. Analysis, however, showed that both the patient and her sister harbored the defective gene and were
P221. CD4+ T cell immune response predicts survival independently from IPI score among Diffuse Large B-cell Lymphoma (DLBCL) patients

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Studies have suggested that of CD4+ T-cell response in DLBCL patients is correlated with a better clinical outcome. Increased infiltration of CD4+ T-cells in B cell tumours has been correlated with longer overall survival in patients. In this study, we aimed to verify this correlation between CD4+ T-cell infiltration and IPI score. Patients diagnosed with DLBCL (between 1999-2001) at a single institution were selected. Immunophenotyping of neoplastic and reactive cellular components was performed by using 5 colour analysis on an FC500 (Beckman Coulter). Patients with >20% CD3+/CD4+ reactive population were considered as CD4+. The CD4+ data was compared with Survival and IPI scores by Mann-Whitney U tests, $x^2$ analysis and Cox proportional hazards model.: 58 patients (34 M/24 F; M: F 1.4) with median age of 55 yrs (range of 33-82yrs) were included. 28/58 (48%) patients were CD4+ and 30/58 (52%) CD4+. The median time for survival was 39 months (range of 1-95 months). 22/58 (38%) of patients had low risk IPI scores (0-1), 17/58 (29%) intermediate risk IPI score (2) and 19/58 (33%) had high risk scores (3-5). There was a significant difference between OS of CD4+ (median=48 mo) and CD4- patients (median=17 mo) with a p value of 0.0039. $x^2$ analysis revealed non-significant differences in IPI distribution in CD4+/- groups (p=0.383). Cox proportional hazards model reaffirmed the significance of CD4+/- with overall survival independent of IPI classification. This data suggests that percentage of CD4+ T cell infiltration of B-cell tumours is of prognostic significance, and is independent of IPI classification. These observations will be of value in designing future clinical trials for immunotherapy among DLBCL patients.

P222. Effects of antioxidants on coronary risk markers in patients with hypercholesterolaemia in the high risk category

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The objective of this study is to investigate the effects of coronary risk markers in patients with hypercholesterolaemia in the high risk category. 28 hypercholesterolaemic patients were categorized into the high risk group according to the National Cholesterol Education Programme Adult Panel Treatment III (NCEP ATP III). Patients were treated with atorvastatin to achieve low density lipoprotein (LDL-c) target (LDL-c <3.0 mmol/L) before being randomised into a double-blinded placebo-control clinical trial, with two treatment groups: (a) tococtrienol (160mg/day) plus slow-release vitamin C (500mg/day) and (b) placebo group – tococtrienol placebo and vitamin C placebo. Blood samples were collected at entry, baseline pre-randomisation (BL), 2 weeks (2w) and 3 months (3m) post-randomisation. Serum fasting lipid profiles (total cholesterol [TC], triglycerides [TG], high density lipoprotein [HDL-c] and
LDL-c), high sensitivity C-reactive protein (hsCRP), interleukin-6 (IL-6) and homocysteine (Hcy) levels were measured at entry, BL, 2w and 3m. Endothelial function by brachial artery flow mediated vasodilatation (FMD) was also measured at BL, 2w and 3m. There were significant reductions of TC and LDL-c levels in both groups at BL compared to entry (p<0.005), reduction of TG levels in tocotrienol group at BL compared to entry (p<0.05) and no significant changes of HDL-c levels were observed in both groups at BL compared to entry. No significant changes were observed on lipid profiles, hsCRP, IL-6, Hcy and FMD levels at BL compared to entry in both groups. In both groups, there were no significant changes of lipid profiles, hsCRP, IL-6, Hcy and FMD levels at 2w and 3m compared to BL. There were no significant differences of percentage delta between tocotrienol plus vitamin C and placebo groups at 2w and 3m post-randomisation. Supplementation of tocotrienol and vitamin C for three months has neutral effects on coronary risk markers in HC patients in the high risk category.

P223. Short term tocotrienol intervention on markers of oxidative stress, inflammation and atherosclerotic lesions in atherosclerotic rabbits

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The objective of this study is to investigate the effects of tocotrienol (TCT) on fasting lipid profiles, markers of oxidative stress, inflammation and atherosclerotic lesion in high cholesterol-diet induced established atherosclerosis in rabbits. 30 male New Zealand White rabbits were fed with 1% high cholesterol-diet (HCD) for 5 months. At 2 months post HCD, they were randomised to either one of the five intervention groups: (a) placebo, (b) TCT 15 mg/kg/day, (c) TCT 30 mg/kg/day, (d) TCT 60 mg/kg/day and (e) TCT 90 mg/kg/day. Serum fasting lipid profile, C-reactive protein (CRP), malondialdehyde (MDA) and 8-iso prostaglandin F_2α (8-isoprostane) levels were measured at B° (baseline pre-HCD), B¹ (1 month post-HCD), B² (2 months post-HCD and pre-TCT intervention), T¹ (1 month post-TCT intervention), T² (2 months post-TCT intervention) and T³ (3 months post-TCT intervention) periods. Aortic vessels were obtained after T³ to assess the atherosclerotic lesions. In all groups, there were significant increments of TC (p<0.05), HDL-c (p<0.05) and LDL-c (p<0.05) levels at B¹ and B² compared to B°. No significant changes of TG level at B¹ and B² compared to B° in all groups. No changes were observed after different doses of tocotrienol intervention on lipid profiles, CRP, MDA, 8-isoprostane and atherosclerotic lesions. There were no changes in oxidative stress, inflammation and atherosclerotic lesions with short term tocotrienol intervention in high cholesterol-diet induced established atherosclerosis in rabbits.

P224. Is the measurement of glycohemoglobin the best method for worldwide monitoring of diabetic patients?

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This study aimed to evaluate the effect of the common abnormal Hb on the detection of glycated hemoglobin (gHb) and hemoglobin A¹c (HbA¹c). Total of 296 EDTA blood samples were obtained from pregnant women including 32 normal Hb, 5 HbH disease, 46 alpha thalassemia trait, 32 beta thalassemia trait, 32 homozygous beta thalassemia, 19 heterozygous HbCS, 61 heterozygous HbE, 30 homozygous HbE, 7 AE Bart’s disease, 2 BF Bart’s disease, 2 HbH disease with HbCS, 22 heterozygous HbE with alpha thalassemia trait, 2 heterozygous HbE with iron deficiency anemia, 4 EA Bart’s disease...
with HbCS, 7 alpha thalassemia trait with iron deficiency anemia and 3 iron deficiency anemia. HbA1c levels were measured by immunoassay on a Hitachi 917 (Roche, Switzerland), an Integra 400 (Roche, Switzerland) and a Dimension RxL (Dade Behring, USA) analyzer as well as by HPLC on D10 (Biorad, USA) analyzer. The determination of gHb was performed with boronate affinity chromatography on a Variant (Biorad, USA) analyzer. There were statistically significant differences in the mean gHb values among normal, HbH diseases and homozygous beta thalassemia. The presence of HbE and homozygous beta thalassemia influenced HbA1c levels measured by Hitachi analyzer and HbH disease, HbE, HbCS and homozygous beta thalassemia affected the mean of HbA1c determined by Dimension analyzer. Similarly, there were interferences in HbA1c levels in HbH disease and HbCS measured by the Integra analyzer as well as in HbE and BF Bart’s disease by D10 analyzer. In 26 samples (19 homozygous HbE, 1 heterozygous HbE, 5 homozygous beta thalassemia and 1 alpha thalassemia trait with iron deficiency anemia) no peak of HbA1c measured with the HPLC method as observed. In conclusion, the presence of common abnormal Hb significantly affected glycohemoglobin test results. Therefore, the measurement of glycohemoglobin may not represent the best method for monitoring the diabetes patients.

P225. Evaluation of ESBL, Amp C, Metallobetalactamases producing gram negative bacterial isolates from body fluids in a tertiary care hospital

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Production of extended-spectrum beta-lactamases (ESBL) and Amp C is the most important prevalent mechanism of resistance to broad-spectrum cephalosporins among species of Enterobacteriaceae. Failure to detect and report ESBL production by gram-negative bacteria in a timely manner may result in a significant delay in appropriate antimicrobial treatment, resulting in increased mortality, morbidity and health care expenditure. This work reveals the prevalence of ESBL producing gram negative bacteria in the body fluids (including pus) of patients admitted to our hospital. The study was done during December 2005-December 2006. A total number of 469 Gram negative bacterial isolates in the body fluids & pus were identified by conventional methods. Antibiogram was done by Kirby Bauer’s disc diffusion method. All the isolates were tested for the production of metallo betalactamases by imipenem and imipenem-EDTA double disc synergy test. Out of 469 Gram negative isolates, total number of ESBL producing isolates were 160 (34.1%) and 36 (7.6%) were metallo betalactamase producers and 35 (7.4%) were Amp C producers. Among ESBL producing strains, E coli had the highest prevalence (37.8%), followed by Klebsiella spp.(32.3%), Proteus spp. (30.6%), and Citrobacter spp. (26.4%). Among Amp C producing strains, E coli (42.8%) had the highest prevalence, followed by Klebsiella spp. (37.1%). Proteus spp. (11.4%) and Citrobacter spp. (8.5%). Surgery (21.8%) and Orthopedics (20.4%) wards had the highest burden. Among 94 imipenem resistant strains, 36 (38.2%) were metallo betalactamase producers. Among them 25 (69.4%) were Pseudomonas spp. And 11 (30.5%) were Acintobacter spp. This work provides evidence for evolution of multidrug resistant ESBL and Amp C and MBL producing isolates in the hospital set-up. It would seem prudent to err on the side of caution and consider that ESBL-producing and Amp C and MBL producing isolates may predict therapeutic failure with extended spectrum cephalosporin drugs and imipenem.
P226. Alpha lipoic acid reduces the ultra-microscopic vascular changes in streptozotocin-induced diabetic rats

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Recently, alpha lipoic acid has gained considerable interest as antioxidant with a therapeutic potential in conditions where oxidative stress is involved. The aim of the present study was to investigate the effects of ALA dietary supplementation in reducing microvascular changes in diabetic rats. Diabetes was induced by a single intravenous injection of streptozotocin (50 mg/kg). The diabetic rats were divided into two groups, one supplemented with alpha lipoic acid (100 mg/kg/day) and another group with no alpha lipoic acid supplement (Non-Suppl group). Normal, non-diabetic rats formed the control group. Following eight weeks of follow-up, all the animals were sacrificed, and electron microscopic examination of the thoracic aorta revealed early atherosclerotic changes in the diabetic animals with no alpha lipoic acid supplement. The diabetic rats that received ALA supplement showed a clear reduction in the microscopic alterations in the aorta. These results suggest that alpha lipoic acid may exert some protective effects to the microvascular changes induce by hyperglycemia, possibly through its anti-oxidant activity.

P227. Diffuse large B-cell lymphoma of the upper aerodigestive tract: Evaluation of prognostic impact by immunohistochemical profiles

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Diffuse large B-cell lymphoma (DLBCL) arising from upper aerodigestive tract (UAT) is characterized as having favourable prognosis. Recent studies showed that DLBCL could be subclassified into germinal center B-cell-like (GCB) and non-germinal center B-cell-like (non-GCB) DLBCL by immunohistochemical staining (IHC) methods, and GCB subtype shows a better overall survival. The aim of this study is to evaluate the expression pattern of UAT DLBCL by IHC, using a panel of antibodies to CD10, Bcl-6, MUM1 and CD138. These markers were positive in 28%, 59%, 69% and 0% of the cases, respectively. EBER in-situ hybridization stain demonstrated that none of the cases were infected by Epstein-Barr virus. Based on the expression pattern of CD10, Bcl-6 and MUM1, there were 34% (11/32) GCB and 66% (21/32) non-GCB DLBCL cases. An alternative subclassification based on expression patterns of GCB markers (CD10 and Bcl-6) and post-GCB markers (MUM1 and CD138) demonstrated pattern A [GCB markers only] constituted 25% (8/32), pattern B [co-expression of both GCB and post-GCB markers] constituted 34% (11/32) and pattern C [post-GCB markers only] constituted 41% (13/32). According to Oh and Park (2006), combined patterns of A and B showed a significantly better overall survival than pattern C. Our series had 59% pattern A and B, and this may be the explanation for a significant favourable outcome reported on DLBCL arising from similar anatomical site, and our findings also further support that there is no association between EBV infection and DLBC.
P228. Negative images of mycobacteria observed in fine needle aspiration cytology (FNAC) smear – A case report.

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A 32-year-old woman presented with a mobile left breast lump, which was subjected to FNAC. The clinical diagnosis was fibroadenoma. The patient was HIV-positive for 4 years with low CD4 counts and cryptosporidiosis induced diarrhea of 6 months’ duration. She was on HAART therapy for 6 months prior to FNAC. The breast lump was 5 cm in diameter, firm and mobile. The FNAC’s smears showed features of a benign proliferative breast lesion, with ill-defined granulomatous reaction and numerous intracellular, rod-shaped unstained structures. These ‘negative images’ stained with Ziehl-Neelsen stain and Mycobacterial infection was diagnosed. A subsequent CT examination of the thorax showed consolidation in the middle and lower lobes with cavitation and bronchiectatic changes, in keeping with an infective process. Though the breast swelling reduced in size after a week of antitubercular therapy, the patient succumbed to her illness two weeks later.

P229. Culture yield and antimicrobial susceptibility pattern of Helicobacter pylori from gastric biopsy specimens

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No single technique is considered ideal for the diagnosis of Helicobacter pylori infection. As it is important to document the infection before therapy is instituted, a combination of diagnostic techniques therefore, improves the accuracy. Microbiology Laboratory of Kuala Lumpur Hospital has been performing cultures and antimicrobial susceptibility tests for Helicobacter pylori since year 2000. The study aims to look at the yield of Helicobacter pylori in our laboratory across a five-year period. Extra step was taken by providing microtainer tube which was filled with isotonic saline (1 ml) to the endoscopy room. Specimens were half -immersed into the solution to create a micro-aerobic condition which is ideal for the bacteria and transported to the laboratory immediately. Standard microbiologic procedures were used for culture and susceptibility tests. The organisms were identified based on conventional test and Vitek® identification system. The antimicrobial susceptibilities were determined based on the CSLI standard. A total of 318 gastric biopsy specimens were received in the Microbiology laboratory from June 2000 to October 2004. 14.8% of the samples yield positive culture for H pylori. The recovery rate of the organism was fairly constant between 21-23% of the samples received per year. All the positive isolates were subjected to antimicrobial susceptibility tests against amoxicillin-clavulanic acid, metronidazole, tetracycline, azithromycin, clarithromycin and amoxicillin. The organisms were very susceptible to tetracycline (97.9%), amoxicillin (97.9%) and amoxicillin-clavulanic acid (93.6%). Susceptibilities to macrolides i.e., azithromycin and clarithromycin were 74.5% and 72.3% respectively. The organisms showed a 30% resistance rate to metronidazole. We found that the yield of Helicobacter pylori in our laboratory was fairly satisfactory. Culture and sensitivity testing were able to assist the clinicians to document the infection apart from histological evaluation. Helicobacter pylori showed variable susceptibilities to the commonly used drugs (range from 70-98% susceptibilities).
P230. Ischemia modified albumin level in patients for myocardial perfusion imaging examinations

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Ischemia modified albumin (IMA) is a novel cardiac biomarker for excluding acute coronary syndrome (ACS) in patients with chest pain in emergency room. Myocardial perfusion imaging (MPI) can evaluate these patients for further management. Little is known about the clinical performance of combination of IMA and MPI in relatively stable patients with CAD. 31 patients (M:F = 27:4; age = 60.8 ± 9.8 years) referred for MPI examinations in National Taiwan University Hospital were included. Venous blood specimens were collected before, after and 1 hour after stress test for IMA and albumin measurements. TnI and hs-CRP were also measured before stress. MPI examination is performed according to 1-day rest/stress protocol. Electrocardiogram-gated single photon emission tomography (SPECT) imaging started 45 min to 1 h after the administration of 99mTc sestamibi which were done at rest and peak stress done by exercise on trademil or dipyridamole intravenous injection methods. Descriptive analyses were done by t test or Chi-square test. IMA level vs ischemia, time, and type of stress were analyzed by 3-way ANOVA. IMA level vs ischemia, left ventricle ejection fraction (LVEF) were analyzed by regression analyses in which IMA was dependent variable and gender, age, LVEF, TnI, hs-CRP, albumin and stress type were independent variables selected by stepwise method. 9 of 31 patients were diagnosed as myocardial ischemia by MPI examination. IMA level of patients with and without myocardial ischemia were not significantly different no matter before, after or 1 hour after stress. But IMA levels were significantly changed in different time points; the patterns of these changes were different from the 2 stress types. The albumin but not LVEF is related with IMA level. Consequently, IMA is influenced by albumin instead of myocardial ischemia or LVEF in patients for MPI examination.

P231. Improvement in oxidative stress in patients with non-familial hypercholesterolemia (NFH) treated with palm-oil derived vitamin E

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The objectives of this study are: (a) To investigate the ox-LDL and F\textsubscript{2} isoprostanes levels in NFH patients compared to normocholesterolaemic (NC) (b) To examine the effects of palmvitee supplementation alone on ox-LDL and F\textsubscript{2} isoprostanes levels in patients with NFH and (c) to determine the beneficial effects of palmvitee supplementation on oxidative stress markers in atorvastatin treated NFH patients. 66 patients with NFH (38 males, 28 females, age ± SD age = 48.0 ± 8.8 years) were recruited and randomised to 3 treatment arms [palmvitee 300 mg/day, containing tocotrienol 40 mg/D (NFHe) or atorvastatin 10 mg/day plus placebo (NFHsp) or atorvastatin 10 mg/day plus palmvitee (NFHse)]. 28 NC (17 males, 11 males, age ± SD age = 44.5 ± 5.6 years) were recruited in parallel. Fasting plasma levels of ox-LDL and F\textsubscript{2} isoprostanes were measured by enzyme linked immunoassay (Mercodia, Uppsala, Sweden and Cayman Chemicals, USA respectively). Oxidative stress markers was measured at baseline (BL) and follow-up visits up to 9 months except for NFHe group which was followed up until 3 months. Levels of ox-LDL and F\textsubscript{2} isoprostanes in NFH patients were higher than NC subjects (p<0.0001 and p<0.0001 respectively). In NFHe group, ox-LDL and F\textsubscript{2} isoprostanes levels were reduced at 3 months compared to BL (p<0.005 and p<0.05 respectively). Only F\textsubscript{2} isoprostanes levels in NFHsp and NFHse groups were reduce at 9 months compared to BL (p<0.0001). NFH patients have higher oxidative stress status compared to NC subjects. Supplementation of palmvitee alone has shown beneficial effects on oxidative stress in NFH patients. Low dose atorvastatin with or without palmvitee reduced F\textsubscript{2} isoprostanes levels in NFH patients. Palmvitee induced improvement in oxidative stress may contribute to the prevention of atherosclerosis.
P232. Testicular injury induced by exposure to electromagnetic field associated with the use of mobile phones: A biochemical and ultrastructural study

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The harmful effect induced by pulsed microwave currents associated with the use of mobile phones is now considered one of the physical injuries. This study was carried out on 60 male albino rats, divided into prepubertal group (one month old) and adult group (7 months old). Each group was subdivided into control group (6 animals) and exposed group (24 animals) the latter was furtherly subdivided equally into 10 minutes exposed group and 20 minutes exposed group according to the duration of exposure to EMF of mobile phone. In this study there were significant increases in the premeiotic testicular enzymes (B-glucuronidase, gamma-glutamyl transpeptidase and glucose 6-phosphate dehydrogenase) and significant decreases in the post-meiotic testicular enzymes (sorbitol dehydrogenase and acid phosphatase) in exposed groups as compared to their corresponding control groups. However the adult exposed groups have shown significant changes in these testicular enzymes when compared to their corresponding prepubertal exposed groups. The histopathological and ultrastructural studies have shown severe damage of the semineferous tubules and shrinkage in the tubular components of the testis that was more obvious in the 20 minutes exposed groups. After 10 minutes of exposure, the prepubertal group showed thickening and splitting of the basement membrane of seminiferous tubules with separation of germ cells while ultrastructural examination revealed few spermatogonia with marked apoptotic changes in the form of chromatin condensation, swollen mitochondria with degenerated cristae. In the 20 minutes exposed prepubertal group there were decrease in the diameter of seminiferous tubules with separation of germ cells away from the basement membrane of the seminiferous tubules, shrunken nucleous of Sertoli cells and abnormal spermatid. Also, there were marked necrotic changes in the germ and Sertoli cells indicated by marked rarification of the cytoplasm. However, the adult exposed groups have shown lesser ultrastructural changes than the prepubertal exposed groups. In conclusion exposure to EMF associated with mobile phones has shown detrimental effect on the process of spermatogenesis particularlu before puberty.

P233. Prevalence of Barrett’s, short segment Barrett’s and IMAGE in biopsies from esophagus and esophago-gastric junction

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The aim of this study is to study the prevalence of Barrett’s, Short segment Barrett’s and IMAGE (Intestinal Metaplasia at Gastro-esophageal junction) in biopsies from esophagus and oesophago-gastric junction. Endoscopic biopsies from lower end of esophagus and esophago-gastric junction were taken from 348 patients after thorough endoscopic examination. Three micron thick sections were stained with haematoxylin and cosin, AB-PAS(pH 2.5), Orcein- AB(pH 2.5), Diastase- PAS and Loeffler’s methylene blue for histological details, presence of SCE (specialized columnar epithelium) and H.pylori. Mean age of patients was 46.8 yrs. M:F ratio was 2.8:1. SCE was found in biopsies from 30/348 (8.62%) patients. Of these 10/30 (33.3%) had Barrett’s, 8/30(26.6%) had short segment Barrett’s and 12/30 (40%) had normal appearance (IMAGE) on endoscopic examination. Presence of SCE could be noted only in 18(60%) cases by H&E stain. Rest of the cases were confirmed after histochemistry. The study highlights the importance of biopsy in conjunction with histochemistry from normal appearing lower esophagus and esophago-gastric junction for diagnosing the possibility of presence of SCE (IMAGE).
P234. Detection of Verotoxin-encoding genes in non-O157 hospitalized patients’ *Escherichia coli* by molecular methods

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Verotoxin-producing *Escherichia coli* (VTEC) are of important pathogens causing serious human enteric diseases and sequels. The objectives of this research study project were to monitor and evaluate verotoxin-encoding genes (VT1 and types of VT2) among human *Escherichia coli* by means of some molecular assays such as PCR and RAPD besides routine procedures, as specific and rapid diagnostic assays. We set up a PCR protocol to detect and identify VTEC in pure cultures of 46 non-O157 *Escherichia coli* positive diarrhea specimens obtained from symptomatic hospitalized patients. Two PCR pairs of primer, specified for VT1 and VT2, were used in the study, generating 348- and 584-bp PCR products (amplicons), respectively. Also, a RAPD protocol, using one pair of 10-mer primers, optimized for the project. *Escherichia coli* were tested for O157 strains by routine methods. In PCR study of a total sum of 46 patients’ stool culture colonies, we found five isolates (about 10.8%) were stx1 and/or stx2 positive (all 5 isolates contained stx2 only and 2 of them included both genes). RAPD data for culture colonies are not shown as it still is in progress. No PCR or RAPD method, utilized directly for diarrhea in this study, was successful. Unexpected, the prevalence of VT genes in *Escherichia coli* was higher than the suggestions, as it was a futuristic research study. The differences between studies can be due to differences in peripheral and hosts’ conditions. The prevalence of VT2 gene was greater than VT1 gene. Finally, direct PCR and RAPD for stool specimens, as mentioned above, were unsuccessful. However, some stool substances such as bile acids, salts, fats etc have been shown to interfere with the molecular assays, needing to more accurate and also expensive methods.

P235. Prenatal diagnosis of alpha thalassaemia in Hospital UKM

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Thalassaemias are recessively inherited blood disorders with profound implications for individuals, families and health care services. They are common in South East Asia and Mediterranean regions. For alpha-thalassaemia syndromes, Hb-H disease and Hb Barts hydrops fetalis are the more serious consequences. To date, with DNA technology it is possible to offer prenatal diagnosis for many genetic diseases including thalassaemias. We report our initial prenatal diagnosis for alpha thalassaemias in Hospital UKM (HUKM) by multiplex PCR with allelic specific primers on six cases; two were Malays and four were Chinese. In two cases, the prenatal diagnoses were done as part of the investigations of the hydrops fetuses. In the four cases they were done for the diagnosis of Hb Barts hydrops fetalis as both parents were known thalassaemia carriers (1-trait). Results of prenatal DNA analysis from the two cases showed that one had a normal genotype (1/) and the other was an alpha-thalassaemia1 trait (1^-seA). Prenatal DNA analysis in the other four cases showed two cases were Hb Barts hydrops fetalis (1^-seA/1^-seA) and the other two cases were alpha-thalassaemia traits (1/-seA and 1^-3.7 seA). Parents of the two cases diagnosed as Hb Barts (1^-seA/1^-seA) were counseled. On termination of pregnancy, the fetuses were found to be severely affected. The cases diagnosed as alpha-thalassaemia traits had full term normal delivery. There were no complications following the diagnostic procedures in all the cases. This initial report indicates that prenatal diagnosis of alpha-thalassaemia can be performed successfully with this PCR method and with the availability of prenatal diagnosis there will be further improvement in the quality of obstetrics care and genetic counseling in our hospital.
P236. The relationship between organ colonization and host mortality in systemic *Candida albicans* infections.

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Systemic *C. albicans* infection is a leading cause of mortality and the organism was most commonly isolated from the blood of patients, followed by *C. tropicalis, C. parapsilosis, C. glabrata* and *C. krusei*. We determined the relationship between fungal load, organ colonization, tissue destruction and mortality rate following infection with a clinical isolate of *C. albicans*. *C. albicans* was cultured in YPD broth (2% bacteriological peptone, 2% D-glucose and 1% yeast extract) at 37 °C for 3 days on a shaking incubator (100 rpm). The culture was supplemented with 10% foetal bovine serum for 3 hours in order to induce germ tubes formation. The yeast cells were harvested, washed, re-suspended with phosphate buffered saline (PBS) and counted with a haemocytometer. Sixty nine female Sprague Dawley Rats (130 – 200 g) were inoculated intravenously with 5 x 10⁷ to 5 x 10⁸ yeast cells/ml and with PBS (control group). The animals were observed daily until sacrificed at 7, 14, 21, 28 and 35 days post infection. The liver, spleen, kidneys, brain, heart and lungs were collected and processed for histopathological examination. Eighty three percent of the rats infected with 5 x 10⁸ yeast cells/ml died within one day after infection. Seventy four percent of the rats infected with 5 x 10⁷ yeast cells/ml died at 2 and 3 days, and 22% of the rats died at 4 and 6 days post infection. The majority of these rats had either eyes or nose bleeding or both. Only four rats survived until the end of the scheduled period of observation. Histopathological examination revealed that all dead but not those that survived or control rats had mild to severe fungal colonization in brains. The kidneys were also colonised and showed extensive tissue destruction. Only the spleens and lungs were mildly infected by *C. albicans* in rats that survived. Multiple foci of necrosis with eosinophilic infiltration and other inflammatory cells were seen in the brains and kidneys of the infected rats. In conclusion, fungal colonization of the brain is a determinant factor for mortality in experimental systemic *C. albicans* infection in rats.

P237. Site specific-methylation of CpG nucleotides in the *hTERT* promoter region can control the expression of *hTERT* during malignant progression of colorectal carcinoma

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Expression of *hTERT* has been recognized an important factor in cellular aging, immortalization, and transformation. Therefore, the regulatory mechanism of *hTERT* expression has been widely studied by many investigators. In this study, to analyze CpG methylation of the *hTERT* promoter as an epigenetic mechanism and its implication in transcriptional regulation of *hTERT*, we performed the site-specific methylation assays of the *hTERT* promoter region and expression assays for the *hTERT* gene in colorectal carcinoma. As a result, we were able to observe an increased pattern of *hTERT* expression according to the malignant progression of colorectal carcinoma. Additionally, we could find that *hTERT* expression was induced when the P1 and P2 region of *hTERT* were sufficiently hypermethylated and, oppositely, the G1 region of *hTERT* was hypomethylated. Importantly, we could find three specific CpG sites (7th CpG of P2 and 11th and 2nd~10th CpGs of P1) closely related with the increasing of *hTERT* expression. These findings may provide important clues to deducing the expression mechanisms of *hTERT* in colorectal carcinoma.
P238. Study of the effect of aluminium on the levels of serum, liver and brain high molecular weight alkaline phosphatase.

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Elevation of high molecular weight alkaline phosphatase (HMW ALP) in the sera may indicate bile duct malignancy. The major aim of this project was to compare the level of this isoenzyme in sera, liver and brain tissues of aluminium treated rats. Rats were injected with aluminum salt. They were killed at indicated times and sera were collected. Liver and brain tissues were removed and homogenized. The homogenates were centrifuged and the supernatant was loaded on gel filtration chromatography with sephacryl S300. The level of HMW ALP was elevated in the sera significantly (p<0.05) but was reduced in the brain and liver. In vitro experiments showed that aluminium has non-competitive inhibitory effects on the activity of this isoenzyme. The elevation of HMW ALP in the sera may indicate malignancy of bile ducts as reported earlier and the reduction in the liver and brain may suggest the inhibitory effect of aluminium on liver and brain HMW ALP, which needs more investigations.

P239. A study of the effect of natural honey on the wound healing process in rat.

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Wound healing is the restoration of physical integrity to internal and external structures and involves intricate interactions between the cells and numerous other factors. Appropriate treatment/care is essential to acceleration of the healing process, prevention of infection and chronicity of the wound. Different means and approaches have thus far been used to this end. The aim of this study was to evaluate the effect of natural honey (NH) on the wound healing process. Two model skin wounds were created in the paravertebral area, 1.5mm from midline on the back of rats; 15mm full-thickness of longitudinal wound and 5mm diameter full thickness of round wounds thereafter were evaluated through measuring the length and area of the healed region, histopathological features on different days, and conducting tensiometery experiments after complete wound healing. The longitudinal wound healing (%) on days 3, 6, 9, 12, 15 in control group changed in NH-treated group from 10.13%, 31.88%, 52.46%, 78.75%, 100% to 18.57%, 39.85% (P<0.05), 69.29% (P<0.01), 98% (P<0.001), 100% respectively. The round wound healing (%) on days 3, 6, 9, 12, 15, 18 in control group changed in NH-treated group from 9.88%, 21.25%, 52.13%, 69.63%, 88.21%, 100% to 25.3% (P<0.01), 48.14% (P<0.001), 70.3% (P<0.001), 94% (P<0.001), 100%, respectively. Stress (maximum tensile force causing skin rupture) changed from 13.10 Newton (N) in the control group of longitudinal wound to 17.29N, also from 11.11N in the control group of round wound to 19.26N (P<0.05) in NH-treated group. Strain (tissue length under maximum strain) changed from 9.50mm in the control group of longitudinal wound to 24.26 mm(P<0.01), also from 10.34mm in the control group of round wound to 19.4 mm(P<0.05) in NH-treated group. Grossly, disappearing rate of induced ulcer denoted more valuable NH effectiveness on round group (P<0.01) in comparison with on longitudinal group(p<0.05). Light microscopic examination (H&E & Masson’s trichrome stain) revealed proportionally marked reorganization of injured area. Our findings suggest that NH may have accelerated the skin wound healing process in rat and increased tissue strength through stimulating collagenization.
P240.  Identification and characterisation of two differentially expressed genes in acute myeloid leukaemia

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Acute myeloid leukaemia (AML) is a malignant proliferation of abnormal blasts cells of the myeloid lineage. Patients initially responding to treatment may eventually relapse with the disease returning. Relapsed AML can be categorised into early relapse (<one year; poor prognosis) or late relapse (>one years; good prognosis). Currently there is no marker to distinguish between these two groups. Subtractive hybridization can be used to isolate for highly expressed genes in AML samples. Two clones (P-A3 and P-A4) were subtracted from a pair of good and poor prognosis AML patients. The identities of the clones were determined by automated DNA sequencing. Primers were designed based on their nucleotide sequence. Semi-quantitative RT-PCR assay was carried out using the two sets of primers on reversed transcribed RNA extracted from AML samples, including the original AML samples these genes were isolated from, and also an apparently healthy human sample. The transcription level of the target genes of interest were measured relative to a housekeeping gene, beta-2-microglobulin ( 2M). We found that P-A3 and P-A4 were expressed at approximately two-fold higher levels in one of the original samples. P-A4 expression level was low in the healthy human sample but high in AML samples. Expression level was also higher in samples with poor prognosis. This correlated with the anti-apoptotic function of the P-A4 protein. The expression of P-A3 on the other hand, was higher in the healthy human sample compared to AML samples. Its expression level was decreased in poor prognosis samples. The decrease in expression may have inhibited its role as a cell cycle regulator. Thus, we showed that both P-A3 and P-A4 were differentially expressed in AML samples with different prognosis and therefore may have potential as prognostic markers for the management of AML patients.

P241.  Serological survey of Neospora caninum infection in cattle in Kerman, Iran

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Neospora caninum is an intracellular parasite which causes abortion in cattle worldwide. This study was conducted in South East Iran to determine the seroprevalence of Neospora caninum in cattle. The aim of this study was to study the seroprevalence of Neospora caninum in cattle in the province of Kerman in South East Iran. For this propose blood samples were collected from 285 cattle in the province of Kerman for determining the seroprevalence of Neospora caninum. Serum samples were analyzed for antibodies against N. caninum antigen using a commercially N. caninum iscom ELISA kit (Svanova Biotech AB, Sweden). Antibodies to N. caninum were found in 36 of the 285 (12.6%) sera based on ELISA test results. This study is the first report of Neospora infection in this area. With regard to seropositivity, no significant difference was observed among the origin, sex and age (P > 0.05).
P242. Study of Sarcocystis infection in slaughtered cattle in Kerman, Iran

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Sarcocystis is one of coccidian parasite that causes cyst in human and livestock. This parasite is widespread, and it can infect various livestock. Although infection with sarcocystis in most of cases is without any sign and detection is only made after slaughtering or necropsy, but in infected animal it has signs such as losing weight, anemia, and abortion and in acute cases, death. The aim of this study was to determine the prevalence of Sarcocystis in slaughtered Cattle in Kerman, Iran. The present study was carried out on 480 slaughtered cattle during spring, autumn, summer, and winter between 2005-2006 in Kerman abattoir. Sarcocystis have two type cysts: Macrocyst and microcyst. At first, for viewing the macroscopic cyst carcasses have been investigated. Then samples were taken from esophagus, diaphragm, inter costal and heart, but no case had macroscopic cysts. Then samples were examined with two methods for investigating carcasses in terms of microscopical presentation of the sarcocystis microcyst. These methods included: (1) Gimsa staining, (2) Peptic digestion. If result of Gimsa staining was negative, we would use Peptic digestion. But in this study, all of samples in Gimsa staining method were positive. A positive case was detected with a microcyst on slide. Base on the obtained results: There were no macroscopic infection in samples (%0), and the frequency of microscopic cyst was 100%, there was no significant differences between male and female, mature and immature (maturity appointed >18 month), and also seasons.

P243. Seroepidemiologic study of Chlamydia pneumoniae in infarct patients, Yazd, Iran.

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Objectives: Myocardial Infarction (MI) is one of the leading causes of death in the world. Today, it is well known that some bacteria and viruses play a vital role in MI manifestation. C. Pneumoniae is considered to be one such bacterium. The general purpose of this cross-sectional study was to determine the correlation between MI and C. Pneumoniae infection. Methods: 103 infarcted patients along with 75 healthy people as control with similar demographic status were selected for this study. Initially, IgG and IgM titres were done using ELISA technique for detecting antibodies against C. Pneumoniae and then the serum was further examined for determination of sugar, CRP, cholesterol and triglyceride levels. Simultaneously, demographic features such as blood pressure, family history and addiction to smoking were recorded. Results: Results showed that 92 (89.3%) of serum samples from patients and 58(77.2%) of controls were positive for IgG, whereas only 2 patient’s serum samples and none of the controls were positive for IgM. Relation of IgG levels with sex was statistically insignificant, but this relationship with age was significant. (P=0.03).History of blood pressure in patients was recorded and had no significant relationship with IgG levels. Conclusion: Although the number of cases studied was limited, it can be concluded that C. Pneumoniae infection is prevalent in Yazd and this silent infection could be the causative factor of acute M I.
Effects of HESA-A on healing of cutaneous wound due to burning in rats

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Background: Wound healing is the restoration of physical integrity to internal and external structures and involves intricate interactions between the cells and numerous other factors. Appropriate treatment and care is essential to acceleration of the healing process, prevention of infection and chronicity of the wound. Different methods and approaches have thus far been used to get this end. The aim of this study was to evaluate the effect of HESA-A (a novel drug of marine-plant origin containing active biological ingredients patented by Dr. Ahmadi) on the wound healing process.

Methods: 1 cm wounds was made by contacting a 1 cm hot metal plate on midline part of vertebral area in rats’ back for 8 seconds. The rats were treated with HESA-A at concentration of 5% (a blend containing 5% of the drug and 95% of chow). The healing process was evaluated through measuring the length and area of the healed region on different days and conducting tensiometry experiments after complete wound healing.

Results: 1. The percentage of reduction in wound area was measured in test group comparing to control on days 3, 6, 9, 12, 15, 18 and 21. From the days 18\textsuperscript{th} - 21\textsuperscript{st} the difference between test and control group was significant (P≤ 0.001). 2. Stress (maximum tensile force causing skin rupture) changed from 15 Newton in the control group to 22.75 Newton (P<0.01) in the group treated with 5% HESA-A. 3. Strain (tissue length under maximum strain) increased from 11.6 millimeter in the control group to 22.5 millimeter (P<0.001) in the group treated with 5% HESA-A.

Discussion: Our findings suggest that HESA-A may have accelerated the wound healing process in rat and increased tissue strength through stimulating collagen formation.

High cyclooxygenase –2 expression in cervical intraepithelial neoplasia and squamous cell carcinoma of the cervix compared to normal cervix

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Cyclooxygenase-2 (COX-2) is known to suppress apoptosis, promote angiogenesis and tumor invasion. The relationship between COX-2 and neoplastic transformation of epithelial cells has been established. The expression of COX-2 is up regulated in various malignancies such as colon, lung, breast, and squamous cell carcinoma of the cervix. The objective of this study was to determine the relationship between the expression of cyclooxygenase-2 in normal cervix, CIN and squamous cell carcinoma of the cervix. Archived tissue blocks of normal cervix (54), cervical intraepithelial neoplasia (CIN) (79) and squamous cell carcinoma (SCC) (56) histopathologically diagnosed from year 1998 to 2003 in Pathology Department of HUSM and HKB were included in this study. All tissue section was subjected to immunohistochemistry using COX-2 antibody to estimate the percentage of the expression of COX-2 protein. The results were correlated with clinical and histopathological diagnosis. Our results showed that there were significant increase in expression of COX-2 with the progression stage of the disease (p<0.001). We also found that COX-2 expression was significantly associated with presence of lymph node metastasis, parametrial involvement and tumor differentiation (p<0.05).
**P246. Tumor angiogenesis as a prognostic marker for squamous cell carcinoma of the cervix**

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Angiogenesis is the development of new blood vessels from preexisting capillaries. It is essential in tissue development, reproduction and wound healing. Tumor angiogenesis is mainly evaluated on the basis of microvessel density (MVD). This can be measured using factor VIII related antigen (FVIIIIRA). The objective of this study was to investigate the relationship between microvessel densities with tumor differentiation, deep stromal invasion, parametrial invasion, vascular space involvement and presence of lymph node metastasis. Another objective was to investigate the relationship between MVD, tumor differentiation, deep stromal invasion, parametrial invasion, vascular space involvement and lymph node metastasis with the prognosis of the patients. Archived tissue blocks of 56 samples of squamous cell carcinoma (SCC) of cervix histopathologically diagnosed from year 1998 to 2003 in Pathology Department of HUSM were included in this study. All tissue sections were subjected to immunohistochemistry approach to determine the microvessel density using FVIIIIRA antibody. Then results were correlated with clinical and histopathological diagnosis as well as the outcome of the patients. Follow-up of the patients were done by reviewed the patients folder at Registry Department of USM on December 2006. We found that there were significant correlation between the expression of microvessel density with tumor differentiation, presence of lymph node metastasis, parametrial involvement, vascular invasion and deep stromal invasion (p<0.05). In univariate analysis, Kaplan-Meier log rank test showed that microvessel density, tumor differentiation, lymph node status and stromal invasion were significant prognostic markers in overall patient’s survival analysis (p<0.05).

In summary, the study suggests that higher number of microvessel density in cervical carcinoma is associated with a poorer prognosis.

**P247. Angiogenesis in cervical intraepithelial neoplasia and the risk of recurrence**

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Angiogenesis is the development of new blood vessels from preexisting capillaries. Tumor angiogenesis is mainly evaluated on the basis of microvessel density (MVD). Recent studies in premalignant condition in the breast, colon and cervix have shown that angiogenesis occur before tumor invasion. Therefore it can be assumed that angiogenesis could be a key step in progression to an invasive carcinoma. The objective of this study was to determine the relationship between the microvessel density in normal cervix and cervical intraepithelial neoplasia. Another objective was to investigate whether microvessel density can predict the risk of recurrence in cervical intraepithelial neoplasia (CIN) patients. Archived tissue blocks of 54 normal cervices and 79 samples of cervical intraepithelial neoplasia (CIN I, II, III) of cervix histopathologically diagnosed from year 1998 to 2003 in HUSM were included in this study. All tissue sections were subjected to immunohistochemistry approach to determine the microvessel density using FVIIIIRA antibody. The expression of MVD was determined with a digital image analyzer. The results were correlated with histological diagnosis and the outcome of the patients. Follow-up of the patients were done by reviewed the patients folder at Registry Department of USM on December 2006. Our result showed that there were significant differences (p<0.05) between MVD in normal samples and CIN II, III groups. The microvessel density is significantly increased in CIN II, III samples compared
to normal tissue, which suggest that angiogenesis occurs in higher dysplasia. Our results showed that there are increased numbers of microvessel density in patients who have recurrence of CIN compared to patients with no recurrence. However the results was not statistically significant (p=0.058). In view of the lack of known prognostic indicators, a finding of potential interest is the increasing range of MVD seen within each histological group. Such a distribution could have prognostic significance for disease progression.

P248. Analysis of canine haemograms using the Abbott Cell-Dyn CD 3500 haematology analyser

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Objective: Analysis of the automated cell count is an essential tool in canine haematological diagnostics. Abbott Cell-Dyn CD 3500 (CD 3500, Abbot Diagnostic Division, Mountain View, CA) is a electrical-impedance light-scatter haematology analyser, which produces a full blood count with white blood cell differential. CD 3500 is available with multiple preprogrammed settings for the more common types of domestic animals. We compared CD 3500 to the laboratory’s current instrument, Serono Baker 9120 (Baker Diagnostics, Pennsylvania, USA). Methods: Blood samples from 70 dogs were analyzed on the CD 3500 and the results compared with those obtained by a Serono Baker 9120 electronic resistance cell counter and a 400-cell manual differential leukocyte count. Results: Erythrocytes count (RBC), packed cell volume (PCV), haemoglobin (Hb) concentration, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet number (PLT), mean platelet volume (MPV), total leukocyte count (WBC) and differential leukocyte count (DLC): neutrophils, lymphocytes, monocytes, eosinophils and basophils were determined and compared. Comparisons of WBC, RBC, Hb, PCV and PLT were excellent between two analysers (r=0.99, 0.97, 0.96, 0.95 and 0.93) and good for MCV and MCH (0.82 and 0.86). Only MCHC and MPV showed poor correlation (r=0.14 and 0.43). Evaluation of DLC indicated also excellent correlation with the manual reference method for neutrophils (r=0.93) and lymphocytes (r=0.94), and good correlation for monocytes (r=0.87). Poor correlation was found for eosinophils (r=0.63) and basophils (r=0.40), probably due to relatively low absolute numbers of these cells in canine blood. All correlations were statistically significant (p<0.05), except MCHC and MPV. Conclusion: Acceptable correlations (r>0.80) were achieved between the two test systems for all values except MCHC, MPV, eosinophils and basophils. During the evaluation period, CD 3500 was easy to handle and appeared well suited for routine canine blood cell analysis.

P249. Laboratory based therapeutic drug monitoring – A case study in drug and research laboratory, HKL

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Therapeutic Drug Monitoring (TDM) is relatively a new scope in medical testing service in Malaysia. Historically TDM testing was handled by the Pharmacist since its inception. Still being the scenario in many government hospitals throughout the country, the diagnostics part was passed on to the Department of Pathology, Hospital Kuala Lumpur in the year 1993. This laboratory gained its accreditation in 2004. Consolidation of test platforms and availability of trained personnel in the
current set up has eliminated the need for extra laboratory. Fluorescent Polarization Immunoassay (FPIA) and Microparticle Enzymatic Immunoassay (MEIA) principles are mainly used for all the testing where currently 19 analytes are offered. The laboratory is participating in two international quality assurance programme, RCPA and RIQAS to monitor the quality of our testing mechanisms in addition to internal quality control which utilizes the first party control. Latest testing methodology is constantly being evaluated to keep up to the current trend and changes in testing, in line with the requirement of accreditation. In conclusion, TDM medical testing can be better executed by direct monitoring of a technical personal which enables proper test handling, accurate and precise results produced. Only a slim line is observed between therapeutic range and toxicity in most of the drugs. Thus quality and accuracy of the results can be assured through internal quality control, participation in external quality assurance programme and technical competence of the personnel involved in testing process and supervision. TDM which is still under its adolescent age can mature into a strong tool for clinical evaluation and patient management.

P250. An evaluation of intraoperative frozen section biopsy in Philippine Heart Centre: A 10-year retrospective study

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Background: Frozen section is a very helpful ancillary procedure to the surgeon in guiding him in his decision intraoperatively. Indications include: to establish the presence & nature of lesion, determine adequacy of surgical margins and to establish whether the tissue obtained contained diagnosable material. The accuracy of frozen section varied depending on the author and type of tissues. Objective: To review and evaluate the accuracy of frozen section in the Philippine Heart Center from 1994-2005. Methodology and Results: This retrospective study where we reviewed 114 lesions that underwent frozen section from 1994-2005. The most common site sent for frozen section diagnosis was the mediastinum, followed by the lung and breast. There were 47% true positive, 54% true negative and 2.7% false negative. There were no cases of false positive frozen section diagnosis. Cases with false negative diagnosis includes 3 cases of mediastinal tumors which were diagnosed thymoma on frozen section but was signed out as lymphoma on permanent sections. There was also a single case of breast tissue with a deferred diagnosis. Our study revealed that our frozen section diagnosis has a very good sensitivity (94.5%) and specificity (100%) in diagnosing tumors. Conclusion: We conclude that frozen section is very accurate in diagnosing mediastinal, pulmonary and breast lesions. However, frozen section has some limitations. We, therefore, recommend good communication between the surgeon and pathologist to avoid misdiagnosis.


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Objective: To assess antiviral potentials of Marine flora and fauna using rapid assays. Introduction: The worlds land and sea represent a limitless reserve of natural, biological and potentially active useful pharmaceutical products. Renewed interest in from natural reserves, advances in chemical separation and analysis have identified hundreds of unique exotic substances form flora and fauna more rapidly. Methods: Extracts of marine flora and fauna (n=221) from 10 collaborating centers, of Department of Ocean Development National project, were assessed for their antiviral potentials
using rapid assays. Rapid assays included in the study were reverse transcriptase (RT) inhibition, HBV DNA and E.Coli DNA polymerase inhibition assays, aimed to assess their antiviral activities against HIV and Hepatitis B Virus. The inhibitory activity was ascertained using tritiated thymidine triphosphate. A 50% or more reduction in the radioisotope uptake count between the control and the test was taken as positive inhibitory activity. **Results:** 24.35% and 15.83% of extracts possessed RT and E.coli DNA polymerase inhibitory activity respectively. 42.72% of the 35 extracts showed HBV DNA polymerase inhibition activity. **Conclusions:** The study shows the availability of flora and fauna with anti microbial activity in general and antiviral potentials in particular, that needs to be harnessed and commercialized.

**P252. Evaluation of Sysmex UF1000i and Iris IQ200 automated microscopic urinalysis systems.**

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Objective: Routine microscopic examination of urine is a time-consuming and laborious process. This study compares the performance of the automated urinalysis analysers, Sysmex UF1000i and Iris IQ200 systems with manual microscopy. **Methods:** Urine samples sent for routine manual microscopy examination were additionally analysed using the UF1000i and IQ200. Inter-day imprecision was measured using manufacturer-supplied QC material. Linearity was assessed using dilutions of blood and urine with saline. Timing studies to assess analytical time with each platform were performed. **Results:** Between-day CVs were Manual Microscopy 26-27%; UF1000i 2-8% and IQ200 around 7%. Using Manual Microscopy as the gold standard, sensitivity(%)/specificity(%) on different systems were: RBC UF1000i 96/47, IQ200 92/64; WBC UF1000i 90/71, IQ200 80/83; Bacteria UF1000i 86/48, IQ200 35/82; Yeast UF1000i 89/89, IQ200 79/95; Cast UF1000i 66/85, IQ200 74/97; Crystal UF1000i 57/94, IQ200 64/98. The Prevalence (P) and the Negative Predictive Value(%)/Positive Predictive Value(%) for each system were: RBC (P:27%) UF1000i 97/40, IQ200 95/49; WBC (P:38%) UF1000i 92/66, IQ200 87/75; Bacteria (P:66%) UF1000i 64/76, IQ200 40/79; Yeast (P:4%) UF1000i 99/26, IQ200 99/43; Cast (P:8%) UF1000i 97/26, IQ200 98/67; Crystal (P:3%) UF1000i 99/22, IQ200 99/53. Average analytical time calculated, in minutes, were Manual Microscopy 1.52, UF1000i 0.87, and IQ200 1.14. Linearity met manufacturer specifications. **Conclusion:** The Sysmex UF1000i automated urinalysis system showed superior performance compared to the Iris IQ200 system in terms of sensitivity and analytical time. It offers fast, sensitive and precise microscopic urine analysis, with walk-away convenience and interfacing capability to LIS.

**P253. Viral spread in a mouse model of enterovirus 71 (EV71) encephalitis**

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The aim of this study was to investigate the mode of EV71 spread into the brain and spinal cord of a murine model using an adapted viral strain that was first isolated from a fatal human case. After several serial passages in mouse brains, we were able to adapt the virus to produce central nervous system (CNS) infection in 14-day-old mice by intracerebral, intraperitoneal, subcutaneous, intramuscular (IM) and oral routes. Following infection, limb paralysis was seen in most animals, often followed by death in 5 to 7 days. Virus titration, immunohistochemistry (IHC) and **in situ** hybridisation (ISH) confirmed presence of virus in the CNS, skeletal muscles, and brown adipose tissues. The distribution
of virus in the CNS closely paralleled that seen in human EV71 encephalitis. To determine the mode of viral spread into and within the CNS, groups of 2-week old ICR mice were injected with $10^5$ TCID$_{50}$ adapted strain into the right hind limb and masseter muscles. Mice were sacrificed at various times after inoculation and the spread of virus in CNS tissues was again studied by virus titration, IHC and ISH. After hind limb IM inoculation, viral antigens or RNA was initially found exclusively within motor neurons of the right anterior horn area, then the lower spinal cord, followed by the upper cord and brain, suggesting direct neural spread via the sciatic nerve into CNS. Dorsal root ganglia and autonomic ganglia were generally not involved. The first neurons to be infected after inoculation into the masseter muscles were in the brainstem. The findings suggest that viral entry into the CNS is via peripheral nerves, and spread within the CNS is by neural pathways. This mouse model is proposed as a suitable model for further studies including pathogenesis studies and anti-viral drug testing against acute EV71 infection.

P254. **Quantitation and distribution of viral antigens in acute human Nipah virus encephalitis**

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Nipah virus infection, an emerging zoonotic paramyxoviral infection is predominantly associated with acute encephalitis. Tissue damage in the central nervous system is believed to be due to two concurrent pathogenic mechanisms viz., direct infection and vasculitis-induced microinfarction. In this study, we investigated the extent of direct infection of brain parenchymal cells (mainly neurons and glial cells), and its relative contribution to tissue damage. Brain tissues from 16 autopsies of acute Nipah encephalitis were examined and quantitated for the presence of viral antigens after immunohistochemistry. Representative formalin-fixed and paraffin-embedded tissue sections from different parts of the brain were stained by an anti-Hendra antibody in a standard immunohistochemical assay. Areas positive for viral antigens were identified by light microscopy, image-captured into the computer and analyzed using the Leica Q-win software to study its distribution and surface area. The cerebral cortex grey matter was much more likely to be positive for viral antigens compared to the white matter (6 % versus 0.3 %; p=0.00). Indeed, viral antigens were overwhelmingly found in neurons. The frontal, temporal, parietal, occipital lobes were found to have decreasing amounts of antigen in this order (range: 8.4 % to 0.1 %), but this was not statistically significant. In the brainstem, the pons appears to have a highest viral load (7.3 %). In conclusion, the Nipah virus is confirmed to have a predilection for neurons and hence it is not surprising that the cerebral grey matter and other neuronal areas are most likely to have the highest viral load. There is a suggestion that the neurons in the frontal lobe and pons may be most susceptible to infection.
P255. Experimental Tioman virus infection in pigs.

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Tioman virus (TioPV) is a recently described paramyxovirus isolated from bats found on Tioman Island, Malaysia in 2001. Recent serological evidence suggested that it could cause human infection but so far its clinical manifestations, if any, have not been established. Based on its close genetic and antigenic similarity to the Menangle virus (MenPV), it is hypothesized that TioPV could cause infection in humans and pigs. MenPV had been reported to cause an influenza-like illness in two humans and congenital abnormalities in pigs in Australia. In the present study, pigs were experimentally infected with TioPV by a subcutaneous route and sequentially sacrificed to study the pathology, tissue tropism and virus shedding using a combination of light and electron microscopy, immunohistochemistry, in situ hybridisation, virus culture and real-time PCR. The pigs developed neutralising antibodies against the virus following infection, although they either showed no symptoms or only mild pyrexia. Evidence of virus was found in the tonsil, thymus, lymph node and spleen, mainly in lymphoid cells and tonsillar epithelium. Warthin-Finkeldy-type giant cells were also found the thymus. Tissue viral load appears to be maximum about 1 week post-inoculation and declined very soon after that. Virus was isolated from the oral cavity, suggesting the oral route as a potential route for viral transmission. The findings suggest that pigs are susceptible to TioPV and that it could act as an intermediate/amplifying host for human transmission.

P256. Demographic distribution of primary central nervous system tumour in Hospital Kuala Lumpur from year 2004 to 2006

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Brain tumours have the twelfth highest incidence in males and the fourteenth highest incidence in females among all cancers in Peninsular Malaysia. The crude annual incidence rate of brain tumours in Hospital Kuala Lumpur was 1.4 per 100,000 population. Primary brain tumours are different from tumours arising in other sites in that a benign lesion may result in death due to compression of vital structures. This study aimed to determine the demographic distribution of primary central nervous system tumours in Hospital Kuala Lumpur over a period of three years from 2004 to 2006. A retrospective cross-sectional study design using universal sampling method was chosen with a sample size of 459. The data was obtained from request forms for histopathological examination of patients with primary central nervous system tumour sent to the Histopathology Unit, Department of Pathology, HKL from 2004-2006. The highest frequency of primary central nervous system tumours are in the age group of 41-50 years old (n=97, 21.1%). The frequency of females is slightly higher (n=237, 51.6%) as compared to males (n=222, 48.4%). Malays had the highest frequency of primary central nervous system tumours (n=290, 63.2%). Meningioma was the most frequently encountered histological type of primary central nervous system tumour in HKL (n=172, 37.5%), followed by astrocytoma (n= 84, 18.3%) and glioblastoma multiforme (n= 74, 16.1%). Headache was the most common presenting symptom (n=131, 28.5%). There is a significant relationship between age group and the histological type of primary central nervous system tumour (p<0.001). There is also a significant relationship between gender and the histological type (p<0.001). However, there is no significant relationship
between race and the histological type of primary central nervous system tumour (p=0.210). Among the demographic factors studied, age and gender do have a significant relationship with the histological type of primary central nervous system tumour but race does not.

P257. **The value of fine needle aspiration cytology in the diagnosis of difficult breast lesion: The diagnostic clue**

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**Background:** Fine Needle Aspiration Biopsy (FNAB) of the breast has earned its reputation in the diagnosis of a wide spectrum of breast lesions. Fresh FNAB aspirated material processed into cell block and examined cytologically under light microscopy and further subjected to a panel of relevant immunohistochemical stains provide valuable diagnostic clues for difficult breast lesions. **Method and Design:** We report a case of a 51 year old Malay lady, who presented to us with a history of rapidly enlarging Left breast mass within a year duration. Clinically there was an associated hypercalcaemia (serum calcium 5.17mmol/L) which requires pamidronate for biochemical control of the suspected paraneoplastic hypercalcaemia (serum parathyroid hormone not raised 0.4 pmol/L). There was a huge left breast mass within the upper outer quadrant measuring 15x14x8cm. FNAB performed and material sent for cell block processing. **Results:** A list of differential diagnoses was considered under Category 5 (C5) for a malignant lesion. The possible diagnoses were Ductal Carcinoma (specialized variant of Cribriform type), neuroendocrine carcinoma (small cell neuroendocrine carcinoma), basal phenotype ductal carcinoma and solid variant of mammary adenoid cystic carcinoma with basaloid features. Hematoxylin & Eosin stained sections of the cell block complement the cytology features of the prepared smears. Immunohistochemical stains done on the cell block sections show diffuse positive staining for estrogen receptor (>90%), progestrone receptor (50%), cErb B2 (overexpressed), E Cadherin (3+) and CK7 (4+). Negative staining was observed in chromogranin, synaptophysin, neuron specific enolase, TTF-1, smooth muscle actin, vimentin and CK 20. Therefore, the final cytology report was validated as malignant breast lesion; consistent with ductal Carcinoma (NOS). Postoperative surgical pathology report and review from a referral center confirmed the above final diagnosis. **Conclusion:** This case reaffirms the role of FNAB in providing rapid diagnosis and proven as a reliable method of fresh sample procurement for cell block and immunohistochemical staining. FNAB can be regarded as a valuable method in the provision of rapid, accurate and confirmatory diagnosis in a wide spectrum of breast lesions. FNAB serves and facilitates the subsequent surgical and oncology management of these patients.

P258. **Nosocomial infection among intensive care unit and high dependency ward patients in a public hospital in Malaysia**

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Nosocomial infections (NI) continue to be a major public health concern throughout the world. Infection is defined as nosocomial when patients acquired infection in the hospital after 48 hours admission, confirmed by culture results and treated with antibiotics. To describe the pattern of infections and antibiotic resistance pattern in ICU and HDW patients in a public hospital in Malaysia, a cross sectional study using retrospective data was conducted at the Microbiology Unit in a public hospital in Malaysia.
from January to December 2003. A total of 377 positive cultures were isolated from 104 patients. Of these patients, 185 episodes of NI were identified. The infection rate of ICU and HDW patients from the total admission was 2.98%. The age range was from 13 to 80 years (mean = 50.71 ± 17.51) with the 61-70 years age group predominating the episodes of NI. The male to female ratio was 2:1, the majority were Malay (51%), followed by Chinese (28.8%), Indian (18.3%) and others (1.9%). The most common specimen came from tracheal aspirate (45.1%), followed by blood (29.4%) and pus (8.5%). Respiratory tract infection (42.7%) was the most frequent infection identified, followed by bloodstream infection (29.2%) and peritoneal infection (7.6%). *Acinetobacter* species (27.8%) was the most common bacteria isolated, followed by *Pseudomonas aeruginosa* (18%) and *Staphylococcus aureus* (12.9%). Methicillin-resistant *Staphylococcus aureus* accounted for 72.7% of all *Staphylococcus aureus* isolated. The proportion of extended spectrum beta-lactamase producers was 62.8% among *Klebsiella pneumoniae* and 40% in *Escherichia coli*. Among the Enterobacteriaceae, *Klebsiella pneumoniae* was highly resistant towards ceftazidime (72.7%) and cefoperazone (64.9%) while *Escherichia coli* was resistant towards cefepime (25%). Gentamicin (86.6%) and ciprofloxacin (79%) resistance was noted in *Acinetobacter* species. Among gram positive bacteria, coagulase-negative staphylococci were highly resistant towards oxacillin (91.2%), gentamicin (76.5%) and erythromycin (73.5%). Effective infection control measures practiced contributed to low infection rate among ICU and HDW patients. Respiratory tract infection was noted as the most common infection, whilst *Acinetobacter* species was the most frequently isolated organism. The occurrence of NI was highest in elderly patients.

P259. Effects of ontensification of traditional farming system on the environment and bio-safety of human population—Nipah virus experience

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A new virus named Nipah virus (NiV) belonging to Paramyxoviridae family was isolated from a human case and was confirmed to be responsible for the disease in both humans and pigs in late 1998. The pig disease was characterized by a pronounced respiratory and neurological syndrome and sudden death. The outbreak which appeared in pig farm workers in Tambun, Perak spread southward to Negeri Sembilan and Selangor. Between October 1998 and May 1999 an excess of 265 viral encephalitis cases among pig farm workers with 105 human deaths were recorded. On confirming NiV as the etiology mass culling of diseased and in-contact pigs was instituted to eradicate the disease. The culling of pigs successfully controlled the human epidemic in all states. During this phase I control program an estimated 901,228 pigs from 896 farms were destroyed. The phase II control program involved screening of 82,000 samples from all remaining farms in the country to detect antibodies against NiV by an indirect ELISA. Another 50 farms that involved 172,750 infected pigs were depopulated. Neutralizing antibodies to NiV were detected in both insectivorous and frugiverous bats. The human NiV outbreak in Malaysia was speculated to have had as a necessary condition of the “jump” of NiV from a wildlife reservoir in fruit bats to pigs reared under intensive farming conditions. The movement of infected pigs from farm to farm and then, interstate and close contact of people with infected pigs could have caused the human outbreak. The need for preparedness and an early warning system to handle any emerging infectious disease in future is vital. The importance of efficient and effective disease diagnostic capabilities in the veterinary and medical laboratories at national and international level must be emphasized. Intensification efforts in any farming system must be planned together with their effects on environment, the flora and fauna, bio-security and sanitation, and bio-safety of human populations.
P260. HIV/Tuberculosis co-infection: Liver biopsy findings

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**Background:** HIV and tuberculosis co-infection in an individual constitutes a serious diagnostic and therapeutic challenge. The diagnosis of tuberculosis using hypersensitivity skin reaction such as the Mantoux test could turn out to be a poor predictor in an immunocompromised individual, and the yield of mycobacterium tuberculosis from sputum or gastric washings is also poor. We undertook this study as an assessment of organ involvement in this disease. **Methods:** Prospective postmortem liver biopsies were done on 67 patients who died of complications of HIV/TB co-infections in Jos University Teaching Hospital. **Results:** A total of 67 biopsies were analysed comprising of 33 males and 34 females. Majority (39) had pulmonary tuberculosis (PTB) while 26 had disseminated tuberculosis (DTB). Only 2 had tuberculous meningitis (TBM). Fourteen (36%) patients with PTB, 10 (38%) with DTB and one (50%) with TBM had hepatic granuloma with caseation. The spectrum of histological diagnosis was granuloma 25 (37.3%), chronic hepatitis 13 (19.4%), non-specific reactive hepatitis (NSRH) 11 (16.4%), steatosis 9 (13.4%), cirrhosis 7 (10.4%), while 1(1.5%) had normal histological picture. The age of the patients ranged from 18 years to 55 years with a mean of 35.5 ± 8.4 years. **Conclusion:** This study showed that the liver is frequently involved by TB and other opportunistic infections in HIV/TB co-infection irrespective of whether it’s PTB, DTB or TBM. Therefore, liver biopsy may be beneficial in the management protocol of HIV/TB co-infection.

P261. Contamination of cooling tower of centralized air condition with legionella: Serological evidence of exposure to college students

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A study of Legionella in cooling towers of research institute in Malaysia was carried out in the year 2005. A total of 66 samples were analysed for the presence of Legionella and 38 samples were found to be positive for *Legionella pneumophila*. To assess the level of exposure to Legionella through centralized air-conditioning, newly registered college student of the institute was followed-up over time. Antibody against *Legionella pneumophila* serogroup 1 and serogroup 2-15 was measured using in-house ELISA format. A cohort of 31 newly registered college students was examined at registration and was repeated 6 months after enrolment. For the *Legionella pneumophila* serogroup 1 ELISA, the mean antibody increased from 0.277 ±0.312 at registration to 0.359 ±0.473 6 months later (p = 0.010). Similar there was increased in anti-serogroup 2-15 antibody from 0.64 ±0.266 to 1.019 ±1.012 (p = 0.007).
P262. Immunoglobulin class switching in human B cells induced by *Plasmodium falciparum*

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The immunoglobulin gene expression particularly in switching region of heavy chain in human B cell after stimulated with *Plasmodium falciparum* crude antigens were investigated. This *in vitro* study mimicked natural falciparum malaria when immune cells exposed to antigens release from lytic infected RBC. Human B cells from 3 healthy donors were cocultured with crude antigens obtained from Thai uncomplicated falciparum malaria and a cerebral malaria (CM) patients, and a laboratory isolate of *P. falciparum* for 4 days. The B cells from each donor cocultured with IL-4 and anti-CD40 were used as positive control. After incubation extracted RNA were determined the occurrence of Ig class switch recombination (CSR) from IgM, to IgG and IgE by detecting activated-induced cytidine deaminase (AID) mRNA expression, germline gene transcripts (GLT), switch circle transcripts (CT), β mRNA and ε mRNA by RT-PCR. The result showed all stimulated B cells could detect CSR with the different patterns of AID mRNA, GLT, CT and mRNA and mRNA expression among 3 donors and the crude antigens. β, ε-GLTs and β mRNA expression were observed in all stimulated and unstimulated B cells from all donors whereas expression of Iβ-Cµ and Iε-Cµ CT varied among the different stimulation factors and donors. There was no occurrence of CSR indicated by AID mRNA expression and CT in unstimulated B cells. Our preliminary finding indicates that crude *P. falciparum* antigens could stimulated CSR to IgG and IgE in human B cells *in vitro*. However, purified crude *P. falciparum* antigens and monoclonal antibody to B cells are needed to gain better understanding in mechanisms of CSR by *P. falciparum*.

P263. A novel ferritin –H sequence variant in African patients with oesophageal squamous cell carcinoma

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**Background:** Oesophageal cancer is one of the most fatal cancers with a multi-factorial aetiology. One of these factors seems to be the accumulation of iron. Iron is known to catalyse the formation of reactive oxygen species (ROS) and is thought to promote oesophageal carcinogenesis through ROS- mediated oxidative damage. Protection against iron-induced oxidative stress is provided by either antioxidant enzymes or by ferritin. Ferritin is the main iron storage protein in all living species and consists of two functionally and genetically distinct subunits, termed H (heavy) and L (light). The structure of ferritin genes and proteins are highly conserved, probably due to the critical role of ferritin in the maintenance of iron homeostasis. Changes in ferritin are important not only in the classic diseases of iron acquisition, transport, and storage such as iron overload, but also in diseases characterized by inflammation, infection, injury, and repair. **Methods:** We screened 117 oesophageal cancer patients and 130 control subjects for mutations in the coding regions of *ferritin-L* and *ferritin-H* by PCR, SSCP, RFLP and subsequent sequencing. **Results:** In the present study, we identified a novel
sequence variant, 249 T>G (Leu 83 Leu) in the ferritin-\(H\) gene. The mutation was detected in 10 (7.7%) oesophageal cancer patients and 3 (2.3%) of control subjects and this was statistically significant, \(P = 0.049\).

**Conclusion:** Oesophageal cancer which is highly prevalent in South Africa is a complex disease likely resulting from polymorphisms of multiple interacting genes and gene-environment interactions. Further functional analysis will show whether the 249 T>G variant in the ferritin-\(H\) gene contributes to oesophageal carcinogenesis.

**P264. A Pathology Department cervical cancer screening clinic: A decidedly non-traditional activity**

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Hong Kong in 1995, despite being a world financial hub, and with the latest medical technology available, had no organised cervical cancer screening programme. Pap smears were taken in an opportunistic manner and those who benefited mostly were women who were well educated and or in the higher socio-economic group. As a community project, a free cervical cancer screening service was set up at the new Sir YK Pao Cancer Centre, Prince of Wales Hospital under the auspices of the Department of Anatomical and Cellular Pathology, Chinese University of Hong Kong. The Clinic provided a Pap smear for all eligible women and also undertook to refer those with an abnormal result for treatment. Funding for the Clinic was provided by Non-Governmental Organisations (NGO). The additional Pap smears from the Clinic placed a strain on the Cytology Department. The resulting delay in reporting smears lead to patient anxiety. When the problems were discussed with the NGO additional funds were provided and this allowed liquid based cytology to be implemented and the purchase of an automated slide reading system FocalPoint® (formerly AutoPap), manufactured by TriPath. After this all Pap smears, both conventional and liquid based, were subjected to initial screening with the FocalPoint®. This greatly improved Pap smear examination and additionally, there was a marked improvement in staff morale. Operating a Pap smear clinic was a very non-traditional activity for a pathology department but an unexpected benefit, of immense value, was the goodwill it generated between the department and the community.

**P265. Regulators of apoptosis in human breast cancer**

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**Objectives:** Tumor cells with DNA damage undergo programmed cell death called apoptosis. Bcl2, BAG-1 and P53 gene products have been linked to apoptosis. The purpose of the present study was to investigate expression of Bcl2 expression in breast tumors and compared it with established clinicopathologic prognostic factors, BAG-1 and mutant P53. **Methods:** Tissue samples obtained from 165 breast cancer patients were stained by immunohistochemical localization on paraffin embedded tissue sections for Bcl2 (Novocastra, UK), BAG-1 (Dakocytomation, CA, USA) and P53 (Dako, Denmark).

**Results:** Fifty seven percentage patients showed Bcl2 cytoplasmic positivity with staining intensity of 1+ in 44% (72/165), 2+ in 8% (13/165), 3+ in 5% (09/165). Bcl2 was correlated with clinicopathological parameters by grouping the patients according to age (<48 years younger age and >48 years older age) and lymph node (LN) status. In younger age group, Bcl2 showed inverse correlation with tumor
size (P=0.036), LN (P=0.036), Stage (P=0.001) and Grade (P=0.013), and no such observations were found with older age group. When grouped according to lymph node (LN) status, Bcl2 showed an inverse correlation with ER positivity (P=0.044) only in LN positive patients. In LN negative patients apart from clinicopathological parameters Bcl2 inversely correlated with BAG-1 (P= 0.015) and positively correlated with P53 (P=0.022). Survival curves determined by the Kaplan-Meier method demonstrated that Bcl-2-positivity was found to be associated with favorable prognosis (P=0.04) in lymph node negative patients. **Conclusion:** The data revealed that Bcl-2 over expression may be a favorable prognostic factor in lymph node negative breast cancer.

**P266. Erythrocyte markers of oxidative insult in the acute phase of graded traumatic head injury in humans**

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**Objective:** Acute oxidative stress following a traumatic head injury (HI) has been implicated to induce severe secondary brain damage and to influence the clinical outcome of HI patients. This study was performed to evaluate and compare the oxidative changes in patients with varying severity of head injury in the early posttraumatic period using the erythrocyte markers. **Methods:** Head injury (HI) patients were divided into two groups based on their Glasgow Coma Scale (GCS) scores recorded at admission to the hospital on the day of trauma itself. Accordingly, the study included 30 severe HI (SHI, GCS scores 8 or less) and 25 Mild HI (MHI, GCS scores more than 8) patients. Thirty age and sex matched healthy individuals were included in this comparative study as controls (NC). Venous blood samples were withdrawn from NC and HI patients (within 24 hours of trauma onset). Erythrocyte oxidative changes were studied by estimating thiobarbituric acid reactive substances (TBARS) and glutathione (GSH) levels and by assaying the activity of superoxide dismutase (SOD). **Results:** Erythrocyte TBARS levels were significantly higher and GSH levels were significantly lower in SHI and MHI patients as compared to NC. SOD activity was significantly increased only in SHI patients and remained unchanged in MHI patients as compared to NC. As compared to MHI patients, erythrocyte TBARS levels were significantly higher, GSH levels were significantly lower and SOD activity was markedly elevated in SHI patients. **Conclusion:** Early oxidative changes were found to vary relatively with the severity of trauma in HI patients. These early changes could have a significant impact on the neurological recovery of HI patients.

**P267. Evaluation of wide broad spectrum antibiotics resistance of E. coli and Klebsiella**

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**Introduction:** Treatment with wide broad spectrum antibiotic against bacteria can lead to resistance. The resistance can be seen in two types, chromosomal alterations due to changes in structure of receptors of specific drugs. Plasmid resistance usually produces enzymes that can result in destruction of antibiotic. In fact ESBLs are enzymes from plasmid with resistance to the generation of cephalosporin, monobactams and new penicillins. The reason for the formation of ESBLs plasmid is the point mutations which are produced due to high amount consumption of broad spectrum antibiotic. **Objective:** for achieving a new strategy for diagnosis of for treatment of plasmid resistance of ESBL on E. coli and
**Materials and Methods:** 218 samples were collected from patients referred to Al-zahrah hospital. JUMS, Esfahan, Iran. The patient specimens were used for detecting of ESBLs enzymes by combined and double disks tests, to check for TEM, SHV genes by PCR method in the *E. coli* and *klebsiella* isolated organism. **Results:** 218 patients specimens, 148 (68%), 70 (32%) were obtained positive culture of *E. coli* and *Klebsiella* respectively. **Out of 132** *E. coli* and *Klebsiella* species were showed ESBLs Enzyme 76 (50%) and 49 (70%) respectively. **In addition, the** *E. coli* and *Klebsiella* 31%, 20% TEM and 15%, 30% SHV were showed plasmid genes respectively. **Conclusion:** the result of this study revealed that the rate of ESBLs is higher than in similar studies in other countries.

**P268. Prospective & retrospective study of cases of carcinoma prostate – their histological typing with special reference to Gleason’s microscopic grading system and its relationship to PSA levels**

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**Introduction:** Carcinoma of the Prostate is the most common internal malignancy among men in the US. Although most prostate cancers are slow growing and remain clinically unrecognized, their course is often unpredictable because of the considerable heterogeneity of the histologic grade and a host of other factors that affect tumour growth. **Objectives:** 1. Study of prospective and retrospective cases of carcinoma prostate. 2. Histological typing of carcinoma prostate cases. 3. Grading adenocarcinomas as per Gleason’s microscopic grading system. 4. Statistical correlation if any, between high serum PSA levels with high Gleason score. 5. Comparing our results with that of other similar studies in literature. **Method:** 100 cases of carcinoma prostate were studied (January 98 to September 05). Samples included needle biopsies, TUR and prostatectomy specimens. Sections were routinely processed and stained by H&E. **Observations:** We graded the specimens across the full spectrum of Gleason score (GS) 2-10, and also did specific grouping of grades, that separated the cases into more clinically meaningful categories. These categories included GS 2-4, GS 5-6, GS 7 and GS 8-10. Out of 100 cases studied, 45 were GS 8-10, 17 cases were GS 7, 24 cases were 5-6, 12 cases were GS 2-4. 2 cases were primary small cell carcinoma, oat cell type. Correlation coefficient between the Gleason score and PSA levels was found to be statistically significant at p = < 0.001, r = 0.43. It signified that with increasing Gleason score, the serum PSA levels showed a statistically significant increase. **Conclusion:** We attribute the large percentage of cases (45%) in GS 8-10 to the fact that for lack of effective screening programme in many developing countries, most cases report late in the disease course with obstructive symptoms. These cases were associated with a higher serum PSA levels as well. As 75% of our cases were diagnosed on TUR chips, it indicates that majority of prostatic carcinomas were incidentally diagnosed on routine TUR of prostate. A confirmed pre-operative diagnosis of malignancy is usually not available with the surgeons, thus planned radical prostatectomies were almost non-existent, as was evident from our study.
P269. Apoptosis-induced chromosomal breaks within the scaffold attachment region (SAR) of the mixed lineage leukemia (MLL) gene

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Chromosome rearrangements such as additions, deletions, translocations and inversions are commonly observed in different types of cancer. Various mechanisms have been proposed. Recently, the apoptotic nuclease was proposed to be involved in chromosome translocations in leukemia. In leukemia, the Mixed Lineage Leukemia (MLL) gene on chromosome 11q23 has been found to translocate with many partner genes, where the breakpoints mapped within an 8.3 kb MLL breakpoint cluster region (bcr). Furthermore, deletions at chromosome 11 q23 are also well reported in nasopharyngeal carcinoma (NPC) although the breakpoint has not been mapped to the gene level. This study aims to analyse apoptosis-induced chromosomal breaks and rearrangements in NPC and leukemia cell lines. NPC and leukemia cell lines were induced to undergo apoptosis by overgrowing the cells to high densities followed by revival in fresh medium to rescue them. The leukemic cells were also induced to undergo apoptosis by treatment with low and high concentrations of etoposide (VP-16). Nested Inverse Polymerase Chain Reaction (IPCR) was employed to detect the chromosomal breaks and rearrangements in the MLL bcr. Our results show that both the cell density-induced apoptosis in NPC and leukemic cells generated chromosomal breaks near the 3’ end of the MLL bcr. Both the 3’ and 5’ breaks were located centromeric to the MLL bcr region. However, no translocation of other genes to the break sites was detected. Comparison of these DNA breakpoints revealed a common region of DNA breaks (3’ end of MLL bcr near to exon 9), which mapped within the Scaffold Attachment Region (SAR). Our results suggests the possibility that chromosome rearrangements could be dependent on chromatin structure but independent of cell type and apoptotic stimuli.

P270. Evaluation of CHROMagar® for MRSA screening

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Introduction: The Society for Healthcare Epidemiology of America has recommended that active surveillance for methicillin-resistant Staphylococcus aureus (MRSA) from the nares of those at risk is one of the strategies in the control of nosocomial transmission of MRSA. Therefore, it is important that a laboratory uses a rapid and highly sensitive test to screen for MRSA for prompt initiation of infection control measures. MRSA CHROMagar® (BBL) is a selective and differential medium for qualitative detection of nasal colonization by MRSA that is recently made available in Malaysia. The Microbiology Laboratory of Kuala Lumpur Hospital has been screening MRSA carriers using the conventional sheep blood agar which requires 48 hours for identification. The introduction of a selective and differential medium would be considered appropriate. Objective: The study was carried out to determine the sensitivity and specificity of CHROMagar® media in comparison to the current method used. Methodology: 100 nasal swab samples were screened for MRSA. The samples were plated on both sheep blood agar and CHROMagar®. After 24 hours of incubation, colonies suspicious of Staphylococcus aureus on sheep blood agar and mauve-coloured on CHROMagar® would be subjected to tube coagulase and oxacillin susceptibility tests. In the absence of colonies, the plates were further incubated for another 24 hours. Results: MRSA were detected from 9 samples (9%). All the isolates were detected from both sheep blood agar and CHROMagar®. All mauve-coloured colonies on CHROMagar® were identified as MRSA. All the organisms were positive at 24 hours of
incubation. Further incubation did not improve recovery of organisms on either medium. The cost per test using CHROMagar® was 3 times higher than the conventional method. **Conclusion:** The sensitivity and specificity of CHROMagar® in detecting MRSA was 100% and further confirmation by coagulase test and oxacillin disc were deemed unnecessary. Our results suggest that in a centre with no financial constraint, the use of CHROMagar® in the screening for MRSA from nasal samples yield excellent and rapid identification.

P271. Expression of cell cycle regulatory protein , cyclin D1 in carcinoma of prostate

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The detection of prostatic cancer, its clinical staging and the prediction of its prognosis remain topics of paramount importance in clinical management. This study aimed to detect the expression of cell cycle related protein cyclin D1 in prostate cancer and to correlate the immunoreactivity of this protein with clinicopathological prognostic parameters: tumour size, clinical stage, PSA level, histologic type, Gleason score, mitotic count vascular and neural invasion and degree of necrosis. Cyclin D1 expression in 30 prostatic cancers was determined by immunoreactivity staining of section cut from paraffin tissue blocks. Brown staining of tumour cell nuclei was regarded as positive immunoreactivity. The immunohistochemical expression of cyclin D1 was observed in 11 tumours (35%). The immunohistochemical was statistically associated with poorly differentiated histologic type (P=0.036) and degree of necrosis (P=0.05). There is increasing evidence that cyclin D1 expression is important in tumogenesis and tumour progression in prostatic cancer. This study demonstrated that cyclin D1 expression could predict the poor prognosis of prostatic cancer. Further studies with the larger sample sizes and survival analysis are suggested to search for biological characteristic and the role of cell cycle related proteins in carcinogenesis of prostatic cancer.

P272. Expression of β-catenin and matrix metalloproteinase 7 (MMP-7) in colorectal carcinoma

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Colorectal cancer (CRC) is the most common gastrointestinal malignancy in Malaysia. -catenin protein is a member of the cell membrane-bound adhesion complex and one of the key regulators in the transduction of Wnt signalling pathway. -catenin may be involved in CRC tumourigenesis because it binds to the product of the tumour suppressor gene APC. Matrix metalloproteinase 7 (MMP-7) is the smallest member of the matrix metalloproteinase (MMP) family. The protein is capable of degrading various extracellular matrix (ECM) proteins and was found overexpressed in colon cancer and many other human cancers. The current study was carried out to determine the frequency of -catenin and MMP-7 expression in colorectal carcinomas, and correlate the expression of these proteins with race,
age, gender, histological grading, Dukes’ staging and nodal status. We also evaluated association between 
β-catenin and MMP-7 expression in these tumors. Expression of β-catenin and MMP7 was detected in 93 and 80 archival CRC specimens, respectively, using immunohistochemistry. Immunostaining of these proteins was performed using standard streptavidin-biotin-peroxidase system. Overexpression of β-catenin and MMP-7 proteins were detected in 68.8% and 38.8%, respectively. Our data showed that elevated levels of MMP-7 (P<0.05) were significantly correlated with poorly-differentiated tumors but not with race, age, gender, Dukes’ staging and nodal status. Overexpression of MMP-7 protein was detected frequently in poorly-differentiated (84%) and moderately -differentiated (56%) compared with well-differentiated (21%) tumors. However, increased expression of of β-catenin was not associated with patient’s characteristics and clinico-pathological parameters. No statistical association was found between β-catenin and MMP-7 expression. The above observations show that upregulation of MMP-7 may be an important event in tumor differentiation.

P273. Reference values for acid-base status in clinically healthy dogs

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Objective: The accurate determination of the acid-base status as well a the status of oxygenation is an extremely important procedure in veterinary medicine. The evaluation of acid-base status provides important information especially in relation to the urinary, respiratory, digestive and endocrine systems. Much of the published work on blood gas analysis is based on human sampling. The goal of this study was to establish reference intervals for acid-base values and blood gases in clinically healthy dogs using the Stat Profile Critical Care Xpress (Nova Biomedical, Waltham, Ma, USA) multiprofile analyzer with application of biosensor-based methods. Methods: Blood was collected from 30 healthy adult dogs various breeds and sex into a commercial preheparinised syringe. Samples of blood were taken from the femoral artery in anaerobically conditions. After collection, the closed syringe was immersed in a mixture of ice and water. The acid-base values were determined within 2 h of blood sampling. Descriptive statistics, including 95% confidence intervals were calculated. Results: Mean blood values were: pH 7,392 ±0,045, pCO₂ 29,22 ±2,37 mmHg, pO₂ 99,69 ±16,74 mmHg, PCV 39,33 ±6,15 L/L, Hb 118,1 ±14,1 g/L, A 112,92 ±2,94 mmHg, a/A 0,88 ±0,13, A-aDO2 16,72 ±8,9 mmHg, HCO₃ 17,81 ±1,73 mmol/L, BE-ecf -7,37 ±2,38 mmol/L, BE-b -5,23 ±2,38 mmol/L, respiratory index 0,19 ±0,12, SBC 20,17 ±1,9 mmol/L, TCO₂ 18,71 ±1,74 mmol/L, pO₂/FIO₂ 476,9 ±80,1 mmHg, SO₂ 96,89 ±1,48 %, and lactate 2,61 ±1,89 mmol/L. Conclusion: Reference interval obtained on the Stat Profile Critical Care Xpress provide valuable baseline information for assessing new acid-base parameters and for interlaboratory comparisons. Differences compared with previously published reference values can be attributed largely to differences in methodology. Acid-base values and blood gases determined in this study can be considered reference data for health control and disease diagnosis.
P274. **Reference values for co-oximetry in clinically healthy dogs**
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**Objective:** Co-oximetry is a complex and reliable laboratory method that measures hemoglobin pigments by dedicated multi-wavelength spectrophotometry. Four species of hemoglobin typically circulate in animals blood: oxyhemoglobin, reduced or deoxyhemoglobin, methemoglobin and carboxyhemoglobin. Measurement and interpretation of co-oximetry are important in clinical practice. Determination of total and relative hemoglobin concentrations is important in the assessment of oxygen transport and in the evaluation of anemia and methemoglobinemia. The aim of this study was to establish reference values for co-oximetry in clinically healthy dogs using the Stat Profile® Critical Care Xpress (CCX) (Nova Biomedical, Waltham, Ma, USA) multiprofile analyzer with co-oximetry module.  

**Methods:** Blood was collected from 27 healthy adult dogs various breeds and sex into a commercial preheparinised syringe. Samples of blood were taken from the femoral artery in anaerobic conditions. After collection, the closed syringe was immersed in a mixture of ice and water. CCX co-oximeter aspirates approximately 115 µL of whole blood. The lysed sample is drawn into an optical cuvette, and the absorbance of light is measured at 7 predefined wavelengths. The co-oximetry (tHb-total hemoglobin, HHb-deoxyhemoglobin, O₂Hb-oxyhemoglobin, MetHb-methemoglobin, COHb-carboxyhemoglobin, SO₂-oxygen saturation, O₂Ct-oxygen content, O₂Cap-oxygen capacity) were determined within 2 h of blood sampling. Descriptive statistics, including 95% confidence intervals were calculated.  

**Results:** Mean values (±SD) for canine blood were: tHb 165.5 ±38.59 g/L, SO₂ 98.81 ±0.53 %, O₂Hb 96.47 ±1.29 %, COHb 2.10 ±0.98 %, MetHb 0.25 ±0.13 %, HHb 1.15 ±0.48 %, O₂Ct 22.06 ±4.81 vol%, O₂Cap 22.38 ±4.99 vol%. Our reference values are similar with values in the CCX manual, except COHb which is higher.  

**Conclusion:** Co-oximetry values determined in this study can be considered reference data for health control and disease diagnosis.

P275. **Osteomyelosclerosis and bone densitometry – A case report.**

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A 59 year-old woman presented in 2006 with symptomatic hypochromic, microcytic anemia, having been investigated elsewhere 5 years previously for a similar presentation without a conclusive diagnosis. In the interim period, she had been transfusion-free. At the initial presentation to our hospital, her Hb was 6.5 g/dl, TWBC 10,100/ul, platelets 355 000/uL. Physical examination was significant for pallor and massive splenomegaly. Iron studies were normal The blood film revealed leucoerythroblastic anemia with tear drop cells. Hb analysis revealed: Hb E 24.2 %, Hb F 1.2 %, with no H inclusions detected. The bone marrow aspirate was dry. The bone marrow trephine sections showed extensive fibrosis with markedly increased reticulin and endophytic new bone formation. Plain Xray of the pelvis was normal. Bone densitometry at the hip revealed a T score of +2.9 and a Z score of +3.6; bone densitometry at the lumbar spine revealed a T score of +1.4 and a Z score of +2.5. This case illustrates that while plain radiography may be insensitive to detect new bone formation in osteomyelosclerosis, this pathological change may be demonstrated by bone densitometry. The finding of Hb E trait in this patient is most likely incidental.
P276. Immune thrombocytopenic purpura complicating polycythemia rubra vera – A case report

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A 32 year-old woman presented with frequent headaches and dizziness and was diagnosed to have polycythemia rubra vera (PRV) in 1994. She was on a regime of periodic venesection until 2000. She was then given hydroxyurea for the next 2 years which was later discontinued. She remained well without any treatment for the subsequent 4 years. During this period she continued to have persistent mild neutrophilia, but normal hemoglobin and platelet counts. In July 2006, she developed mild thrombocytopenia which progressively worsened, reaching a nadir of 8000 /μL after 5 months. Concurrent with this, she developed petechial rashes and complained of menorrhagia. She denied any preceding viral illness and was not on any new drugs. Physical examination revealed moderate splenomegaly without any significant peripheral lymphadenopathy. She declined a repeat bone marrow examination. Anti-platelet antibody assay was negative. She was treated presumptively for immune thrombocytopenic purpura with prednisolone 1mg/kg. She improved dramatically with a rise in platelets to 108,000/μL which later stabilized, enabling the tapering off of steroid therapy after 4 months. PRV is a clonal disorder now thought to be related to a mutation in the JAK2 gene whereas ITP is an autoimmune disorder. These disorders are pathophysiologically unrelated; their temporal relationship in this patient is, albeit interesting, probably a coincidence. It does, however, underline the fact that different, unrelated hematologic disorders may sometimes co-exist in the same patient.

P277. Hairy cell leukemia – A case report

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Hairy cell leukemia (HCL) is an uncommon hematologic malignancy. We present a case of HCL who presented to our hospital with persistent fever. The patient was a 38 year-old Indian man who had moderate hepatosplenomegaly and marked lymphocytosis, but anemia, thrombocytopenia and neutropenia. Imaging revealed extensive enlarged lymph nodes in the mediastinum, axillae, abdomen and right inguinal region as well as multiple splenic nodules with splenomegaly, leading to an initial clinical suspicion of malignant lymphoma. Bone marrow aspiration was dry; trephine biopsy showed an infiltrate of lymphoid cells. Biopsy of the right inguinal lymph node showed a similar feature compatible with the diagnosis of hairy cell leukemia. Later review of his peripheral blood film showed typical hairy cells and leucoerythoblastic anemia. Flow cytometry of peripheral blood indicated that the circulating lymphoid cells expressed CD11c, CD19, CD20, CD22, CD25, FMC-7 and HLA-DR, consistent with hairy cell leukemia. He was treated with a single 5-day course of cladribine and achieved partial remission. He was subsequently lost to follow up after documentation of relapse 2 years later. This case illustrates the importance of morphology in the diagnosis of this condition.
P278. Prevalence of MRSA among health care staff working in Afshar General Hospital, Yazd, Iran

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*Staphylococcus aureus* is a common cause of pyogenic infection. Between 20% and 30% of people carry *S. aureus* in their nose and may also carry the organism on their skin. Methicillin-resistant strains cause the same infections as sensitive, but are particularly associated with hospitalization, exposure to invasive procedures and treatment in intensive care. **The aim of this study was to determine the prevalence of MRSA among Health care staff working in different wards of the Afshar General Hospital from June to Oct. 2005 in the city of YAZD/IRAN**

**Methods:** Samples from nose of 185 Health staff (67 male and 118 female) working in 7 different wards were swabbed and inoculated on the MSA medium. Following incubation at 37°C for 24 hrs, the colonies were further examined for *S. aureus* and the antimicrobial sensitivity testing was performed using 0.5 McFarland suspension of the isolated organism and a standard methicillin disk was embedded on the medium. After 18 hrs. incubation, the zone of inhibition was measured and noted. **Results:** A total of 185 health staff working in 7 different wards at the Afshar General Hospital was participated in this survey. 21 samples (11.4%) were positive for *S. aureus*, in which 10 (47.6%) were found to be MRSA. To wards; ICU and Gynecology were all negative for MRSA. In comparison, person of CCU; Radiology and operation room the most contaminated.

**Conclusion:** The result show that 10 (47.6%) of hospital workers were colonized for MRSA. Although this result is satisfactory, but it is necessary to emphasize that transmission to patients may threaten their life. Therefore, following cleaning up the carriers, intensive care and control program be implicated to stop further transmission.

P279. Urothelial carcinoma with plasmacytoid feature: A case report

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Urothelial carcinoma with plasmacytoid feature is extremely rare. Only 7 cases are described in the literatures and the recent World Health Organization classification. Most of which are associated with poor prognostic outcomes. The authors report a case of urothelial carcinoma with plasmacytoid features diagnosed in a 72-year-old Thai male patient with histological and immunohistological findings and our literature reviews.

P280. Study of parasitic infections in luminal contents and tissue sections of appendix specimens

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**Introduction:** Appendicitis has a worldwide prevalence. Its aetiology is still much to be debated. Parasites, both helminths and protozoa have been suggested to cause acute appendicitis. Studies have demonstrated that parasites were present in the appendix specimens removed from surgery. **Objective:** The objective was to determine the prevalence of parasitic infections in a double study examining both the tissue sections and also the luminal contents in appendices
removed during appendectomy. **Material and Methods:** One hundred appendectomy specimens were taken from Batu Pahat Hospital. Sections from the body and tip of the appendix were taken for histopathological analysis. Tissue specimens were sectioned at 4 µm and stained using haematoxylin and eosin staining method. The sections were classified on their degree of inflammation as per standard criteria. Luminal contents were stored in both 10% neutral formalin and polyvinyl alcohol and stained by both trichrome and modified Ziehl-Neelsen methods. **Results:** Sixteen percent of the cases were positive for parasites. Most of the parasites were detected in the luminal content. In cases that were positive for parasites, 81.25% occurred in patients aged 30 years old or younger. In addition, acute appendicitis was seen in 81.25% of cases that were positive for parasites. Helminths were not detected in the study, only protozoa were present. Unusual parasites that had rarely been reported were detected; this includes Blastocystis hominis, Isospora belli and Microsporidia. **Conclusion:** Detection of parasites in 16% of the cases was considered significant as previous studies did not show such a high incidence. Examination of the luminal content has proven to be valuable as most cases of parasites infection were detected in the luminal extract. Statistical analysis did not show association between acute appendicitis and parasitic infection. In conclusion, a larger sample size should be used in future studies to be able to draw absolute conclusions.

**P281. Differential expression patterns of molecular markers p73 and its isoform p73A in normal, dysplastic and malignant cervical epithelia.**

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**Introduction:** The p73 gene is over expressed in many types of tumours and there is low expression in some normal tissues. The p73 isoform is expressed in normal squamous epithelium and there is loss of expression in invasive cancers. The aim of our study was to determine the differential expression of p73 and its isoform p73α in normal, dysplastic and malignant cervical epithelia. **Material and Methods:** We analysed 48 patients attending the Obstetrics and Gynecology OPD at GH Kuala Lumpur of which 20 were normal and 28 were patients with carcinoma cervix. Immunohistochemistry was done with antibodies to p73 and p73α and staining intensity was scored and graded. Statistical analysis was performed with the chi square test. **Results:** Normal squamous epithelium of cervix showed a uniform and intense staining for p73 in the parabasal cells. Intense staining pattern of p73α immunostain was seen in basal layer of the normal ectocervix. In invasive cervical cancer, almost all cases stained positive with p73 and no specific tumour host interface relationship. Expression of p73 was intense in well differentiated tumours and in the basal cells of dysplastic epithelia of the cervix. Loss of expression in the basal cells lining the invasive front of the invasive carcinomatous islands and in poorly differentiated tumours was seen. **Conclusion:** Different isoforms of p73 are expressed differently in normal and malignant epithelia, hence different isoforms of p73 enhance or suppress neoplastic cell growth. The down regulation of p73 expression in poorly differentiated tumours raises the hypothesis that the loss of expression of p73 is associated with progression and invasion of tumour. The ease of retrieving p73 and uniformity of expression patterns of p73 make it a more reliable marker for cervical carcinoma.
P282. The prevalence of macroprolactin in patients presenting with hyperprolactinaemia at Dr George Mukhari hospital

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Background: Macroprolactin, also known as the big big prolactin, is most frequently a complex of monomeric prolactin and immunoglobulins, mostly IgG with a molecular mass of around 150-170 kDa. Macroprolactin has no biological activity in vivo but it retains immunoreactivity leading to falsely elevated prolactin in many commonly used prolactin immunoassays. Unrecognized hyperprolactinaemia due to the presence of macroprolactin often leads to diagnostic confusion, unnecessary investigation and therapy. Recently, a screening test using polyethylene glycol has been used to identify the presence of macroprolactin. Objectives: To determine the prevalence of macroprolactinaemia in hyperprolactinaemic samples at Dr George Mukhari Hospital using the polyethylene glycol precipitation method, with a view of implementing the routine screening for macroprolactin in hyperprolactinaemic samples in our laboratory. Methods: Hundred and fifty blood samples i.e. fifty from healthy individuals as controls, fifty from pregnant women in all stages of gestation and an additional fifty from the hyperprolactinaemic samples in our laboratory were sampled for macroprolactin screening using the polyethylene glycol precipitation method. Prolactin concentrations were measured by Immulite 2000 DPC before and after treating blood samples with polyethylene glycol. Macroprolactin was diagnosed when prolactin recovery was < 40% post polyethylene glycol treatment. Recoveries between 40 and 50% to be subjected to gel filtration chromatography to confirm or refute the presence of macroprolactin, whereas recoveries > 50% being identified as being due to true hyperprolactinaemia. Results: Four percent (2 out of 50) of our healthy control subjects had macroprolactin and all were women with normal prolactin concentrations before polyethylene glycol treatment. Sixteen percent of our pregnant women had increased prolactin levels explained by macroprolactin. Fifty percent were in the first trimester of pregnancy and 3% in the last trimester. Of our hyperprolactinaemic patients, 26% (13 out of 50) had macroprolactin as the predominant Immunoreactive isoform. Most of the patients with macroprolactinaemia whose hospital records were available (62,5%), presented with symptoms that could be associated with hyperprolactinaemia. In 25% hyperprolactinaemia was a coincidental finding and only one patient presented with a microadenoma which was surgically removed but hyperprolactinaemia persisted post removal. Conclusion: Macroprolactinaemia is a common finding in patients with hyperprolactinaemia. It cannot be differentiated from true hyperprolactinaemia by clinical features alone. Polyethylene glycol precipitation method allows easy identification of macroprolactin in routine clinical practice. There is no doubt that by introducing this testing protocol to detect hyperprolactinaemia due to macroprolactin, our laboratory will contribute to improving patient care and the best use of health service resources. All hyperprolactinaemic samples must first be screened for macroprolactin, if confirmed positive, repeat hormone or neuroimaging studies and unnecessary treatment with bromocriptine should be avoided.
P283.  Microsatellite instability in gastric cancer in a Malaysian population

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Background: Microsatellite instability (MSI) due to defective DNA repair by MLH1 and MSH2 genes has been implicated in various cancers including colorectal and gastric cancers in a Malaysian population. Method: Eighty-two cases of resected gastric cancers diagnosed between 1998 and 2006 were retrieved from the files of Hospital UKM and Hospital Seremban. There were 52 males and 30 females (54 Chinese, 19 Malays, 9 Indians; age range 28 – 83 years, median 69 years) with 55 intestinal and 27 diffuse cancers by the Lauren classification. All 60 cases from HUKM were available for immunohistochemistry (IHC) and PCR analysis. Tumour sections were stained with 2 antibodies, MLH1 (1:50 dilution) and MSH2 (1:150) with loss of nuclear staining of tumour cells for either antibody taken as positive for MSI. PCR analysis was performed on extracted DNA from paired normal and tumour samples using panel of 5 MSI markers namely BAT25, BAT26, D2S123, D5S346 and D17S250. Novel peaks in tumour samples in 2 or more markers were regarded as evidence for MSI. Data were analysed using Genescan 3.1 and analysis software. Results: Six of 60 (10.3%) cancers showed MSI by IHC, three cases each by MLH1 and MSH2 gene. PCR and Genescan analysis confirm the IHC findings apart from detecting 2 additional cases negative by IHC giving a total of 8/60 (13.3%) cases positive for MSI. All 8 cases were Chinese (6M/2F) and all cases but one of intestinal type. Conclusions: The prevalence of MSI in gastric cancer was 13.3% involving mainly intestinal type cancers and this compares well with published results. The study demonstrates the utility of IHC in detecting most cases of MSI although occasional positive cases would only be detected by PCR analysis.

P284.  Depressed salivary immunoglobulin-A among adolescents newly recruited to tennis academies

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Introduction: Studies have shown a relationship between the frequency of upper respiratory tract infections and physical exertion associated with endurance-type sports. Immunoglobulin-A (IgA) is a major component of physiological barriers, that form part of the innate immune system. IgA is central to mucosal immunity, offering protection against infection. Reduced salivary IgA content has been widely reported in association with endurance-type sports, but not with racquet-based sports. The aim of this study was to compare the salivary IgA/protein ratio in samples from new arrivals at two tennis academies with those of well established students at the same specialist training centres. Method: A cross-sectional comparison of salivary IgA levels among new and established students at two specialist tennis-academies (England, UK) at the beginning of an academic year was performed. Male and female adolescents (ages 13-14 years) participants were grouped as; new (n=42) and well-established (n=19) students. IgA and total protein concentrations were determined in saliva samples from subjects collected at three points throughout a day during the first month of the new academic year. Laboratory analysis was performed using the Bradford protein assay and an immunoturbidimetric IgA assay, run on a Cobas Mira. IgA/protein ratios were compared between the two groups using non-parametric statistical tests. Results: There was a significant difference (p=0.002) between the new and established students. The median (inter quartile range) IgA/protein ratio for new students was 51.4 (35.4–80.1)
mg/g as compared to 81.8 (46.2–120.2) mg/g for the established students. **Conclusion:** The lower salivary IgA/protein among the new students, as compared to the established students, suggests that salivary IgA/protein measurement is potentially useful for the mucosal-immunity monitoring of new arrivals at intensive tennis academies for young people. This simple test may be of use in monitoring the general health of young athletes.

**P285.** Quantitative variations of leukocyte subpopulations in students suffering allergic rhinitis
Ahwaz University of Medical Sciences

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Allergic rhinitis, which is generally named hay fever, is the most common allergic disease in the world with prevalence of 0.4% to 40%. Many researches have been shown in this disease immune system change. The aim of this study was to investigate leukocyte subpopulations in students suffering allergic rhinitis in Ahwaz University of Medical Sciences. **Thirty patients with allergic rhinitis and thirty non allergic and healthy students (control group) were studied. Allergic patients and control group were selected randomly among student girls of Ahwaz University of Medical Sciences that resident in accommodation. Blood samples were taken, stained with hematological method. WBC count and the percent of lymphocyte, neutrophil, monocyte, eosinophil were determined. The data from two groups were compared and statistically analyzed using SPSS software and t test. The results showed the mean percent of eosinophils increased statistically in test group compared with control group (p<0.034). The mean percentage of lymphocyte and neutrophils and WBC counts were not changed significantly in test group in comparison with control group. However, the mean percentage of monocyte decreased in test group compared with control group (p<0.006).** With regard to increase peripheral blood eosinophils in students suffering allergic rhinitis, it is possible background of other allergic disease such as asthma in these patients occur.

**P286.** A survey on frequency type of recurrent infection and blood cell count in 1-7 years old children at Abuzar Hospital of Ahwaz in 1994-2005

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**Background:** Each living creature should make different defense such as innate and acquired immunity in order to become safe from microorganism’s invasion. **Findings have shown that people with recurrent infections, most of the time are immunocompromised. Objectives:** The goals of this study are including a) study the frequency type of recurrent infection b) study of blood cells counts in patients and compare them with the range of normal population. c) study the relationship between white blood cells with variables such as age/sex/birth body weight and…. in patients with recurrent infections. **Material and Methods:** In this cross study near 100 patients with recurrent infections between 3800 hospital files of patients in Abuzar Hospital in Ahwaz at age 1-7 years old selected. Data including type of infection, WBC count, Europhile percentage, lymphocyte percentage, RBC count, sex, age, birth body weight were collected and compared with normal ranges. **Results:** Puenomi infection frequency among children was highest (42 cases), then UTI (29 cases), Meningitis (24), Otitis (3) and Sinusitis (2 cases). WBC count, lymphocyte percentage, neutrophile percentage, RBC count in patients with recurrent infections had a great increased in comparison with normal ranges in Abuzar Hospital (p value<0.05). Most of
the patients were boys (63%) and had normal weight. Nearly 65% of patients were breast milk in their infancy. The analysis showed there is no relationship between white blood cells with the variables. Conclusion: Most of the patients were suffered from pneumonia, Meningitis, UTI and the changes in WBC were in neutrophiles. It seems that the patients have deficiency in humeral immunity. Further studies including immunoglobulins (IgG/IgM/IgA) assay, lymphocyte B count and complement system (C$_3$/C$_4$/CH50) recommended.

P287. A survey on lymphocyte subpopulations in beta major thalassemia patients

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It is accepted that the immune alterations in patients with thalassemia major are secondary to the continuous transfusion-related antigenic stimulation together with iron overload. The aim of this study was to evaluate immune system of patients with beta-thalassemia major using flow cytometry method. A panel of monoclonal antibodies against T lymphocytes and its subsets, B cell, NK cell in peripheral blood of 25 patients with beta-thalassemia major refer to Thalassemia and Hemoglobinopathia Research Center of Shafa Hospital were used. The results compared with peripheral blood of 30 normal people. The results showed T lymphocytes reduced significantly in patients with beta-thalassemia major in comparison with normal group (P-value< 0.5). This alteration also was significant in CD4+ T lymphocytes. CD8+ T lymphocytes, B lymphocyte and NK cells did not alter significantly. It seems there is deficient in T lymphocyte population in beta-thalassemia major patients. More investigations in related to CD4 + T lymphocytes such as Th1 and Th2 cytokines are suggested.


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There have been many instances of mass disasters reported in history where in large number of people were killed. The most difficult task for the investigating agencies is to establish the identity of victims in situation of mass disaster. Biometric is a science of using biological properties to identify individuals. It has been the most effective, strongest and precise method to identify individuals of mass disaster when other means are not available. The common biometric techniques like finger printing, physical identification, DNA, and dental records have been successfully used to identify victims of mass disaster. Several other promising biometric techniques based on computer system have also been developed like finger print readers, iris scanner, face imaging devices, hand geometry reader and voice readers. Various biometric techniques along with use of Interpol victim identification form and issues that could help in successful and accurate identity of the victims of the mass disasters has been discusses in this paper.
P289. Drug-resistance mutations and subtype strains among HIV-infected infants from Rio de Janeiro, Brazil.

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Objective: the aim of the study was to analyze drug resistance and subtype diversity in the HIV perinatally infected pediatric population. Methods: drug resistance analyses were conducted in a group of 19 HIV infected infants born in Rio de Janeiro State, Brazil, under highly active antiretroviral therapy, through HIV-1 genotyping kit (Visible Genetics Inc., Toronto, Canada). Phylogenetic analysis was done using the neighbor-joining method, Mega program (Kimura 2 parameters). Results: mutations in the pol region conferring resistance to NRTIs, NNRTIs and PIs were detected in 14, 4, and 10 children, respectively. Eleven from the 14 children presented viral resistance to all NRTIs. Phylogenetic analysis indicated that 13 infants were infected with a B subtype strain, 3 were infected with a F1 subtype, 2 were infected with an inter-subtype recombinant B/D and 1 child presented an inter-subtype recombinant B/F strain. According to these subtypes, the most common TAMs distribution was as follows: M41L: 10 samples (8 B, 1 B/D and 1 B/F); D67N: 8 samples (7 B; 1 B/F); T215F/Y: 10 samples (9 B; 1 B/D). The discriminatory mutation M184V was seen in 11 samples being 8 B, 2 B/D and 1 B/F. The B/F inter-subtype recombinant sample presented two mutations of special meaning: H208Y and K103N. The H208Y mutation is prevalent in the B subtype specimens harboring M184V and the TAMs M41L, D67N, L210W and T215Y and has an accessory role in the NRTI resistance. The K103N mutation is most associated with NNRTI cross-resistance, conferring high level resistance to efavirenz. The viral resistance profile observed in the sample presenting both mutations was compatible with this profile except for ddI resistance. Conclusion: our results drive attention to the value of resistance testing for all HIV-infected infants as well to the need to consider subtype diversity in treatment strategies.

P290. Seroprevalence of toxoplasmosis among students at the Faculty of Medicine and Health Sciences UPM: A cross-sectional study.

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Toxoplasma gondii is a protozoan parasite of warm-blooded animals that causes one of the most common parasitic infections in humans. Toxoplasmosis is not a notifiable disease under Communicable Disease Act 1988 and hence its prevalence is based on regional studies. The objective of this study is to determine the seroprevalence of toxoplasmosis among students of the Faculty of Medicine & Health Sciences UPM and its associated factors. This cross-sectional study was conducted to evaluate the seroprevalence of anti T. gondii antibodies among 97 healthy students. ELISA kit was used to detect the presence of specific IgG and IgM antibodies. Socio-demographic background and risk factors associated with seropositivity were investigated using self-administered questionnaires. Blood samples were drawn from the respondents via venipuncture by trained personnel after obtaining written consent from them. The samples were centrifuged to separate serum and stored at -80°C prior to testing for anti-Toxoplasma antibodies (IgG and IgM) by ELISA. The O.D. readings of these antibodies were measured by using DYNATEX Microplate Reader (USA). Prevalence of IgG positivity for this study was 14.4% (n=14), while examination of specific IgM antibodies was negative. There were no
significant differences between genders or between ethnicity (p = 0.380 and p = 0.530, respectively). An increased consumption of satay/barbequed food (0.023%) and unpasteurised milk (0.022%) was observed in serologically positive individuals. No significant relationships were observed between anti-Toxoplasma IgG antibodies and personal hygiene, working with soil, consumption of fast food and untreated water and exposure to cats. Consumption of satay/barbequed food and unpasteurised milk were associated with toxoplasmosis seropositivity in this study. Specific preventive measures to handle the associated factors were recommended.

P291. Report on molecular identification of Candida orthopsilosis isolated from blood culture

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The incidence of candidemia and invasive candidiasis has increased markedly due to the increasing number of immunocompromised patients. There are 5 major medically important species of Candida with their frequency of isolation in diminishing order, namely C. albicans, C. parapsilosis, C. tropicalis, C. glabrata and C. krusei. In addition, there are numerous other species of Candida which differ in their genetic makeup, virulence properties, drug susceptibilities and sugar assimilation capabilities. In this report, an unusual Candida species was isolated from blood culture of two leukaemic patients. Conventional culture and biochemical tests identified the Candida species as C. parapsilosis. Using fungal-specific oligonucleotide primers ITS1 and ITS4, we managed to amplify the ribosomal RNA gene and its internal transcribed spacer region from the genomic DNA of isolates. The PCR products were then purified and subjected to automated DNA sequencing. Upon DNA sequence analysis using BLAST and CLUSTAL softwares, the isolates were identified as Candida orthopsilosis, strain MCO456. C. orthopsilosis is a new species recently identified in 2005, being morphologically indistinguishable from C. parapsilosis. This report highlights the importance of complementing traditional culture and biochemical-based identification methods with DNA-based molecular assays such as PCR as the latter is superior in terms of its discriminatory power and speed.

P292. HPV genotypes in cervical biopsies from Malaysian women

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Human papillomavirus (HPV) infection is universally accepted as the major cause of dysplastic and cancerous lesions in the uterine cervix. The distribution of HPV genotypes has become important with the introduction of HPV vaccination. To gather data on the prevalence of HPV genotypes among Malaysian women, 113 archived cervical biopsies obtained from women (mean age 42.5 years) attending a private hospital were examined by PCR-sequencing for all known HPV types. HPV-positive samples were re-examined by a multiplex PCR for 6 common high risk types (16, 18, 45, 52, 31 and 33) and samples showing low viral load were re-tested by real-time PCR. Overall results from all 3 methods of testing showed the presence of HPV in 51 (45.1%) of the biopsies. The percentage positive by histological diagnosis was 14.3% for normal histology, 25% koilocytosis, 36.4% CIN1, 51.7% CIN 11, 76.0% CIN 111/CIS, and 50% (one of two) adenocarcinoma. In descending order of frequency, the genotypes identified were 16, 18, 52, 45, 33, 11, 58, and 81. As expected, types 16 and 18 were predominant but together, contributed to only 56.8% of HPV genotyped. Although the number of
samples examined was small, these findings suggest that currently available vaccines against HPV types 16 and 18 may not be as effective for Malaysian women as indicated by data reported from clinical trials conducted in other parts of the world.

**P293. Chondrogenic differentiation of human adipose tissue derived stem cells in three dimensional culture system.**

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Damaged or lost cartilage in consequence of traumas and diseases may progress in the serious decreasing of the life quality of affected individuals in all age groups. Mature cartilage has a very limited capability for self-healing due to intrinsic properties. Small defects are regenerated by the migration of chondrocytes. Full-thickness damages are healed by the formation of fibrocartilage, which is biologically and mechanically inferior in comparison with hyaline cartilage. In many cases osteoarthritis is developed. The stem cell therapy offer possibility to solve this problem. There are some sources of adult mesenchymal stem cells (MSCs), including adipose tissue. The aim of this study was to verify the chondrogenic potential of human adipose tissue derived MSCs in vitro, obtained from healthy donors. Collected cells were expanded in vitro. The pellet culture system was used to induce chondrogenic differentiation. Pellets were formed by centrifugation of 1.10^6 cells at 1500 rpm for 10 minutes in polypropylene tubes. After centrifugation fresh DMEM/F12 medium with 10% foetal bovine serum was added. Chondrocytes from the second passage cultured as pellets were used as a control. The culture medium was refreshed every third day during 21 days. After 21 days, the pellets were prepared for histological analysis (Hematoxilin and Eosin, Alcian blue and Masson’s trichrome). For immunohistochemistry, an anti type II collagen monoclonal antibody was used. The cartilage-specific gene expression was examined by real-time PCR. The spontaneous chondrogenic differentiation was observed in all samples. Immunohistochemistry showed the production of collagen type II. Presence of proteoglycans was observed, as well. Real-time PCR confirmed production of collagen type II. Obtained results proved that human adipose tissue derived MSCs have chondrogenic potential in vitro and may play an important role in cartilage tissue engineering. Supported by the grants APVT-20-003104, UK 35/2007 and VEGA 1/4252/07.

**P294. Actinomyces–Like Organism (ALO) infection pattern among intrauterine contraceptive devices (IUCD) users in Malaysia**

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This study determined the infection of Actinomyces–like organisms (ALO) in Malaysian women who have used Intrauterine Contraceptive Devices (IUCD). Cervical smears from 6549 patients from 51 clinics of National Population and Family Development Board, Malaysia (LPPKN) were studied. A cervical smear was taken before IUCD insertion to confirm respondents were free from any infection including ALO infection. After IUCD insertion, cervical smear was repeated at six month intervals and stained by the Papanicolaou (PAP) staining method. Smears also were done for culture, gram stain and wet slide. The stained smears were routinely examined for the presence of any pathological inflammatory
cellular changes and the existence of ALO infection. ALO were diagnosed by the presence of dense blue or purple balls of aggregates with radiating filamentous hyphae using low and high objective light microscope. Out of 6549 respondents, 1488 (22.7%) were new cases, 5029 (76.8%) old cases while no information are available in the remainder 32 (0.5%) cases. The youngest patient seen was 18 years of age while the oldest was 57 years with mean age 39.4 years and mode age 42 years. A total 636 out of 6549 cases studied had infection. Total 257 (3.9%) were infected by ALO. This group consisted of 29 (11.3%) new cases and 228 (88.7%) follow up cases. Respondents infected as early 21 years old. Infection rate was shown to be rising in women of 35 years and above 31.1% amongst age group 40 to 44 years. A total of 4159 (63.5%) were diagnosed as normal smear, 2217 (33.9%) as benign, High Grade Squamous Intraepithelial Lesions (HGSIL) 4 cases (0.1%), Low Grade Squamous Intraepithelial Lesions (LGSIL) 10 cases (0.2%), Atypical Squamous Cells of Undetermined Significances (ASCUS) 22 cases (0.3%). 86 cases (1.3%) were diagnosed as reactive, 13 cases (0.2%) inconclusive and 38 (0.6%) unsatisfactory. The prevalence rate of ALO infection associated with the used of IUCD was 3.9% which was comparable with other reported studies. The results of this study reflected a significant association between ALO infection with age, duration (new and follow up cases) and also showed an association with Pap smear diagnosis.

P295. HLA Antibodies Detection by Complement Dependent Lymphocytotoxicity (CDC) Assay, Enzyme Linked Immunosorbent Assay (ELISA) and Beads based Flow (BBF) Assay in Transplantation candidates.

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The Human leukocyte antigens (HLA) system is important in immune regulation, transplantation, and transfusion. Studies have shown that the presence of anti-HLA antibodies has been associated with hyperacute, acute and chronic graft rejection. Various methodologies are used for detecting anti-HLA antibodies. This study evaluated the performance of conventional Complement Dependent Lymphocytotoxicity (CDC) assay, Enzyme Linked Immunosorbent assay (ELISA) and beads-based Flow (BBF) assay in the detection of preformed HLA antibodies in transplantation candidates. We included 30 awaiting transplantation patients who screened for panel reactive antibodies (PRAs) level by CDC PRA and 14 CDC cross matched pretransplant patients in this study. All sera were then tested sequentially using Enzyme –linked immunosorbent assay (ELISA) and beads-based Flow cytometric assay (BBF). For CDC PRA screening samples, the results showed that both ELISA and BBF assays were able to detect more positive sera in non-sensitized group 27.8% and 38.7% respectively as compared to CDC assay. The agreement for the three assays in Group I (PRA 50%) was 61.1%, 85.7% and 80%, respectively. In CDC-cross match samples, CDC assay was able to detect highest number of positive ser (71.4%). However, combination of these assays allowed detection of 78.6% positive sera. This study suggests that ELISA and BBF are reasonable alternatives for detecting HLA antibodies. These assays can also be used for established the role of non-complement binding antibodies which have been reported to be associated with inferior graft survival.
P296. Clinical utility of anti-cyclic citrullinated peptide autoantibodies (anti-CCP) in Malaysian patients with rheumatoid arthritis

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Background: Anti-cyclic citrullinated peptide autoantibodies (anti-CCP) is a new diagnostic marker for rheumatoid arthritis (RA). It is highly specific for RA, can be detected early in the disease and has prognostic potential. Objective: To determine the sensitivity and specificity of anti-CCP in patients with rheumatoid arthritis. Method: 537 serum samples from rheumatology clinics (Putrajaya, Taiping, Ipoh and Selayang Hospital) were tested for the presence of anti-CCP and rheumatoid factor (RF). These included 171 patients diagnosed with RA, 53 from other rheumatic diseases and 313 healthy controls. Patient demographic data, clinical diagnosis, radiographic information and other laboratory data were recorded. Results: Anti-CCP antibodies were detected in 76.6% (131/171) patients with RA, 13.2% (7/53) in patients with other rheumatic diseases and 8.6% (27/313) in healthy controls. The sensitivity and specificity of anti-CCP reactivity at the recommended cut off values were 76.6% and 86.8% respectively. These was higher than that for RF (52.0%) and (73.6%). However, the presence of both anti-CCP and RF improved the specificity to 94.3%. Conclusion: The detection of anti-CCP is very useful in the diagnosis of RA. However, when used concomitantly with RF, it can improve the diagnostic ability significantly. It is anticipated that, anti-CCP assays will be introduced into routine laboratory practice, which will have a significant impact on early diagnosis of RA thus improving patient care and cost efficiency of diagnostic procedures.

P298. Prevalence and anti-biogram pattern of coagulase negative staphylococci in clinical specimens in Shahid Mohammadi Hospital

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Background: Coagulase Negative staphylococci (CONs), commonly known as part of normal biota of human body (skin and mucosa), have become a predominant pathogens over the last decades. Due to the vast use of antibiotics for therapy or prophylaxis, those CONs strains acquired in the hospitals have become resistant to various antimicrobial agents. According to this regard, the aim of this study was to determine frequency and anti-biogram pattern of CONs in clinical specimens transferred to Shahid Mohammadi Hospital laboratory. Methods: A descriptive cross-sectional study was carried out from December 2005 to June 2006 on 5063 clinical samples. Identification of CONs was performed by routine microbiological methods using gram staining, catalase and coagulate tests. Susceptibility of microorganisms to 15 antibiotics was determined by Disc Diffusion Method. Results: Among 1573 positive cultures, 17.5% of isolates were CONs. 73.9% of isolates belonged to OPD and 26.1% were obtained from hospitalized patients. Most of CONs were isolated from Internal (38.9%) and Internal emergency wards (34.7%). Among different clinical samples, CONs were mainly isolated from urine cultures (78.6%) in women. Vancomycin (100%) and Imipenem (94%) were found to be the most effective antibiotics. Conclusions: Due to the increasing prevalence of CONs infections, and multi-resistant strains, it is of great important to attention to frequency and anti-biogram pattern of this microorganism.

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Background: Body fat estimation in children is a subject of growing interest in clinical and paediatric medicine due to its growth in epidemic proportions and health, socio-economic and pathological consequences in later life. The aim of this study was to assess the prevalence of obesity among school children and a comparison of body fat measurement by Body Mass Index (BMI), anthropometric equations & skinfold thickness (SFT) and bioelectrical impedance analysis (BIA) methods. Methods: A cross-sectional study of 309 randomly selected school children (male=147; female=162) from three schools in the district of Serdang, Malaysia. Height, weight, waist & hip circumference and skinfold thickness of biceps, triceps, subscapular and suprailiac regions were measured and body fat estimated using the Siri and body density equations of Jackson & Pollock. BMI was calculated (weight / height squared) and thereafter percentage body fat estimated using the Deurenberg formula. A Tanita bioelectrical impedance analyser was also used to estimate body fat independently. Methodological differences among the various methods were statistically analysed. Results: Comparison between the means of the various methods used showed that the fat percentage from the BMI and the BIA had a mean difference of 0.671 whereas that between BMI and skinfold estimates was 3.086. The mean difference between BIA and the skinfold estimates was 3.757 which indicated a significant difference between the two methods. BMI and skin fold estimates showed similarity at maximum values of body fat percentage. Conclusions: While BMI, BIA and skinfold equation estimates may be independently dependable as reasonably good predictors of body fat, significant differences exist between the methods, due to factors like inter-tester variability, hydration status of subjects, age and gender.

P301. Identification of rpoB mutations associated with rifampicin resistance in Malaysian isolates of Mycobacterium tuberculosis

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Rifampicin resistance is used as a marker for multi-drug resistant tuberculosis (MDR-TB). Its rapid and accurate detection is important for effective TB control. In this study, 86 confirmed TB isolates from clinical specimens were examined by the amplification of the rpoB gene followed by reverse line blot hybridization (RLBH) for mutations in the cluster I region, and by single strand conformational polymorphism (SSCP) for mobility shifts caused by nucleotide changes. All strains showing the presence of mutations in the 2 assays, strains with discrepant results as well as 20 randomly selected strains with no observed mutations were further examined by DNA sequence analysis covering clusters 1-111 as well as rifampicin minimum inhibitory concentration (MIC) determinations. Both SSCP and RLBH assays detected mutations in 26 out of the 86 isolates tested, with a concordance rate of 92.3%. All mutations were in the cluster 1 “hot spot” region and comprised 11 Ser456Leu (codon 531 of the E. coli codon numbering system), 5 His451Tyr (codon 526), 3 His451Arg (codon 526), and 1 each of Gln438Leu (codon 513), Asp441Val (codon 516), Ser447Leu (codon 522), His451Asp (codon 526), Ser458Pro (codon 533), del. 444 (AAC at codon 519), and 9 bases (TCGGCGCTG) deletion involving codons 456-458 (531-535). All of these strains had rifampicin MICs greater than 256 µg/ml, except for the strain with a deleted codon 444 which had a MIC of 1.50 µg/ml of rifampicin. The 20 strains with no mutations showed wild type DNA sequences in the “hot spot” region that is consistent with negative results in the PCR-SSCP and RLBH assays and rifampicin MICs of less than 1.0 µg/ml. The PCR-SSCP assay allowed rapid screening of strains for genotypic resistance while DNA sequence analysis provided definitive identification of mutations associated with resistance.
P302. The genetic diversity of virulence genes in Legionella pneumophila

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Legionella is an intracellular parasite that is commonly found in fresh aquatic environments around the world. Legionella is a common cause of community acquired pneumonia and an occasional cause of nosocomial pneumonia. Legionella infection can range from mild flu-like sickness to acute fatal pneumonia and is invariably acquired from an environmental source. The majority of cases of Legionella infection are caused by L. pneumophila, particularly serogroup 1. Many known virulence determinants of Legionella are L. pneumophila specific. In this study, the genetic diversity of isolated virulence genes among different serogroups of L. pneumophila was investigated by low-stringency southern hybridization with specific DNA probes. Nine open reading frames (ORFs) in L. pneumophila strain Philadelphia genome sequences representing different virulence genes were studied for their distribution among 41 L. pneumophila isolates of different serogroups from Malaysia and the U.S.A. These ORFs have either a known or putative protein domain and/or function such as autolysin similarity, tetratricopeptide repeat, adenylate cyclase activity, zinc metalloprotease activity, ectonucleoside triphosphate diphosphohydrolase activity, dot/icm effector similarity and involvement in lipopolysaccharide (LPS) biosynthesis. Eight of the nine ORFs studied were shown to be distributed among different serogroups of L. pneumophila. However, the orf3 of LPS biosynthesis locus was found only in L. pneumophila serogroup 1. The U.S L. pneumophila reference strain showed distinct southern hybridization pattern of all the 9 virulence genes compared to L. pneumophila isolates from Malaysia. The results of this study revealed the presence of distinctive genetic variation of the virulence genes between L. pneumophila serogroup 1 and other serogroups, and revealed a correlation between the virulence factors studied and the phenotype of L. pneumophila serogroup 1 as a more dominant infectious agent.

P303. The efficiency of electropositive filter membranes for the concentration of male-specific coliphages in water.

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Male-specific (F+) coliphages are used as a viral indicator for faecal pollution in water and food as they have been shown in numerous studies to be highly correlated with human enteric viruses. The concentration of these viral particles in a water sample is an important pre-requisite to their detection and quantification. Concentration methods include membrane adsorption-elution using electropositive and electronegative filters, ultracentrifugation, and adsorption to various inorganic/organic compounds. The effectiveness of each method depends on the sample type, type of filter, components of the eluent, volume of sample and the structural configuration of the phages in water. In this study, we investigated the efficiency of a new electropositively charged alumina fibre filter (Virocap) using tap water and well water spiked with MS2 coliphages. Filter eluents were pooled for phage enumeration using the APHA double agar layer (DAL) assay with the Escherichia coli HS(Famp)R host. Using the Virocap filter, the recovery of MS2 was 64±3% and 49±3% for tap and well water respectively and these rates were about 5% higher than corresponding rates obtained by filtration with conventional nitrocellulose filters. In addition, Virocap filtration was found to be more efficient in acidic conditions and phage recovery improved with increased number of elution steps.
P304. Presence of the icmX gene among environmental isolates of Legionella species

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Legionella are ubiquitous in the aquatic environment but at least 20 species have been implicated in human infections. As more than 90% of Legionnaires’ disease has been attributed to L. pneumophila, this species has been most widely studied for virulence determinants. Among proteins in the Dot/Icm transporter system, the IcmX protein has been identified as being essential for phagosome trafficking and intracellular growth. Early studies reported that the icmX gene was uniquely found in L. pneumophila but more recent findings suggested that the gene might be conserved in the Legionella genus. We examined 55 isolates of legionella-like organisms from water cooling towers in various parts of Malaysia, for the presence of the icmX gene. Species identification was by 16S rDNA sequencing.

For the PCR amplification of the icmX gene, we designed primers based on the DNA sequence of icm VWX available at GenBank (Accession no. U07354). In addition to the 55 isolates, 17 reference strains were similarly examined. The icmX gene was found in all 49 strains of L pneumophila as well as 8 of 23 non-pneumophila species comprising L. longbeachae, L. gormanii, L. anis, L. gresilensis and L. busanensis. A phylogenetic tree constructed with all icmX gene sequences showed considerable inter- and intraspecies variability.

P305. Molecular study of the hepatitis C Virus (HCV) in haemodialysis patients.

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Hepatitis C virus (HCV) has been estimated to infect approximately 170 million people worldwide. In Malaysia, the seroprevalence of anti-HCV among adult blood donors is reported to be 1.49% to 3.0%. A high proportion of HCV infected patients progress to chronic hepatitis and its complications including liver cirrhosis and hepatocellular carcinoma. Intravenous drug abuse and blood products are the primary means of transmission, but HCV infection also occurs frequently in haemodialysis (HD) patients as a result of their frequent exposure to blood or HCV-contaminated medical equipment. In Malaysia, the prevalence of anti-HCV antibody in HD patients is 17% (12th Report of the Malaysian Dialysis and Transplant Registry, 2004). The objective of this study is to determine HCV genotypes, viral load and HCV quasispecies in HD subjects. A total of 40 HD subjects were studied and 71 HCV patients with no known renal disease served as controls. With PCR-RFLP, all HD patients examined were infected by genotypes 1a, 1b or 3a. Similarly, 69 of 71 control subjects were also infected by these genotypes. However, the HD group had a greater number of genotype 1 isolates than genotype 3 (60% vs. 40%), while the control group had more of genotype 3 than genotype 1 (63.4% vs. 35.2%). The difference was statistically significant (p=0.016). HCV viral load was studied using an optimized in-house quantitative real-time PCR (RTm-PCR) with a detection limit of ~5x10^2 IU/ml and analytical specificity of 100%. The mean viral load for the HD and control groups was 2.63x10^5 and 2.82x10^5 IU/ml respectively. There was no difference in HCV RNA concentration between these two groups by t-test analysis (p=0.930). With respect to the HCV quasispecies studies, no difference was observed in all the quasispecies variability parameters including complexity, diversity, synonymous substitution (Ks), non-synonymous substitution (Ka) and Ka/Ks ratio between HD and control subjects. None of the above parameters correlated with gender and HCV RNA viral load. Only one parameter, Ka/Ks
ratio, was positively correlated with disease duration. In conclusion, HCV genotype distribution is different in the HD and control groups. There is no difference in HCV RNA level and HCV quasispecies variability between the two groups.

P306. **Dendritic cell count in patients with newly diagnosed and relapsed non-Hodgkin lymphoma**

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Dendritic cells (DC) are antigen presenting cells that are found to be affected in patients with haematological malignancies, either quantitatively or functionally. Severe reduction of myeloid DC (MDC) and plasmacytoid DC (PDC) has been observed in patients with B lineage malignancies like acute lymphoblastic leukaemia. This on-going study aims to determine the correlation between DC count with biology, clinical characteristics and treatment outcome in patients with newly diagnosed and relapse non-Hodgkin Lymphoma (NHL). To date, there has been no similar study addressing these important issues. Full blood count and DC quantitation were performed on blood samples taken from patients with newly diagnosed and relapsed NHL at diagnosis and after three cycles of chemotherapy from November 2006 until May 2007. DC were quantified by three-colour flow cytometry using FACScan cytometer (Becton-Dickinson, New Jersey, USA). Twenty-one patients with a mean age 52 ± 12 years were recruited into this study. Seventy-six % of the patients were male. Majority (71%) had clinically aggressive disease according to WHO Classification of the NHL. Newly diagnosed cases constituted 71% of the patients. At diagnosis, MDC numbers increased to 5.5 ± 14.8 x 10^7/L (normal range: 6.58 x 10^6 - 3.28 x10^7/L) but dropped to 2.0 ± 1.6 x 10^7/L after three cycles of chemotherapy (p=0.972). Meanwhile PDC numbers were 0.9 ± 1.7 x 10^7/L at diagnosis and 0.7 ± 0.7 x 10^7/L after three cycles of chemotherapy (normal range: 4.51x10^6 – 2.04x10^7/L, p=0.507). There was no significant difference in the numbers of MDC and PDC according to the disease status (newly diagnosed or relapse) and aggressiveness of the disease. The results in this preliminary study indicated that there are changes in the numbers of myeloid and plasmacytoid DC in patients with NHL when the disease is active and in remission. Increased knowledge in the kinetics of DC in NHL would provide valuable information regarding the role of DC in lymphomagenesis and the most appropriate timing of DC vaccination.

P307. **Need for universal patient-protection algorithm against deleterious effects of hyper-pressured ETT cuffs on tracheal mucosa**

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Inadvertent intubation with hyperpressured endotracheal cuffs lead to moderate to severe tracheal mucosal pathology and is a major cause for resection and reconstruction. A heightened level of awareness and proper vigilance during anaesthesia with meticulous attention to specifications on the endotracheal tube can significantly improve higher levels of patient safety during anaesthesia. One type of endotracheal tube circulating internationally was highly suspected of requiring excessive intra-cuff pressures (approximately 70-80 cm H20) to provide an adequate tracheal seal. An alert was raised with the manufacturer but progress towards investigation and corrective measures were delinquently slow. In the wider interest to protect the patient, a universal algorithm to defend against the deleterious effects of hyperpressured ETT cuffs can be effected if each anaesthesia and ICU documents intra-cuff
pressures of tubes against the brands are used as regularly as possible and tracheas routinely examined endoscopically following extubation. When lesions are spotted they should be recorded, notified and treated appropriately and in a timely manner to avert adverse prognosis. To offer patients their rightful level of protection, a universal algorithm for intra-cuff pressure monitoring and the provision of more information to the patient including the possible misadventures of tracheal stenosis is necessary as a benchmark for care prior to anaesthesia and ICU intubation.

P308. Histopathological pattern of Barrett’s oesophageal biopsies at the University of Malaya Medical Centre

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Although Barrett’s oesophagus (BE) has long been regarded as a disorder of Western populations, it is now increasingly recognised in Asia. BE has been aetiologically linked with gastrointestinal reflux disease (GERD) and is regarded as an important precursor for oesophageal adenocarcinoma. However, there remain many controversies with regards its histogenesis, pathogenesis and diagnostic criteria. This study records the histological features of oesophageal biopsies regarded clinically as BE encountered in gastrointestinal practice at a large tertiary hospital in Malaysia. Over a 5-year period, 80 of 139 oesophageal biopsies endoscopically suspected to be BE were regarded histologically to be consistent with BE due to the presence of metaplastic columnar epithelium. The patients ranged from 15 to 93 years in age (mean 55 years) and showed an equal male:female ratio. Of the 80 biopsies, 71 (88.8%) had evidence of active inflammation. Only 21 (26%) showed the presence of goblet cell metaplasia and 7 (8.8%) exhibited epithelial dysplasia. 8 biopsies revealed only columnar cell change without goblet cell metaplasia, dysplasia or inflammation. Helicobactor pylori was not detected in any of the biopsies. These findings underscore the problems of defining BE by complete intestinal metaplasia. Although Helicobactor pylori related gastritis is common in Malaysia, it does not appear to be linked with BE. An extension of the study using immunohistochemistry may help to define this entity in the Asian setting.

P309. Subcutaneous sporotrichosis: A case report

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Sporotrichosis presents as chronic infection in both sporadic and epidemic settings caused by a thermally dimorphic fungus, Sporothrix schenckii. This study reports a case of subcutaneous sporotrichosis in a farmer with a one month history of solitary, painless subcutaneous swelling on the left leg. Ultrasonography study revealed a heterogenous echogenic mass with cystic changes. An aspiration biopsy was performed and cytological findings were consistent with chronic infected cyst. Fungal cultures of excised mass yielded colonies of S. schenckii. The patient was followed-up post-operatively for six months. He was not prescribed with anti-fungal agent and did not have any recurrence.
P310. Epitheliod sarcoma: A report of 2 cases.

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Objective: Presentation of classical features of two subtypes of rare malignancy. Method: Two cases of epithelioid sarcoma reported at Hospital Queen Elizabeth, Kota Kinabalu, within last six months were retrieved from case records. Two subtypes of epithelioid sarcoma were diagnosed. Clinical, histological and immunohistochemical features of these cases are being presented here. Conclusion: Both these cases were clinically diagnosed as benign. Though rare, clinical features should alert clinician to think of differential.

P312. Ethanol induced ultrastructural changes in rat cerebellum

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Ethanol induced oxidative impairment of tissues is interested purpose of gene therapy. The most important antioxidant enzyme is superoxiddismutase (SOD), especially mitochondrial Mn-SOD. Previous experiments shown, that tissues sensitive to ethanol induced oxidative stress are cerebellum cortex and Purkinje cells. Wistar rats (n=27) were randomly divided into four groups: Control, Alcohol, SOD, Alcohol + SOD. Plasmid pcDNA3 containing the gene for mitochondrial Mn-SOD (300µg in 100µl saline; SOD, Alcohol + SOD) or the same volume of saline (Control, Alcohol) was administered intramuscularly into the musculus biceps femoris of left hindlimb once a week. At the end of the study rats were sacrificed by decapitation in anesthesia. Blood and organ samples were collected for biochemical and histological analyses. Morphological analysis shown swollen mitochondria and impairment of myelin sheaths of nerve fibres in all groups besides water group. Our antioxidative therapy has not protective effect on ethanol induces cerebellum impairment. In addition to SOD supplement of in the form of plasmid pcDNA3 caused pathological changes in mitochondria and myelin structure. This result indicates elevated activity of SOD without appropriate elevated activity of catalase or glutathione peroxidase – enzymes, that detoxify SOD product – hydrogen peroxide. Reactive oxygen species induced in alcohol uptake play a crucial role in swollen mitochondria and myelin impairment. It is possible, similar to Down syndrome, that hydrogen peroxide is decomposes non-enzymatically into hydroxyl radical. It is more reactive than superoxide anion. Next experiments will be devoted to using of combined antioxidant gene therapy with application of SOD and catalase.
P313. Vitamin C and alcohol binding to phospholipid monolayers.
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The main components of cell membranes are phospholipids and proteins. The mechanism of toxic effect of etanol chronic uptake has been studied intensively. The simple model of the biological membrane is provided by well-controlled lipid monolayers at the air - water interface. The Maxwell displacement current technique (MDC) provides novel approach to conformation study of the membrane models. The effect of alcohols is interaction with membrane molecules, mainly with the lipid head group and consequent changes in physical – chemical properties of the membrane. The aim of our study was to examine structural and mechanical changes of dipalmitoyl-phosphatidylcholine (DPPC) monolayer as a simple model system of a cell membrane in different environments. As subphases of Langmuir films as a membrane models were used pure water, ethanol and methanol solutions and vitamin C. Monolayers properties are investigated by surface pressure analysis (including mechanical properties evaluation) and the Maxwell displacement current measurement, the dipole moment projection calculation. Surface pressure –area isotherms show similar behaviour of the DPPC monolayer on alcohol – water mixtures independently on presence of vitamin C. At both subphases (ethanol – water and methanol – water) the elasticity modulus of the monolayer decreases leading to the loss of membrane elasticity. We observe small or negligible binding of methanol molecules on oxygen bonds of DPPC. Thus antioxidant, vitamin C, has no significant effect. For ethanol – water mixtures is observed recovery of electrical properties in presence of antioxidant agent. We suppose vitamin C control of DPPC – ethanol molecules interaction. We found other effect of vitamin C on phospholipide membrane as antioxidant. With connection of various techniques may be investigated structural and mechanical properties of lipids and enzymes.

P314. Renal primitive neuroectodermal tumour – A case Report
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Objective: Presentation of a rare malignancy at rare site. Method: A case of primitive neuroectodermal tumour of left kidney in a young lady who presented with 6 months history of left loin pain, was diagnosed recently at Hospital Queen Elizabeth, Kota Kinabalu and succumbed to death within two months of postoperative period. A detail clinical presentation, radiological, histomorphological and immunohistochemical features are being presented. Conclusion: Awareness of rare malignant lesion of kidney.
P315.  Dendritic cells and cytokine levels as predictors of acute graft-versus-host disease in allogeneic hematopoietic stem cell transplantation

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Introduction: Graft versus host disease (GVHD) is an important complication of allogeneic peripheral blood stem cell transplantation (PBSCT) and is also one of the main causes of transplant related morbidity. Dendritic cell (DC) counts and cytokine dysregulation in the recipient have been shown to influence the development of GVHD. Methods: Serial fresh whole blood was obtained from 5 patients undergoing allo- PBSCT for various hematological disorders at pre-conditioning, followed by intervals of 1, 14, 21, 30, 60 and 90 days post-PBSCT. DC analysis was performed by 3-colour flow cytometric analysis and corresponding circulating cytokine levels of IL-10, IL-6, IL-4, IL-2, TNF- and IFN- were measured by cytometric beads array analysis. Results: Two of the 5 patients studied had no evidence of GVHD. Both of them showed sustained or increments in DC counts following PBSCT accompanied by low cytokine levels as compared to preconditioning values. One patient who successfully engrafted but later developed chronic GvHD showed a sharp increase in DC counts at D+14 and a rapid decline after D+30, accompanied by a 5-fold increase in IL-6 level at day +14 returning back to baseline level after day+21, while the other cytokines were maintained at baseline levels. Two patients died following PBSCT. One developed hyperacute GvHD and expired on D+27, and the other patient suspected to have developed skin GvHD expired after >100 days post-PBSCT due to severe lung infection. Both patients showed diminishing dendritic cells count after day +14 onwards with elevated cytokine levels. The patient with hyperacute GvHD showed 5-fold increase for all cytokines analysed but the patient with severe infection had elevated levels for IL-10, IL-6, IL-2, and IFN- only. Conclusions: Reduced DC counts accompanied by increased cytokine levels, in particular IL-6 within the first 21 days after transplantation is associated with the occurrence of GvHD.

P316.  Human Plasmodium knowlesi infection: A rare presentation with severe thrombocytopenia

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Malaria remains the most common vector-borne parasitic disease in Malaysia despite a decline in annual number of cases. There are chances of missing the diagnosis of malaria due to high prevalence of dengue in the region. However, blind screening of all thrombocytopenic samples might be a possible way to detect clinically unsuspected malaria cases in the accident and emergency department. CASE REPORT: Severe thrombocytopenia is common in isolated falciparum, mixed falciparum & vivax infection. However it is not reported in the case of plasmodium knowlesi. We hereby report a case of severe thrombocytopenia (platelet count < 20 x 109/L) with plasmodium knowlesi infection. This patient’s working diagnosis was dengue fever. Dengue serology was repeatedly negative for IgM and IgG. Suspicion of malaria infection only arose while examination of full blood picture showing schizont stage of plasmodium malariae. Later it was confirmed by thin and thick smears of the blood. The blood sample was subjected to PCR and the result for four human species of plasmodium including plasmodium malariae was negative. The diagnosis was confirmed by PCR for the RNA of plasmodium knowlesi. This patient responded well to anti-malarial therapy and his platelet count recovered.
completely (330 x 109/L) with in one week. Our aim is to highlight two important messages; that is one must not ignore malaria as one of the causes of thrombocytopenia presuming it is now not common in Malaysia and plasmodium knowlesi infection can easily be missed and diagnosed as plasmodium malariae or plasmodium falciparum depending upon the stage of parasite seen in the peripheral blood. This is a rare case of thrombocytopenia due to even rarer plasmodium knowlesi infection. Due to similarities in the morphology we recommend that all diagnosed cases of plasmodium malariae should be subjected to PCR specific to plasmodium knowlesi.

P317. Attitude of blood donors responding to call of transfusion medicine unit after positive screening test

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Every donated unit is tested for infectious diseases. Most blood donors in the Unit Transfusion Medicine Unit (UPT), USM are called upon by letters & telephone calls in case of any positive screening test results. However we have noticed over the years that many of these donors do not respond to the letters or even telephone calls. These cases are then referred to health department according to guidelines. Some studies have shown that there are donors who have practiced deferrable risk behaviors. Reports from previous studies have shown that some donors use blood donations as a means of testing their results for infectious diseases. These ‘potential test seekers’ tended to be younger, single, unemployed and first-time donors. We have studied indirectly the attitude of blood donors towards a letter/call from UPT USM. In a retrospective study we reported 502 cases of positive screening results (208 for Hepatitis C, 209 for Hepatitis B and 85 for VDRL) during January 2005 – December 2006. Out of 208 positive cases of hepatitis C only 140 (67.30%) responded to our letters/calls sent to the address/phone number provided at the time of registration. Sixty one donor (29.33%) defaulted and did not respond at all. 7 (3.37%) missed their follow-up after responding initially. Similarly out of 209 positive donors for hepatitis B, 86 (41.47%) defaulted. In VDRL group 28 (32.95%) defaulted their blood bank attendance. Poor attitude of blood donors can be due to poor knowledge about health awareness & importance of the screening tests carried out in blood donors. Other possible explanation is felony by the donors as such by hiding proper information at the time of counseling. There is a need to review the effectiveness of the present deferral system, which is based on the goodwill of the donor to disclose personal health risk factors.

P318. Demographic distribution of primary central nervous system tumour in Hospital Kuala Lumpur from year 2004 to 2006

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Brain tumours have the twelfth highest incidence in males and the fourteenth highest incidence in females among all cancers in Peninsular Malaysia. The crude annual incidence rate of brain tumours in Hospital Kuala Lumpur was 1.4 per 100,000 population. Primary brain tumours are different from tumours arising in other sites in that a benign lesion may result in death due to compression of vital structures. This study aimed to determine the demographic distribution of primary central nervous system tumours in Hospital Kuala Lumpur over a period of three years from 2004 to 2006. A retrospective cross-sectional study design using universal sampling method was chosen with a sample
size of 459. The data was obtained from request forms for histopathological examination of patients with primary central nervous system tumour sent to the Histopathology Unit, Department of Pathology, HKL from 2004-2006. The highest frequency of primary central nervous system tumours are in the age group of 41-50 years old (n=97, 21.1%). The frequency of females is slightly higher (n=237, 51.6%) as compared to males (n=222, 48.4%). Malays had the highest frequency of primary central nervous system tumours (n=290, 63.2%). Meningioma was the most frequently encountered histological type of primary central nervous tumour in HKL (n=172, 37.5%), followed by astrocytoma (n= 84, 18.3%) and glioblastoma multiforme (n= 74, 16.1%). Headache was the most common presenting symptom (n=131, 28.5%). There is a significant relationship between age group and the histological type of primary central nervous system tumour (p<0.001). There is also a significant relationship between gender and the histological type (p<0.001). However, there is no significant relationship between race and the histological type of primary central nervous system tumour (p=0.210). Among the demographic factors studied, age and gender do have a significant relationship with the histological type of primary central nervous system tumour but race does not.

P319. Nosocomial infection among intensive care unit and high dependency ward patients in a public hospital in Malaysia

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Nosocomial infections (NI) continue to be a major public health concern throughout the world. Infection is defined as nosocomial when patients acquired infection in the hospital after 48 hours admission, confirmed by culture results and treated with antibiotics. To describe the pattern of infections and antibiotic resistance pattern in ICU and HDW patients in a public hospital in Malaysia, a cross sectional study using retrospective data was conducted at the Microbiology Unit in a public hospital in Malaysia from January to December 2003. A total of 377 positive cultures were isolated from 104 patients. Of these patients, 185 episodes of NI were identified. The infection rate of ICU and HDW patients from the total admission was 2.98%. The age range was from 13 to 80 years (mean = 50.71 ± 17.51) with the 61-70 years age group predominating the episodes of NI. The male to female ratio was 2:1: the majority were Malay (51%), followed by Chinese (28.8%), Indian (18.3%) and others (1.9%). The most common specimen came from tracheal aspirate (45.1%), followed by blood (29.4%) and pus (8.5%). Respiratory tract infection (42.7%) was the most frequent infection identified, followed by bloodstream infection (29.2%) and peritoneal infection (7.6%). Acinetobacter species (27.8%) was the most common bacteria isolated, followed by Pseudomonas aeruginosa (18%) and Staphylococcus aureus (12.9%). Methicillin-resistant Staphylococcus aureus accounted for 72.7% of all Staphylococcus aureus isolated. The proportion of extended spectrum beta-lactamase producers was 62.8% among Klebsiella pneumoniae and 40% in Escherichia coli. Among the Enterobacteriaceae, Klebsiella pneumoniae was highly resistant towards ceftazidime (72.7%) and cefoperazone (64.9%) while Escherichia coli was resistant towards cefepine (25%). Gentamicin (86.6%) and ciprofloxacin (79%) resistance was noted in Acinetobacter species. Among gram positive bacteria, coagulase-negative staphylococci were highly resistant towards oxacillin (91.2%), gentamicin (76.5%) and erythromycin (73.5%). Effective infection control measures practiced contributed to low infection rate among ICU and HDW patients. Respiratory tract infection was noted as the most common infection, whilst Acinetobacter species was the most frequently isolated organism. The occurrence of NI was highest in elderly patients.
P320. Renal allograft biopsy pattern over a 6-year period at the University of Malaya Medical Centre

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Biopsy of the allograft after renal transplant is a common procedure for early detection of preventable causes for possible graft failure. While protocol biopsies are not conducted at the University of Malaya Medical Centre, allografts are nevertheless liberally biopsied whenever there is any unexplained rise in serum creatinine. We endeavoured to study the pattern of renal allograft biopsy at our institution to establish a baseline for better understanding of the range of conditions which led to biopsy. All renal allograft cases which were biopsied between January 2001 and December 2006 at the University of Malaya Medical Centre were retrieved from the Department of Pathology archives. A total of 255 biopsies were carried out on 109 patients during that period. Of these, there were 71 males and 38 females. Ethnically, 77 were Chinese, 16 Malays and 16 Indians. Ages ranged between 18-65 years in the males and 17-69 in females. The average rate of biopsies per case did not differ between male (2.28) and female (2.34) patients. Interestingly, the rate of biopsies/case seemed to be higher in the younger age groups. In males, the highest rate of biopsies/case (4.67 biopsies/case) was seen in the 20-29 years age group and in the females (6.00 biopsies/case) in the 10-19 years age group. Tubulointerstitial rejection, Banff type 1, was the most commonly encountered pathological entity and constituted 40.4% of the histopathological diagnoses followed by borderline rejections 18.4%. In 15.7%, no pathology was detected while 15.3% had insufficient tissue for histopathological diagnostic assessment. Vascular, Banff type 2, rejection, calcineurin inhibitor toxicity, chronic allograft nephropathy, recurrent focal segmental glomerulosclerosis and IgA nephropathy were among other histopathological diagnoses. The findings of this study indicated that male Chinese were the largest group with renal allograft biopsied during the 6-year period implying that renal transplantation was most common amongst male Chinese. While gender did not seem to influence the decision for biopsy of the allograft, the procedure appeared to be performed more frequently in younger than older patients. Expectedly, tubulointerstitial rejection formed the most common underlying aetiology leading to allograft biopsy.

P321. Immunohistochemical expression of Bcl-2 versus Bax in hepatocellular carcinoma

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B-cell lymphoma-2 (Bcl-2) family members play important roles in the regulation of apoptosis. The twenty or so family members manifesting either anti-apoptotic or proapoptotic properties are capable of forming heterodimers which nullify each other’s properties leading to a nett effect of anti or proapoptosis. Bcl-2 is the prototype of the antiapototic family members with Bax the proapoptotic counterpart. A study was initiated to assess whether apoptosis was a predominant feature in hepatocellular carcinoma (HCC). Immunostaining for Bcl-2 (Dako:Clone 124) was carried out in 36 and Bax (Dako:A3533) in 38 histologically confirmed HCC. These were compared with labeling of the respective markers in 20 normal liver controls (NL) obtained at post-mortem examination. Labeling for Bcl-2 and Bax were quantitated by identification of positively-stained cells in 2000 consecutive HCC or NL cells for each case and control using a computerised image analysis system (Leica Q550 CW). The mean ± 2SD positivities were computed for Bcl-2 (0) and Bax (37.0) in NL and taken as arbitrary cut-offs. 24 (66.7%) HCC demonstrated Bcl-2 with 11 (28.9%) Bax labeling in excess of NL. These findings indicate that both antiapoptotic Bcl-2 and proapoptotic Bax, were over-expressed, although there appears to be a preponderance of Bcl-2, a finding consistent with a nett gain of the neoplastic HCC cells.
P322. Large liver cell dysplasia: How it differs morphometrically from hepatocellular carcinoma?

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Large liver cell dysplasia (LLCD) can at times be morphologically disturbing and misdiagnosed as hepatocellular carcinoma (HCC), in particular on small core biopsies. In an attempt to assess whether morphometric analysis has an adjunctive role in distinguishing the two entities, a study was conducted at the Department of Pathology, University of Malaya Medical Centre to assess for differences in the nuclear area and circumference of hepatocytes of LLCD and those of HCC. 52 cases of HCC in which 25 showed adjacent LLCD were selected for study. 10 consecutive nuclei were selected on the H&E stained sections from the LLCD and HCC areas. These were manually outlined and measurements made of the nuclear area and circumference using a computer-assisted image analysis system at 400x magnification. Mean area (MeA) of the 250 LLCD nuclei was 74.1 and that of the 520 HCC nuclei 73.2 (p=0.9). Standard deviation of the nuclear areas was 21.6 for LLCD and 26.1 for HCC. Mean circumference (MeC) of the 250 LLCD 33.2 and 520 HCC nuclei was 34.1 (p=0.5), while standard deviation of the nuclear circumferences was 4.6 for LLCD and 6.3 for HCC. Thus, mean nuclear area and circumference of LLCD do not appear to differ significantly from HCC. Nevertheless, the standard deviation of both the nuclear areas and circumferences were slightly lower in LLCD compared to HCC, implying increased pleomorphism of HCC nuclei compared with LLCD. Whether the increased nuclear pleomorphism in HCC can be detected on eyeballing requires further study and clarification.

P323. Survey the seroprevalence of HTLV I/II in hemodialysis patients and blood donors

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Background: Human T lymphocyte Virus (HTLV) is a virus from retroviridae family which is presented as the cause of T cell leukemia, Spastic Paraparesis and tissue Necrotizing lymphadenitis. It is mainly transmitted by contaminated blood products, blood recipients such as Thalassemia, Hemophilia or Hemodialysis patients are at a high risk of infection to this virus. However, immunodeficiency in hemodialysis patients increases this risk. The aim of this study is to survey the seroprevalence of HTLV in hemodialysis patients and blood donors, and to analyze the effectiveness of each risk factor.

Material & Methods: In this descriptive cross-sectional study, first data was collected about demographic features such as age, sex and the frequency of blood transfusion through a year in hemodialysis patients and blood donors (as control group) of Urmia, Iran in 2006. Blood serum was evaluated serologically in both of two groups by enzyme-linked immunosorbent assay (ELISA) for HTLV I/II and finally all collected data was analyzed by descriptive and chi-square tests by means of SPSS software ver11.5.

Results: 114 hemodialysis patients and 2046 blood donors was evaluated and there were 65 men (57%) and 49 women (42.9%) in hemodialysis group. Also there were 1910 (93.4%) men and 136 women (6.6%) among healthy blood donors. Mean age of hemodialysis patients was 45 years old. 3 persons of 114 hemodialysis patients were seropositive for HTLV (prevalence of 2.63%), which two of them were female and one was male. Among all blood donors, 1997 persons (97.6%) were seronegative for HTLV and 49 persons (2.4%) were seropositive in ELISA serologic laboratory test and positive cases was confirmed by western blot. Finally in this group, total seroprevalence of HTLV was calculated 0.34%. Discussion: Because we do not perform blood products screening in our region, blood recipients are at a high risk of HTLV infection. More transfusion times and immunodeficiency in hemodialysis patients has increased HTLV incidence in this group. So screening for HTLV in high risk groups suggested in this study.
P324. Hyperacute rejection of renal allografts- 4 distinct types

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Until recently hyperacute rejection of renal allograft was regarded purely antibody mediated. Studies done in Newcastle, Australia show hyperacute rejection of renal allograft can be divided into 4 distinct types:  

Type 1: Classical pattern. C4d positive C4d staining in peri-tubular capillaries and capillary loops of glomeruli. Prominent margination of neutrophils and presence of thrombi in peri-tubular capillary, vein, artery and glomeruli.

Type 2: T-cell mediated hyperacute rejection. C4d negative.

Type 3: C6d8 cell mediated hyperacute rejection. C4d negative.

Type 4: fibrinoid necrosis of muscular arteries, arterioles and glomeruli. The muscular coat of the arteries and arterioles are C4d positive. The peri-tubular capillaries are normal and C4d negative. Type 2 and Type 3 pattern of hyperacute renal allograft rejection was presented recently (Murugasu 2007). Type 4 pattern of renal hyperacute has not been documented in the medical literature. A 33 year old man received a renal transplant in Royal Newcastle Hospital in October 1982. On the table biopsy showed fibrinoid necrosis of all the glomeruli (10). The patient remained anuric. The day 7 biopsy showed fibrinoid necrosis of all the glomeruli (35). The muscular coat of the lobular arteries and arterioles showed fibrinoid necrosis. The peritubular capillaries were normal. There was no margination of neutrophils or thrombi. There was no evidence of cellular infiltrate in the interstitium. The tubules appeared normal. C4d done on the paraffin fixed tissue showed that the muscular coat of the lobular arteries and arterioles were positive. The glomeruli and peri-tubular capillaries were negative. The hyperacute rejection in this case is antibody mediated inspite of the lack of peritubular capillary deposition of C4d. It appears that the antibody has targeted antigen present in the smooth muscle of muscular artery and arteriole. Angiotensin II Type 1 - Receptor Activating Antibodies in Renal-Allograft rejection have been shown to cause fibrinoid necrosis of muscular artery and C4d deposition in the muscular coat (Dragun et al 2005).

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