

**The 23<sup>rd</sup> Scientific Meeting of the Malaysian Society of Pathologists was held at the Faculty of Medicine, University of Malaya, Kuala Lumpur from 13 to 14 November 1998. Abstracts of the free paper communications follow:**

**Oral presentations:**

**1. Fine needle aspiration cytologic appearances in invasive lobular carcinoma of the breast**

*Gita JAYARAM and \* YIP Cheng Har*

*Departments of Pathology and \*Surgery, Faculty of Medicine, University Malaya, Kuala Lumpur*

Invasive lobular carcinoma (ILC) of the breast, characterised histologically by small neoplastic cells that diffusely infiltrate the breast stroma in single file and targetoid pattern, has been said to be difficult to diagnose on cytology owing to the frequent paucity of cellular material in fine needle aspiration (FNA) samples, the small cell size and often innocuous appearance of the tumour cells. The present study is a detailed cytomorphological analysis of 22 cases of ILC with histological correlation in 19. These 19 cases included 12 cases of pure ILC, 2 cases of ILC with in-situ ductal carcinoma and 5 cases of mixed ILC and IDC. The pattern of ILC was classic in 9 cases, alveolar in 3 and mixed in 7.

21 cases presented with palpable lumps or ill-defined breast nodularity which could be subjected to FNA in the clinic. In one case FNA was done under stereotactic guidance for a mammographically suspicious lesion in which no mass was palpable. The cytologic diagnosis was ILC in 16 cases, invasive carcinoma (unspecified) in 5 and IDC of low nuclear grade in 1. The cytological picture in ILC was characterised by dissociated cells that were usually small or intermediate sized, with eccentric nuclei and a low nuclear grade. Intra-cytoplasmic lumina (ICLs) and signet ring forms were present in some but not all cases. These were confirmed by mucicarmine and alcian blue-PAS stains. Absence of ICLs made it more difficult to correctly cyto-type cases of mixed IDC and ILC and cases with variant patterns of ILC. With increasing experience and awareness of the cytomorphology of variant types of breast carcinoma and in a scenario where the cytopathologist routinely performs and interprets the FNA, the false negative cytodagnostic rate in ILC can be considerably reduced and correct pre-operative cytologic typing can be achieved which would go a long way in instituting prompt and effective treatment protocol.

**2. Prenatal diagnosis of Hb Bart's hydrops fetalis**

*E. George, Mokhtar AB, Hwang CM, Faridah K*

*Unit Haematology, Department of Pathology, Faculty of Medicine, Hospital Universiti Kebangsaan Malaysia, Kuala Lumpur.*

Hb Bart's hydrops fetalis is a serious disorder with a fatal prognosis for the affected fetus. In Malaysia, the homozygosity for the  $\alpha$ -thal 1 ( $\alpha^0$ ) deletion defect ( $--^{SEA}/--^{SEA}$ ) is the most common cause for Hb Bart's hydrops fetalis syndrome. The hallmark of the condition is the total absence of functional  $\alpha$ -globin genes, absence of alpha globin chain synthesis and the presence of Hb Bart's. For prenatal diagnosis of Hb Bart's hydrops fetalis, fetal DNA obtained from chorionic villus sample in the first trimester and from fetal blood in the second trimester are used in PCR based techniques to identify the ( $--^{SEA}$ ) molecular defect. With fetal blood, the phenotype expression can be identified by doing Hb electrophoresis, globin separation and immunocytological tests, Hb Barts and Hb Portland being the predominant haemoglobins in Hb Bart's hydrops fetalis. The absence of  $\alpha$ -globin chains would be seen in the globin separation and immunocytological tests. From June 1994 to December 1997, a total of 48 (32 cordocentesis, 16 chorionic villus sampling) prenatal diagnosis were done for Hb Bart's hydrops fetalis. These studies indicate many couples at risk of producing

a fetus with Hb Bart's hydrops fetalis were not identified prior to conception. Prevention aimed at education, carrier identification, genetic counselling need to be addressed for this serious health care problem.

### **3. Hyalinizing clear cell carcinoma of minor salivary gland: report of a case with review of literature.**

*Madhavan M, Nor Hayati O and \*Rani A.S.*

*Departments of Pathology and \*Surgery, Hospital Universiti Sains Malaysia, 16150, Kubang Kerian, Malaysia*

Hyalinizing clear cell carcinoma (HCCC) is a recently described neoplasm affecting predominantly the oral cavity of the adult female. As the name indicates, it is characterised microscopically by prominent hyalinizing stroma and predominant clear cells. Positive staining for cytokeratin and negative staining for S100 and smooth muscle actin differentiate it from other salivary gland tumours having predominant clear cell component. This immunohistochemical staining pattern also indicates that HCCC is composed only of epithelial cells. It is considered as a low grade indolent neoplasm, because of rare recurrence and metastasis. Because of the lack of awareness, HCCC is often misdiagnosed as poorly differentiated carcinoma, squamous cell carcinoma, acinic cell carcinoma, mucoepidermoid carcinoma and epithelial-myoepithelial carcinoma. We report a case of HCCC in a 40-year-old Malay woman with swelling in the floor of mouth for 2 years, with review of the literature. We believe this is the first case report of this neoplasm from Malaysia.

### **4. Detection of Dystrophin gene deletions in Duchenne muscular dystrophy and Becker muscular dystrophy**

*NOR YATIZAH Mohd. Yatim, \*Kum Thong WONG*

*Histopathology Unit, Department of Pathology, Hospital Kuala Lumpur and \*Pathology Department, University Malaya, Kuala Lumpur*

Duchenne muscular dystrophy (DMD) is an X-linked progressive muscle wasting disorder, with the mild form known as Becker muscular dystrophy (BMD). The gene and its protein product, dystrophin, were identified to the position Xp21.1. Overall, it was found that 57-60% of patients have large intragenic deletions, and about 6% have duplication mutations. In Malaysia there is no data on the pattern and prevalence of deletion/s among our DMD and BMD patients

In this study, nineteen pairs of primers were used in a multiplex polymerase chain reaction (PCR) technique to detect large gene deletions. These primers allowed the detection of about 98% of detectable deletions within the "hot spot" regions as identified among the patients studied in the West. The study showed detectable deletions in eight out of sixteen patients. One patient showed deletion at the 5' end region (exon 8 to 19). The other seven patients showed deletions at the distal end region. One patient's deletion was identified to a single exon 45. Six patients showed multiple exons deletions; exon 45 to 52 (two patients), exon 45 to 50 (one patient), exon 48 to 50 (one patient), exon 49 and 50 (one patient), exon 47 to 51 (one patient). The study shows the detection rate of large gene deletions in DMD/BMD by multiplex PCR is about 50%. This rate is close to a similar study in Singapore.

### **5. Gastrointestinal stromal tumour (GIST) - A clinicopathological analysis of 9 cases**

*Nor Hayati Othman, Meor Zamari Meor Kamal, M Madhavan*

*Department of Pathology, Universiti Sains Malaysia, Kubang Kerian, Kelantan.*

A clinicopathological analysis of nine cases of gastrointestinal stromal tumour (GIST) seen in the Hospital Universiti Sains Malaysia over a period of 6 years was undertaken. The mean age of patients was 55 years. Five (5) of the patients were Malays and 4 were Chinese giving an incidence of GIST among the Chinese population more than ten folds compared to the Malays. [In HUSM the

annual average inpatient population of Malays is 25,000 and Chinese 1700]. There was no significant difference in the sex predilection among these ethnic groups ( $p=0.685$ ), however 66.7% of the cases were males. The tumours were asymptomatic in 11.1% patients while the most common presentation was malaena (55.6%). The most common organ involved was the stomach (55.5%) in which the antrum was the most common (80%) anatomic site involved. The average size of the tumour was 37.78 mm. There were only two cases (22.2%) of malignant GIST. The growth pattern was leiomyomatous in 44.4%, schwannomatous in 44.4% and infiltrative in 11.1%. All were vimentin positive. 55.5% of tumours displayed Neuron Specific Enolase (NSE) and S100 protein positivity indicating neural differentiation implying the potential of malignant transformation. Mitotic count, NSE reaction, differentiation and site in the stomach are significant variables ( $p<0.05$ ) determining behaviour of the tumours. All our patients are still alive and free of disease with a maximum follow-up period of 6 years.

## **6. The pattern of lymph node pathology in a private laboratory**

*Lian-Hua KIM, \*Kai-Soon CHAN, Suat-Cheng PEH*

*Department of Pathology, University of Malaya, Kuala Lumpur and \*BP Clinical Lab, Ipoh, Perak.*

*Background:* Lymph node excision biopsy is commonly carried out in private practice for the investigation of lymphadenopathy. The objective of this study is to elucidate the pattern of nodal pathology seen in a private laboratory practice. *Method:* 137 nodal biopsies for primary investigation of nodal enlargement in one year (1997) from a private laboratory were retrieved. Lymph nodes excised for cancer staging were excluded from this study. The histology was reviewed and agreed upon by both pathologists (KSC and SCP) based on H&E stained sections, and with additional histochemical and immunoperoxidase stains when deemed necessary. Cases of malignant lymphoma were subclassified with the aid of further phenotyping using a panel of monoclonal and polyclonal lymphoid antibodies. *Results:* One case was excluded from this study due to inadequate tissue for further assessment. There were 58 male and 78 female in this series, a male:female ratio of 1:1.3. The remaining 136 cases consisted of 13 (9.6%) Malays, 108 (79.4%) Chinese, 14 (10.3%) Indian and others 1(0.7%), with a ratio of 1: 8.3: 1.1:0.1. The pathology consisted of 17 (13%) malignant lymphomas [6 (35%) Hodgkin's Lymphoma, 11 (65%) Non-Hodgkin's lymphoma], 45 (33%) reactive hyperplasia, 35 (26%) metastatic carcinoma, 19 (14%) tuberculosis, 11 (8%) Kikuchi's disease and 9 (7%) others, (Castleman's disease 2, cat scratch disease 2, Kimura's disease 1, sarcoidosis 1, non-specific lymphadenitis 3). All categories of nodal disease showed an approximately similar ratio of ethnic and gender distribution as above, except for Kikuchi's Disease, of which 100% of patients were female. *Conclusion:* The most common nodal pathology seen in the private laboratory was reactive hyperplasia, followed by metastatic carcinoma. Malignant lymphoma constituted only 13% of cases. The ethnic and gender distribution reflects higher Chinese and almost equal gender utilisation of the private medical laboratory service.

## **7. A comparative study of respiratory cytology specimen and tissue biopsy in the diagnosis of lung cancer.**

*ZANARIAH Alias, NORIZAN Mohd. Annuar.*

*Histopathology Unit, Department of Pathology, Hospital Kuala Lumpur.*

With the increasing incidence of lung cancer, respiratory cytology is becoming the most frequent tool in diagnosis. This study aims at evaluating the respiratory cytologic modalities namely pre- and post-scope sputum, bronchial brushings and bronchial washings in comparison to biopsy which is the confirmatory method of diagnosing cancer. Specimens were obtained via fiberoptic bronchoscopy.

The study showed a pickup rate of 74.5% for biopsy and 67.3% for cytology. Bronchial brushings showed the highest pickup rate (54.5%), whilst pre- and post- bronchoscopy sputum showed the lowest positive yield. Combination of any cytologic modalities and biopsy gave a higher positive result, and especially so in combination with bronchial brushings where the positive result

reached 92.7%. Bronchial washings appeared not to add significantly to the diagnostic yield. However, both pre- and post- bronchoscopy sputum were more specific but less sensitive as compared to other cytologic modalities. Cytologic modalities in this study showed, as indicated in many other studies, their value in establishing and complementing biopsy in making the diagnosis of cancer. Among the cytologic modalities, bronchial brushings showed a better positive yield.

#### **8. CD44 expression in correlation with histological grading of neuroblastic tumours**

*MJ JULIA MUNCHAR, Noor Akmal SHARIFAH, \* Rahman JAMAL, \*\* Lai Meng LOOI and \*AR RAJA KHUZAIHAH*

*Departments of Pathology and \*Paediatrics, Hospital Universiti Kebangsaan Malaysia and \*\*Department of Pathology, Faculty of Medicine, University of Malaya, Kuala Lumpur.*

CD44 is a cell adhesion molecule that plays an important role in the cascade of metastasis and progression of human malignant tumours. In neuroblastic tumours, the non-expression of CD44 is a marker of aggressiveness. The objective of this study is to compare the expression of CD44 with Shimada's histological classification. Paraffin blocks of primary neuroblastic tumours were histologically graded according to Shimada's histological classification into favourable and unfavourable types. Tumour tissue were analyzed with immunostaining using monoclonal antibodies against CD44 epitopes. In this retrospective study, 32 cases of primary neuroblastic tumours were collected. Based on Shimada's histological classification, 13 cases (40.6%) had a favourable histology while 19 cases (59.4%) were of unfavourable histology. Statistically, there was significant correlation between Shimada's histological classification and the presence of CD44 staining (Fischer's exact test,  $p < 0.05$ ). CD44 expression was detected in 81.8% of neuroblastic tumours with favourable histological type while 89.5% of tumours with unfavourable histological type do not express CD44 staining. The sensitivity, specificity and positive predictive value of CD44 pattern for neuroblastic tumours are 69.2%, 89.6% and 81.8% respectively. The analysis of CD44 expression should be recommended as an additional biologic marker in the initial routine staging of the disease.

#### **9. Fine-needle aspiration biopsy of salivary gland lesions: a cytological review of 60 cases with histological correlation**

*Noraidah MASIR, NA SHARIFAH, \*BS GENDEH*

*Departments of Pathology and \*Otorhinolaryngology, Faculty of Medicine, Universiti Kebangsaan Malaysia.*

A study was undertaken to evaluate the correlation between the fine-needle aspiration (FNA) findings and the histological diagnoses in 60 patients who presented with salivary gland enlargement. The results of 60 cases of FNA of the salivary gland which had histologic confirmation were reviewed. The cytological reports were classified into benign and malignant lesions followed by their specific diagnoses. Using the histology as the "gold standard" statistical evaluations were performed on the results. Eight specimens (13%) did not allow adequate cytological evaluation. Among the benign lesions, the cytologic diagnoses were concordant with histologic diagnoses in 95.5% of cases and among the malignant tumours, there was 100% concordance. There were 2 (4.5%) false negative cases. The specificity and sensitivity of FNA cytology were 96% and 80% respectively. The diagnostic accuracy was 96% and the positive predictive value was 100%. FNA is a useful and reliable preoperative technique for assessment of salivary gland lesions. Adequate sampling material and experience in interpretation are important in achieving high diagnostic accuracy.

**10. The use of the Amplification Refractory Mutation System (ARMS) as an effective and economical tool for prenatal diagnosis of  $\beta$ -thalassaemia in Malaysia**

TAN Jin Ai Mary Anne, \*YAP Sook Fan, TAN Kim Lian, \*\*THONG Meow Keong.

*Departments of Allied Health Sciences, \*Pathology and \*\*Paediatrics, Faculty of Medicine, University of Malaya*

$\beta$ -thalassaemia major patients express complete suppression of P-globin chain synthesis or are deficient in globin production. Clinical and health complications in these patients pose a heavy load on blood transfusion and paediatrics services as P-thalassaemia is the most common single gene disorder in Malaysia with a carrier rate of about 3% in the Chinese and Malays. An important approach in addressing this health problem is to offer effective prenatal diagnosis. The implementation of effective prenatal diagnosis programmes depends on the diversity of the ethnic groups and types of  $\beta$ -mutations in a country as over 160  $\beta$ -mutations have been reported.

We established and evaluated the ARMS as a prenatal diagnostic tool for P-thalassaemia in Malaysia. Using ARMS, 101 chromosomes from 51 Chinese families with P-thalassaemia were studied. A total of 13 mutations along the  $\beta$ -globin gene complex was analysed: -29, -28, Cap (+1), Cd 8/9, Cd 15, Cd 17, Cd 19, Cd 26 (Hb E), IVSI #1, IVSI #5, Cd 41-42, Cd 71-72 and IVSII #654. Our results indicated that prenatal diagnosis can be offered to 96% of the Chinese families. Five P-mutations alone (Cd 41-42, IVSII #654, -28, Cd 17 and Cd 71-72) accounted for 92% of P-thalassaemia in the Chinese. The rare p-mutations at Cd 15, Cap (+1) and -29 were detected in 3% of the Chinese. The ARMS which employs direct amplification of a gene sequence followed by simple agarose gel electrophoresis was found to be a rapid, effective and economical protocol for prenatal diagnosis of  $\beta$ -thalassaemia in the Malaysian Chinese.

**Poster presentations:**

**P1. Role of transforming growth factor-beta 1 in breast cancer**

Leslie C. LAI, Yui Su EE, Karin REIMANN and \*Patricia K.C. LIM

*Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, and \*Department of Molecular Pathology, Institute for Medical Research, Kuala Lumpur*

Transforming growth factor-beta (TGF- $\beta$ ) exerts a growth inhibitory effect on mammary epithelial cells and may play a role in the development of breast cancer. Oestrogens also play an important role in breast cancer development. Oestrone sulphate (E<sub>1</sub>S) acts as a large reservoir of active oestrogens in the breast and is converted to the weak oestrogen, oestrone (E<sub>1</sub>), by oestrone sulphatase, which is in turn converted to the potent oestrogen, oestradiol (E<sub>2</sub>), by oestradiol-17 $\beta$  hydroxysteroid dehydrogenase. Although breast cancers initially require oestrogens for their growth a substantial proportion become oestrogen unresponsive. The aim of this study was to assess the effect of the TGF- $\beta$ 1 isoform on growth of and oestrogen metabolism in the hormone dependent, MCF-7, and hormone-independent, MDA-MB-231, human breast cancer cell lines. Cells were grown in supplemented Dulbecco's Modified Eagle Medium for 3 days, then treated with varying concentrations of TGF- $\beta$ 1 and incubated for a further 3 days. The cells were then incubated with appropriate tritiated oestrogen for 4 hours. The amount of tritiated product formed was measured and the cell numbers were determined on a Coulter Counter. In the MCF-7 cell line TGF- $\beta$ 1 significantly inhibited cell growth and significantly stimulated the conversion of E<sub>1</sub>S to E<sub>2</sub>, but had no effect on E<sub>1</sub> to E<sub>2</sub> conversion. In the MDA-MB-231 cell line TGF- $\beta$ 1 significantly stimulated cell growth and inhibited interconversions between E<sub>1</sub> and E<sub>2</sub>, but had no effect on estrone sulphatase activity. The inhibitory effect of TGF- $\beta$ 1 on MCF-7 cells would confer a protective effect in breast cancer, while its ability to increase the amount of E<sub>2</sub> would increase the risk of breast cancer. Which of these effects predominates *in vivo* remains to be explored. The growth stimulatory effect of TGF- $\beta$ 1 on the ER-negative, MDA-MB-231 cell line probably acts through a mechanism independent of the effect of TGF- $\beta$ 1 on oestrogen concentrations since this cell line is hormone unresponsive.

**P2. Telomerase activity and telomere length in tumours**

Angela NG, Lai Meng LOOI and Phaik Leng CHEAH

*Department of Pathology, Faculty of Medicine, University Malaya*

The purpose of our project is to study the mechanism involved in telomerase activation and cell immortalization. Telomeres in eukaryotic organisms are characterized by tandem repeats of 3'TTAGGG' at both the chromosome ends. Due to end replication problems, the telomere region is shortened with each round of cell replication. This loss of telomeric DNA may serve as a mitotic clock in cellular aging and cell senescence. However, telomerase, an enzyme responsible for telomeric repeats synthesis *de novo* enables telomeres to maintain their lengths. In adult humans, telomerase activity has been reported in perpetually replicating cells such as germline cells in ovaries and testes, hematopoietic stem cells, immortal cell lines and cancer cells. This had led to the hypothesis of telomerase participation in cell immortalization.

In our preliminary study, we have studied telomerase activities in various tumours and their mean telomere lengths. Here, we are presenting a TRAP-ELISA (Telomeric Repeat Amplification Protocol) method and a radioisotope method of detection for this purpose. Our preliminary results show that not all tumour cells exhibit telomerase activities. Also, their mean telomere lengths do not always correlate positively with the telomerase activity present. This suggests that telomerase is not the single essential factor leading to cell immortalization. Thus there may be alternative mechanisms involved in this process.

**P3. ABI PRISM 377 automated DNA sequencer – our initial experience**

YH Yu, O Ainoon, SK Cheong and NH Hamidah

*Department of Pathology, Faculty of Medicine, National University of Malaysia, Kuala Lumpur.*

DNA sequencing is one of the essential methods in molecular biology. The technique for DNA sequencing was first introduced by Sanger in 1977 and since then many modifications and advancements have been made. These include automation which, apart from providing solutions for the shortcomings of manual sequencing, has also enhanced the usage of this technique in the study of many genes. Here, we would like to report our early experience in the use of the ABI PRISM 377 DNA Sequencer in analysing genes related to haematological disorders. Using the method based on cycle sequencing using fluorescent dye-labeled terminators we sequenced: 1) the normal G6PD gene, 2) exon 13 of the G6PD gene from a G6PD-deficient neonate to confirm the presence of the G-T at nucleotide 1376 which was determined earlier by PCR-based method using primers that artificially creates restriction enzyme site 3) the 385 bp DNA fragment amplified using BCR-ABL gene specific primers from a known case of chronic myeloid leukemia and a case of acute myeloid leukemia 4) a 800 bp DNA fragment amplified using BCR-specific primers from a normal individual. Results of sequences in the above cases were confirmed by comparing with sequences in the NCBI database (Basic Blast search).

**P4. Value of intracellular myeloperoxidase in differentiating lymphocytes, monocytes and granulocytes**

TAY Siow Phing, CHEONG Soon Keng, Noor Hamidah HUSSIN and Ainoon OTHMAN.

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

A study was undertaken to evaluate the value of intracellular myeloperoxidase in differentiating populations of lymphocytes, monocytes and granulocytes. Lysed whole blood method was employed in this study. Anticoagulated blood from normal individuals was lysed with FACS lysing solution and permeabilized FACS permeabilizing solution respectively, at room temperature for 10 minutes. The cells were then stained with anti-CD45, anti-CD14, anti-IgG<sub>1</sub>, and anti-IgG<sub>2a</sub> and anti-myeloperoxidase (MPO-7) monoclonal antibodies by direct immunofluorescence. The geometric

means of the fluorescence intensity were measured using FACSCalibur flow cytometer (Becton Dickinson). The intensity of CD14 expression in lymphocytes/monocytes and monocytes/granulocytes was significantly different. Significant differences in the expression of CD45 were shown between lymphocytes/monocytes and lymphocytes/granulocytes. Expression of MPO was significantly different between lymphocytes/granulocytes and monocytes/granulocytes. These results indicate that anti-MPO is able to distinguish statistically normal granulocytes from normal lymphocytes and monocytes, but unable to differentiate normal lymphocytes from normal monocytes.

#### **P5. Double staining of EBER-ISH and CD30 immunostain**

*Lian-Hua KIM, Suat-Cheng PEH, Siew-Pin CHAI.*

*Department of Pathology, University of Malaya, Kuala Lumpur*

**Background:** Epstein-Barr virus (EBV) has been frequently detected in lymphoid malignancies. In Hodgkin's disease (HD), the Reed-Sternberg (RS) cells express CD30 antigen and it is known that EBV infection can up-regulate the expression of CD30. Double staining for EBV by EBER *in situ* hybridisation (ISH) and immunostain against CD30, enables the demonstration of co-expression of EBER and CD30. **Methods and Materials:** A known EBV-associated HD case was chosen for this study. We performed double staining with different antigen retrieval methods such as microwaving or Proteinase K digestion for CD30 on the paraffin embedded tissue section. Proteolytic digestion for the EBER-ISH technique was with Proteinase K. 4-Nitroblue-Tetrazolium chloride/5-Bromo-4-Chloro-3-Indolyl-Phosphate (NBT/BCIP) which gives dark blue/black coloration and 3-Amino-9-Ethylcarbazole (AEC) which gives a red coloration were used as the substrates for EBER-ISH and CD30 immunostain respectively. Counter stain was Haematoxylin. We worked on different sequence of staining steps: 1) EBER-ISH followed by CD30 staining with microwave pretreatment, 2) EBER-ISH followed by CD30 staining without pretreatment, 3) CD30 staining with microwave pretreatment followed by EBER-ISH and 4) CD30 with Proteinase K pretreatment followed by EBER-ISH without further enzyme digestion. **Results:** Method (1) produced the best result with CD30 staining up the cytoplasmic membrane red and the EBER positivity in the nucleus with dark blue/black colour. These were very distinguishable in the background of light blue stain of Haematoxylin. The double staining showed that vast majority of the RS cells were EBER<sup>+</sup> and CD30<sup>+</sup>. Methods (2), (3) and (4) gave clear EBER positivity but weak CD30 staining. **Conclusion:** EBER-ISH followed by CD30 staining with microwave pretreatment is the method of choice, and can be used for demonstration of co-expression for the investigation of HD in future studies.

#### **P6. The pattern of malignant lymphoma in East Malaysia**

*S.P. CHAI, S.C. PEH, M.Y. LIM, \*H.R. Gudum*

*Departments of Pathology, University of Malaya and \*Universiti Malaysia Sarawak*

**Background:** Malignant lymphoma is a highly heterogenous group of malignant disease. This study aimed to elucidate the pattern of malignant lymphoma in East Malaysia patient population. **Materials and Methods:** 105 cases of reconfirmed malignant lymphomas from the East Malaysia biopsy material were retrieved from the files in the Department of Pathology, University of Malaya in the period between 1981 to 1983. With the use of a panel of lymphoid antibodies, the diseases were classified using Rye classification for Hodgkin's disease (HD) and modified Kiel classification for non-Hodgkin's lymphoma (NHL). **Results:** Of the 105 cases, 60(57.1%) were from Sarawak, 45(42.9%) from Sabah. There were 64 (61%) male, 41 (39%) female, a male-female ratio of 1.6:1. Their ages ranged from 2 to 78 years. 12 (11.4%) were children (<15 years old) and 98 (88.6%) adults. There were 16 (15.2%) Malay, 13 (12.4%) Chinese, 1 (1%) Indian, 34 (32.4%) Sarawak and 41(39.0%) Sabah indigenous populations respectively. There were 11(10.5%) HD, 80 (76.2%) B-NHL and 14 (13.3%) T-NHL. The HD:NHL ratio was 1:9. The most common type of lymphoma in children was Burkitt's lymphoma 7 (58.3%). In the adult group, there were 72 (78.3%) B-NHL (49 large cell, 8 Burkitt's lymphoma, 5 Follicular, 2 ALCL CD30+, 2 low grade MALT, 1 Mantle cell and 5 not otherwise specified due to poor morphology), 12 (13.1%) T-NHL and 9 (9.8%) HD. The most common T-NHL was PTCL unspecified (11/12) and the remaining 1 case was T-cell ALCL

HD-like. Majority of PTCL unspecified (10/12) were from Sabah. Of the 9 adult HD, the most common subtype was nodular sclerosis 6 (66.7%). In conclusion, the variety of subtypes of malignant lymphoma seen in East Malaysia was rather similar to West Malaysia except for the very low prevalence of PTCL (3.3%) in Sarawak.

**P7. Upper gastro-intestinal haemorrhage secondary to a gastric lipoma: a case report**

MJ JULIA MUNCHAR, AR HAYATI, \*AY JASMI, \*M ROHAIZAK.

*Departments of Pathology and \*Surgery, Faculty of Medicine, Universiti Kebangsaan Malaysia, Kuala Lumpur.*

A 40-year-old Chinese lady first presented to a private Hospital in August 1997 for massive upper gastro-intestinal haemorrhage. An emergency upper gastro-intestinal endoscopy was performed which showed a bleeding gastric ulcer. At laparotomy, a bleeding ulcer was noted with an associated underlying mass. Undermining of the ulcer and partial excision of the mass was carried out. The histology of the mass was reported as a gastric leiomyoma. She recovered from the operation and was sent home with anti-ulcer drugs. A surveillance upper endoscopy six months later showed the mass had regrown. She was then referred to our hospital for further management. A CT scan performed revealed a large intramural mass in the stomach. The density of the mass was equivalent to that of the subcutaneous fat. Due to the difficulty in excluding a recurrent leiomyoma and the worrying rapidity of its growth, a relaparotomy was performed. Intraoperatively, a huge lobulated mass was found within the wall of the stomach and a wedge resection of the middle third of the stomach with primary anastomosis was carried out. Grossly there was a huge, soft, lobulated mass measuring 15x8x2.5cm in size and occupying the whole circumference of the resected specimen. Histologic examination established the diagnosis of a gastric lipoma. A review of the clinical, diagnostic, pathological and therapeutic aspects of this rare disease is reported.

**PS. Serum ferritin and lactate dehydrogenase in a case of hemophagocytic lymphohistiocytosis**

C.F. LEONG, S.K. CHEONG, \*KANNAHESWARI Y, NH. HAMIDAH, AINOON O

*Departments of Pathology and \*Paediatrics, Hospital Universiti Kebangsaan Malaysia, Kuala Lumpur*

A 40-day-old baby girl presented with intermittent fever, lymphadenopathy, massive hepatosplenomegaly, progressive pancytopenia and features of disseminated intravascular coagulopathy. A bone marrow aspiration was performed and showed florid histiocytic proliferation with marked hemophagocytosis. Based on the diagnostic guideline for HLH proposed by the Familial Hemophagocytic Lymphohistiocytosis Study Group of Histiocyte Society, this patient fulfilled most of the criteria. We have also found the serum ferritin and lactate dehydrogenase (LDH) levels to be very high in this patient. This is in agreement with what has been described by an author, Imashuku *et al.* HLH is a disorder of the monocytic-macrophage lineage. It remains uncertain whether the disorder is a reactive or neoplastic condition.

**P9. True histiocytic lymphoma - a case report**

AB Hamidah, SK Cheong, \*S Fadillah, M Julia and M Ibtisam

*Departments of Pathology and \*Medicine, Hospital Universiti Kebangsaan Malaysia, Kuala Lumpur.*

True Histiocytic Lymphoma (THL) is an uncommon type of non-Hodgkin's lymphoma composed of malignant cells with enzymatic and immunologic features of phagocytic histiocytes. A full appreciation of THL, as a recognizable disease, has been complicated by its rarity, diversity and inconsistent terminology used to describe various histiocytic processes. Whether malignant histiocytosis and the THL are part of the same spectrum of histiocytic and reticulum/dendritic cell malignancies which have been given arbitrary names and definitions, is not known at present. Successful immunophenotyping of THL depends on two important factors. First, the malignant cells react

preferentially with multiple histiocyte associated monoclonal antibodies, and second, the malignant cells fail to react with B- or T-cell monoclonal antibodies except against those antigens that are known to be expressed on cells of monocytic origin. A 38-year-old lady presented with weight loss, fever, left groin pain which radiated to the left leg, hepatosplenomegaly and lymphadenopathy. Staging evaluation revealed multiple paraaortic lymphadenopathy with a left iliac fossa mass; bone marrow aspirate/biopsy revealed only mild plasmacytosis; a lymph node biopsy showed a poorly differentiated malignancy which stained strongly for vimentin, CD68 and Mac 387 but negative for Actin, Desmin, Cytokeratin, EMA, HMB-45, S-100, LCA, B-cell, T-cell, Ki-1 and Leder's stain. A diagnosis of Histiocytic Lymphoma was made based on the detection of specific histiocytic markers. We present this case because it posed difficulties in diagnosis and to reinforce the premise that a single antibody cannot be relied upon for a diagnosis of THL.

#### **P10. Fine needle aspiration cytology of malignant liver tumours in children**

*SHARIFAH NA, WM WAN MUSTAFA, \*A Rahman Jamal, \*\*Zulfikar ANUAR, #Zakaria ZAHARI*

*Departments of Pathology, \*Paediatrics and \*\*Radiology Hospital Universiti Kebangsaan Malaysia and #Department of Surgery, Institute of Paediatrics, Hospital Kuala Lumpur.*

This study describes the cytologic finding observed in 15 cases of hepatolastoma, two cases of hepatocellular carcinoma and one case of undifferentiated embryonal sarcoma of the liver (UESL) in children. The main objective is to analyse the cytomorphologic characteristics of these three different primary malignant liver tumours and to determine whether these findings permit us to make a reliable diagnosis. All diagnoses were confirmed by subsequent histological examination of resected specimens or cell block. A definite diagnosis of hepatoblastoma was made in 14 of 15 cases (93%). Two cases of hepatocellular carcinoma and one case of UESL were also correctly diagnosed on cytology. We conclude that with knowledge of the cellular features and architectural patterns of liver tumours together with relevant clinical and radiological information, a reliable diagnosis (94%) can be made.

#### **P11. Immunohistochemical detection of p53 protein in childhood rhabdomyosarcoma**

*Yusmaila MY, Sharifah NA, \*Rahman JAMAL, Wan Muhaizan WM, \*\*Raja Khuzaiah AR*

*Departments of Pathology and \*Paediatrics, Hospital Universiti Kebangsaan Malaysia and \*\*Department of Paediatrics, Institute of Paediatrics, Hospital Kuala Lumpur.*

Alterations in the tumour suppressor gene p53 are the most frequent observed genetic change in various types of cancer including rhabdomyosarcoma. To investigate the overexpression of p53 and its relation to age, sex and histological type in childhood rhabdomyosarcoma (RMS), an immunohistochemical study was performed. Formalin-fixed paraffin-embedded tissue sections obtained from 15 cases of childhood RMS were immunostained with a mouse monoclonal antibody towards p53. Positive staining was assessed by the presence of brownish nuclear staining of the tumour cells. Nuclear accumulation of p53 protein was detected in 7 of 15 samples. Statistical analysis performed demonstrated no correlation between p53 staining with age, sex and histological type. This suggests that although p53 nuclear immunoreactivity in RMS is high compared to other studies, there is no correlation between p53 overexpression and clinicopathological features of RMS.

#### **P12. BCR-ABL fusion gene in patients with acute myeloid leukaemia**

*Nadirah MUSA, Noor Hamidah HUSSIN, Ainoon OTHMAN and Soon Keng CHEONG*

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

BCR-ABL fusion gene which results from a translocation between the long arms of chromosome 22 and 9 occurs frequently in chronic myeloid leukaemia (CML), less commonly in acute lymphoblastic leukaemia (ALL) and rarely in acute myeloid leukaemia (AML). This fusion gene results in two

major subtypes of distinct fusion proteins, p190 and p210. In this study, 19 cases of leukaemia (8 cases of CML, 3 cases of AML, 8 cases of ALL) and 1 case of essential thrombocytopenia (ET) were studied. This was done by using reverse transcriptase polymerase chain reaction (RT-PCR) as it is quicker, more sensitive and specific. It was found that 2 out of 3 cases of AML revealed the occurrence of BCR-ABL gene with b2a2 rearrangement that resulted in the formation of nucleotides of 310 bp length. These were confirmed by direct sequencing. From previous studies it was found that patients of AML with detection of BCR-ABL gene had lower remission rates and poorer survival. Thus the presence of BCR-ABL gene in AML cases may have prognostic implication.

### **P13. Ki-67 and p53 in non-Hodgkin's lymphoma**

*Ming Tsuey CHEW and Suat Cheng PEH*

*Department of Pathology, University of Malaya, Kuala Lumpur*

Ki-67, a growth fraction expressed in all phases of the cell cycle except in the resting phase (G<sub>0</sub>), has been found to correlate with tumour grades and prognosis in non-Hodgkin's lymphoma (NHL). p53, a tumour suppressor gene which controls the cell cycle of damaged DNA also regulates apoptosis. This study aims at determining the pattern of expression of Ki-67 and p53 in NHL. Forty cases of NHL, 31 B-NHLs (25 high-grade and 6 low-grade) and 9 T-NHLs (all high grade) were retrieved from the files of the Department of Pathology, University Hospital Kuala Lumpur. They were stained for Ki-67 and p53 oncoprotein with immunocytochemical technique. Expression of Ki-67 in more than 25% of the tumour cells were graded as high, and more than 10% for p53 was considered as overexpression.

Of the 40 cases, 17 (43%) showed p53 overexpression. Fourteen were high-grade B-NHLs (14/25, 56%) and 3 T-NHLs (3/9, 33%). Out of the 14 cases of high-grade B-NHLs, 11 were diffuse large cell lymphomas and 3 Burkitt's lymphomas. Twenty of the 40 (50%) cases showed high Ki-67 proliferative index: 14 (14/25, 56%) were high-grade B-NHLs and 6 (6/9, 67%) were T-NHL. None of the cases of low-grade NHL overexpressed p53 or showed high Ki-67 proliferative index. 13/40 cases (33%) showed both overexpression of p53 and high Ki-67 proliferative index. Chi-square test showed a statistically significant association of Ki-67 and p53 with histological grades of NHL,  $p=0.029$  and  $p=0.020$  respectively. However, McNemar's test did not show significant association of p53 overexpression and high Ki-67 proliferative index.

### **P14. Primary squamous cell carcinoma of the thyroid – a case report**

*Muhaizan WM, Phang KS, Sharifah NA, \*Al Amin D.*

*Departments of Pathology and \*Surgery, Universiti Kebangsaan Malaysia, Kuala Lumpur.*

A rare case of primary squamous cell carcinoma of the thyroid is reported herein. A-64 year-old Malay lady presented with a history of a gradually enlarging thyroid nodule for 6 months and underwent total thyroidectomy. Histopathological diagnosis was given as squamous cell carcinoma of the thyroid with complete excision achieved. Possible primary elsewhere was excluded. Postoperative irradiation was given and patient is still alive after 2 years of follow up.

### **P15. Malignant mesothelioma of the pleura : a case report**

*Siti-Aishah M.A., \*Thomas Krausz, S.A. Wahab, \*\*Al Amin M.D., \*\*Haron Ahmad*

*Departments of Pathology and \*\*Surgery, Faculty of Medicine, Hospital Universiti Kebangsaan Malaysia, Kuala Lumpur, and \*Department of Histopathology, Hammersmith Hospital, London*

Malignant mesothelioma of the pleura is a rare tumour. This may be the first reported case in this country. We describe a 53-year-old Malaysian of Chinese origin who presented clinically with a right sided pleural effusion. Thoracoscopy was done and it showed that pleural nodules were present in the right inner chest wall. Extensive soft adhesive forming loculations surrounded the right lung. Histopathological examination revealed the pleural tumour had a partly solid papillary growth

pattern and was composed of epithelioid neoplastic cells. No neutral mucin was seen on special staining. Immunohistochemistry showed positive staining for cytokeratin, calretinin and focally for HMFG2. CEA, LeuM1, Ber-EP4, S-100 were negative. The pleural effusion was persistent and she was referred to the Institute of Radiotherapy and Oncology, Kuala Lumpur for further management.

**P16. Crystalline deposition in the bone marrow of a patient with multiple myeloma.**

*MNY Nik Rus, SK Cheong, \*AW Fadilah.*

*Departments of Pathology and \*Medicine, Faculty of Medicine, Universiti Kebangsaan Malaysia.*

A 48-year-old Malay man presented with 3 months history of persistent right knee pain. Apart from pallor and tenderness over the right knee, he was otherwise unremarkable. Investigations revealed normochromic normocytic anaemia (Hb 5.5g/dL) with rouleaux formation, elevated ESR (124mm/Hr), hypoalbuminaemia and hypercalcaemia. Renal functions were not impaired. Multiple lytic lesions were detected in the lower right femur and upper right tibia-fibula. Serum and urine protein electrophoresis confirmed the presence of IgG kappa M component with markedly high serum paraprotein level (60g/L). Bone marrow aspiration was dry but its trephine revealed abundant non-congophilic crystals and amorphous materials in the marrow spaces. Further immunohistochemical stains showed that these deposits were Periodic Acid Schiff and anti-kappa positive. Amyloid, light chain and immunoglobulin depositions are well recognised in multiple myeloma. However, we have not encountered any report of a crystalline deposit in the bone marrow of patients with multiple myeloma. We believe this case illustrates an unusual deposition of kappa light chains in high concentration leading to crystal formation in the bone marrow.

**P17. Ki-67 expression as evidence of cellular proliferation in diffuse (WHO CLASS IV) lupus glomerulonephritis**

*PL Cheah, R Kunasegaran, LM Looi*

*Department of Pathology, University of Malaya Medical Centre, 50603 Kuala Lumpur*

The hallmark of diffuse (WHO Class IV) lupus glomerulonephritis (DLGN) is the morphological increase in number of cells within glomeruli. It generally assumed that this increase is due to proliferation of glomerular cells but a numerical increase visualised morphologically can be entirely due to decreased obsolescence. In an attempt to obtain proof of glomerular cellular proliferation, 23 histologically reconfirmed cases of DLGN were immunohistochemically stained for Ki-67 (DAKO) antigen expression, using the standard immunoperoxidase method. Kidney tissue from 23 previously healthy young adults who had died from traumatic injuries and were subjected to autopsy within 24-hours of death served as controls. There were 17 Chinese and 6 Malays among the lupus cases. Of these, 20 were females and 3 males. Ages ranged between 10 to 56 years (mean = 31.9 years). The control cases were made up of 9 Indians, 7 Malays, 4 Indonesians, 2 Chinese and 1 Bangladeshi. Among them, 20 were males and 3 females. Their ages ranged between 15-33 years (mean = 23.3 years). 16 (69.6%) out of 23 cases of DLGN and 8 out of 23 control kidneys showed Ki-67 immunopositivity in at least one glomerulus ( $p>0.05$ ). A total of 256 glomeruli in the DLGN group and 2159 in the control kidneys were studied for Ki-67 expression. 37 (14.5%) DLGN and 16 (0.7%) control kidney glomeruli showed at least one mesangial, endothelial or epithelial cell to be Ki-67 immunopositivity ( $p<0.01$ ). These observations confirm that there is indeed cellular proliferation in DLGN and this is at least contributory to the morphological numerical cellular increase observed.

**P18. Hepatocellular carcinoma in Malaysians: the role of p53 mutation**

*PL Cheah, AR Nazarina, LM Looi*

*Department of Pathology, University of Malaya Medical Centre, Kuala Lumpur*

Immunohistochemical detection of nuclear mutant p53 protein serves as a convenient surrogate for identification of p53 tumour suppressor gene mutation. 43 cases of hepatocellular carcinoma (HCC)

diagnosed at the Department of Pathology, University of Malaya Medical Centre, Kuala Lumpur between January 1991 to December 1997 were studied for p53 mutation using a standard immunoperoxidase method. Archival, formalin-fixed, paraffin-embedded material were stained using a commercial p53 (D07-DAKO) monoclonal antibody. Of these 43 cases, 38 were males and 5 females. Ethnically, 33 were Chinese, 5 Malays, 4 Indians and 1 of a minor indigenous racial origin. Their ages ranged from 21-85 years with a mean of 57.1 years. 19 (44.2%) cases did not exhibit any p53 nuclear positivity. Of those which demonstrated p53 immunopositivity, weak staining was seen in 6 cases in <25% and 2 in 26-50% of the tumour nuclei. Moderate intense staining was observed in 1 in <26-50%, 4 in 51-75% and 5 in >75% of tumour nuclei. Strongly positive signals were seen in 1 in 51-75% and 5 in >75% of tumour nuclei. Percentage of tumour nuclei exhibiting p53 positivity, rather than intensity of staining, is generally considered as being more indicative of a mutational event although there is a tendency for intensity to correlate with percentage. Accepting only a stringent cut-off value of >75% of tumour nuclei p53 immunopositivity as indicative of mutation, would imply that p53 mutation occurred in 23.3% of HCC. p53 mutation would then seem to be an event which occurs occasionally but is not a necessary feature in 67.4% of the total number of cases. This finding is interesting and may be due to the generally high hepatitis B carrier rate among the Chinese. What resulted in the male predominance has however still to be explained.

**P19. Bone turnover in post-menopausal women with non-insulin dependant diabetes mellitus (NIDDM)**

*Tengku Norita TENGKU YAZID, \*Khalid ABDUL KADIR, Hapizah MOHD NAWAWI*

*Chemical Pathology Division, Department of Pathology and "Department of Medicine, Faculty of Medicine, Universiti Kebangsaan Malaysia.*

NIDDM is a common chronic metabolic condition and is linked to osteoporosis. It has been shown that there is a two-fold increased risk of fracture neck of femur in diabetics. Improvement of glycaemic control has been shown to decrease bone turnover. The pathogenesis of diabetic osteopaenia has not been well established. We studied 45 postmenopausal NIDDM patients (mean  $\pm$  sd age =  $59.8 \pm 7.0$ , range 50-76 years) and 29 non-diabetic postmenopausal control subjects (mean  $\pm$  sd age =  $55.7 \pm 4.0$ , range 50-65 years). Exclusion criteria include smokers, excessive alcohol intake, renal, liver, endocrine disorders, bone diseases or drug(s) which affect bone metabolism. Serum total alkaline phosphatase (TALP) and bone specific alkaline phosphatase (BSALP) as markers of bone formation, and urinary deoxypridinoline (Dpyr) as a marker of bone resorption were determined. Serum TALP assay was performed by a standard automated method (Roche Integra), BSALP and Dpyr were measured by enzyme immunoassays (Metra Biosystems). The urinary Dpyr was expressed as a ratio to urinary creatinine (Dpyr/Cr). There were significantly higher (a) TALP (mean  $\pm$  sem =  $67.5 \pm 5.7$  vs.  $45.0 \pm 2.5$  iu/L,  $p < 0.005$ ), (b) BSALP (mean  $\pm$  sem =  $20.1 \pm 1.1$  vs  $12.1 \pm 0.6$  iu/L,  $p < 0.0001$ ) and (c) Dpyr/Cr ratio (mean  $\pm$  sem =  $39.9 \pm 21.1$  vs  $7.9 \pm 1.0$  iu/L,  $p < 0.0001$ ) in NIDDM patients compared to control subjects. There was no significant difference in the Dpyr/Cr:BSALP index between NIDDM and control subjects. There was significant correlation seen between TALP and BSALP in the NIDDM patients ( $r = 0.624$ ,  $p < 0.0001$ ) but no significant correlation seen between either TALP or BSALP and Dpyr/Cr ratio in both the NIDDM and control groups. In conclusion, there is absence of the bone resorption: formation uncoupling phenomena in NIDDM. However there is enhanced bone resorption and a parallel increment in the bone formation, suggesting an increased bone turnover in NIDDM which may explain the osteopaenia associated with diabetes.

**P20. The immunohistochemical discrimination of endometrioid adenocarcinomas**

*K Sarojini Devi, AR Nazarina*

*Department of Pathology, University of Malaya Medical Centre (UMMC), Kuala Lumpur*

Adenocarcinomas of endometrioid morphology frequently arise in the endometrium, endocervix and ovary to involve also the adjacent pelvic tissues including the colon, in females. Conversely,

carcinomas of similar morphology may primarily develop in the colon and secondarily involve, most frequently, the ovary. The determination of the primary site of the lesion is a diagnostic problem of importance for subsequent treatment and prognosis. Apart from clinical pointers, immunohistochemical stains, in particular, vimentin and carcinoembryonic antigen (CEA), have been shown to be particularly useful in aiding this discrimination. This retrospective study looked at the vimentin and CEA expression of 91 cases of endometrioid adenocarcinomas, including 34 of the endometrium, 22 of the endocervix, 19 of the ovary and 16 of the colon obtained from a 12-year record, from January 1985 to October 1996, in the Department of Pathology, UMMC. The objective was to document a local corroboration of such immunohistochemical discrimination. Formalin-fixed, paraffin-embedded material were stained using commercial (DAKO) monoclonal vimentin and polyclonal CEA. Vimentin was positive in 19 (55.9%) of the endometrial tumours, 3 (33.6%) of the endocervical tumours, 9 (47.4%) of the ovarian tumours and none (0%) of the colonic tumours. CEA was positive in 2 (5.9%) of the endometrial tumours, 13 (59.1%) of the endocervical tumours, 1 (5.3%) of the ovarian tumours and 12 (75%) of the colonic tumours. Thus, endometrioid carcinomas arising in the endometrium and ovary are more often positive for vimentin than their cervical or colonic counterparts which exhibit a comparable frequency of CEA expression instead. Of note is that none of the colonic tumours expressed vimentin while only <6% of the endometrial and ovarian tumours expressed CEA. In practical terms, vimentin and CEA can be used to differentiate endometrial and endocervical endometrioid adenocarcinomas as well as between ovarian and colonic tumours of similar endometrioid morphology. This, in essence, supports previous findings. Demographic profiles are also presented.

#### **P21. Monstrous cells in prostate biopsies: a mimic of carcinoma**

*Lai-Meng LOOI and Phaik-Leng CHEAH*

*Department of Pathology, University of Malaya, Kuala Lumpur*

In histopathology practice, one has to be constantly aware of mimics of specific entities, particularly of malignancies, as such mimics are important causes of misdiagnosis, leading to serious consequences of mismanagement. In a study of 100 consecutive needle biopsies of the prostate, we encountered monstrous cells in seminal vesicle epithelium in 8 (8%) instances. These cells show enlarged nuclei with marked hyperchromasia and obscure nuclear details. Golden-brown lipofuscin granules are often present in the cytoplasm, and there is lack of immunoreactivity for prostatic specific antigen. This finding cautions us on the importance of being aware of this entity, as its misdiagnosis as carcinoma could have lead to serious mismanagement of a substantial proportion of patients.

#### **P22. Prostatic intraepithelial neoplasia in prostate biopsies**

*Lai-Meng LOOI, Phaik-Leng CHEAH, P Jayalakshmi and \*Sahabudin Raja Mohamed*

*Department of Pathology, University of Malaya, Kuala Lumpur and \*Institute of Urology, Hospital Kuala Lumpur.*

Studies from the West have shown conflicting information on the contribution of prostatic intraepithelial neoplasia (PIN) to raised serum prostatic specific antigen (PSA) levels, although high grade PIN is generally considered to be a precursor to some forms of prostatic carcinoma, particular adenocarcinoma of the peripheral zone. During a prostate awareness programme conducted by the Institute of Urology, Kuala Lumpur Hospital, sextant biopsies of the prostate were performed on subjects with raised PSA levels (>4 ng/mL). Histopathological examination of the first 100 consecutive biopsies from this programme were performed at the Department of Pathology, University of Malaya and revealed 5 (5%) instances of PIN. 3 of these were low-grade and 2 were high-grade PIN. Of the latter, 1 showed the concurrent presence of prostatic adenocarcinoma. This is the first major study into the pattern of prostatic disease in our population. Although the current data on PIN appears similar to the West, this data is preliminary, and numbers are too small for statistical conclusions.

### **P23. Non-Hodgkin's lymphoma – bone marrow and peripheral blood changes**

*LIM Ee Jin and PEH Suat Cheng*

*Department of Pathology, Faculty of Medicine, University of Malaya, Kuala Lumpur.*

47 patients of University Hospital Kuala Lumpur with non-Hodgkin's lymphoma (NHL) were studied retrospectively to determine their marrow and blood changes at diagnosis. Each patient's blood counts and individual white cell absolute counts were recorded. Blood films were examined for lymphoma cells, atypical lymphocytes (al), myeloid precursors **and/or** nucleated red cells (m/nrbc). Marrow smears and trephine biopsies were examined for lymphoma cells, infiltration pattern, lymphocytosis, eosinophilia and reticulin increase (RI), and tissue biopsies for confirmation of diagnosis and subclass using the modified Kiel's classification.

21.3% had lymphoblastic lymphoma (LB), 21.3% had peripheral T cell lymphoma (unspecified) (PTCL-u), 29.8% had B large cell NHL (BNHLLC), 10.6% had Burkitt's lymphoma (BL) and 17% had other subtypes. The overall incidences of patients with one or more abnormal counts (**omAC**), anaemia, leucocytosis, thrombocytopenia, lymphocytopenia and **m/nrbc** at diagnosis were 85.1%, 66%, 27.7%, 25.5%, 43.5%, and 28.3% respectively. The incidence of other blood abnormalities were below 25%. The frequency of marrow involvement (MI) was 46.8%, with diffuse infiltration in 71.4%. The incidences of marrow RI, eosinophilia and lymphocytosis were 40.9%, 44.7% and 21.3% respectively. Presence of **omAC** and anaemia were common in all subtypes. In LB, common features included lymphocytosis and **al** in the blood, diffuse **MI** and marrow lymphocytosis. In PTCL-u, the common features were peripheral lymphocytopenia, marrow RI and eosinophilia. In BNHLLC, peripheral lymphocytopenia and **m/nrbc**, **MI** and eosinophilia were common. In BL, diffuse **MI** and eosinophilia were common. The B- and T-NHLs did not differ significantly in parameters studied. Our findings do not differ much from others except for higher overall incidences of anaemia and diffuse MI, and lower incidence of **MI** in PTCL-u.

### **P24. Non-Hodgkin's lymphoma – correlation between haematological changes and marrow involvement**

*LIM Ee Jin and PEH Suat Cheng*

*Department of Pathology, Faculty of Medicine, University of Malaya, Kuala Lumpur.*

47 patients of University Hospital Kuala Lumpur with non-Hodgkin's lymphoma (NHL), were studied retrospectively to determine the correlation between hematological changes at diagnosis and marrow involvement (MI). Full blood counts and individual white cell absolute counts were recorded. Blood films were examined for lymphoma cells, atypical lymphocytes (al), myeloid precursors **and/or** nucleated red cells (**m/nrbc**). Marrow smears (MS) and trephine biopsies (TB) were examined for lymphoma cells, infiltration pattern, lymphocytosis, eosinophilia and reticulin increase (RI), and tissue biopsies for confirmation of diagnosis and subclass using the modified Kiel's classification.

46.8% of all patients had MI. TB detected MI in 45.7% and MS in 38.1% of cases. Involved cases with simultaneous aspirates and biopsies at diagnosis, lymphoma was detected by trephine **alone** in 16.7% but not detected by MS alone; therefore TB were slightly more sensitive. Multiple cytopenias, anaemia, thrombocytopenia, neutropenia, pb **m/nrbc**s and marrow RI were significantly more common at presentation in patients with MI. Absence of abnormal counts made **MI** unlikely but not impossible. 10 patients had lymphoblastic lymphoma, 10 had peripheral T cell lymphoma (unspecified) (PTCL-u), 14 had B large cell NHL (BNHLLC), 5 had Burkitt's lymphoma and 8 had other subtypes. Greater frequency of RI, pb **al** and decreased frequency of leucocytosis were seen in **MI** by lymphoblastic lymphoma but none of these were statistically significant. Neutropenia, pb **m/nrbc** and RI were more common in PTCL-u with MI. These observations were not statistically significant. In BNHLLC, anaemia, thrombocytopenia, multiple cytopenias, **m/nrbc** and RI were significantly associated with MI. There were too few Burkitt's lymphoma patients for analysis, but RI was more frequent in MI.

**P25. Correlation between mast cell infiltration and histopathological parameters in gastric carcinoma**

*P.Jayalakshmi, LM Looi and \*John T Arokiasamy*

*Departments of Pathology and \*Social and Preventive Medicine, Faculty of Medicine, University of Malaya, Kuala Lumpur.*

Mast cells (MC) have been investigated in tumour pathology of its ability to generate tumour necrosis factor alpha C which is cytotoxic to certain tumour cells as well as a potent stimulator of immune cells. However, other studies have demonstrated that MC enhance tumour growth because MC heparin has potency as an endothelial mitogen and suppressor of lymphocyte activation.

Although MC infiltration in rectal cancer has been reported to be associated with a poor prognosis, its role in other tumours remain controversial. Currently, the prognostic assessment of carcinoma of the stomach, one of the common cancers in Malaysia, is dependent on staging and histological typing. We studied the association between MC infiltration and various histopathological prognostic parameters in 34 consecutive gastrectomy specimens with adenocarcinoma of the stomach. Formalin-fixed, paraffin embedded sections of the tumour border and one tumour free surgical margin were stained for MC, by the standard avidin-biotin complex method using antitryptase monoclonal antibody. MC was countered using a light microscope under x 40 objective. A minimum of 40 fields were studied. The tumours were classified according to the Lauren's system into intestinal (15) and diffuse (19) types. In all the cases, the tumour had infiltrated the muscular wall. Spread to the lymph node was seen in 24 cases. MC were observed at both tumour edge and tumour free surgical margins. There was no statistical correlation between density of mast cell infiltration and histological type and presence of lymph node metastasis. While it would appear that MC infiltration is not related to histological type or lymph node status, we note that the majority of our cases had advanced carcinomas and that this study may not have sufficient discriminatory power to differentiate good and bad prognosis tumours.

**P26. A comparison of pS2 expression with oestrogen receptor positivity, histological grade and lymph node status in infiltrating ductal carcinoma of breast**

*Wan Azura Wan Yaacob, L.M. LOOI, P.L. CHEAH, C.L. LAM and M.H. NG*

*Department of Pathology, Faculty of Medicine, University of Malaya*

Although early studies on trefoil proteins concentrated on the gastrointestinal tract, there has been recent interest in their presence in mammary cells. pS2, the first trefoil protein to be described, was originally identified through its regulation by oestrogen in a breast cancer line suggesting that it may be a marker of hormone responsiveness with a potential role in the selection of breast cancer patients for anti-oestrogen therapy. We have carried out an investigation into the immunohistochemical expression of pS2 in 70 consecutive infiltrating ductal breast carcinomas treated with mastectomy and axillary lymph node clearance to gain insight into (1) its prevalence in invasive breast carcinoma in our local population, (2) its relationship with oestrogen receptor (ER) protein expression and its probability as a co-marker of hormone responsiveness, and (3) whether it may be a marker of tumour aggressiveness through its correlation with tumour histological grade and the presence of axillary lymph node metastasis.

Of the 70 carcinomas studied, 4 (5.7%) were histological grade 1, 40 (57.1%) grade 2 and 26 (37.1%) grade 3 tumours. 45 (64%) showed histological evidence of axillary lymph node metastasis. 40 (57%) carcinomas were ER positive while 31 (44%) were pS2 positive. There was a statistically significant correlation between pS2 and ER expressions (Chi-square test with Yates correction:  $p < 0.005$ ). There was no correlation between pS2 expression and histological grade ( $p > 0.1$ ) and the presence of lymph node metastasis ( $p > 0.1$ ). Our findings support the views that pS2 should be seriously considered as an alternative or co-marker of endocrine responsiveness in invasive breast cancer and that pS2 does not influence breast cancer biology in terms of invasiveness and potential for metastatic spread.

**P27. Correlation of acute atherosclerosis and hypertension in molar pregnancy**

Azizah A., \*Hayati A.R., Wahidah A.

*Department of Pathology, Hospital Kuala Lumpur and \*Department of Pathology, Universiti Kebangsaan Malaysia Kuala Lumpur.*

38 cases diagnosed as molar pregnancy in Hospital Kuala Lumpur were studied. The curettage samples were processed into hematoxylin and eosin slides. The sections were studied for the presence of acute atherosclerosis in the decidual vessels. All the cases were complete moles. Only eight out of the 38 cases (8.4%) showed the presence of acute atherosclerosis. None of the cases had hypertension.