

The 21st Annual Scientific Meeting of the Malaysian Society of Pathologists was held in Kuala Lumpur from 30th August to 1st September 1996. Abstract of the free paper communications follow: .

#### Oral presentations:

##### 1. C-reactive protein in neonatal sepsis

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C-reactive protein (CRP) is one of the most important acute phase proteins and the serum content in healthy people including children is usually in a small amount of less than 10 mg/l. Serum CRP level is significantly elevated in various diseases and infections especially of bacterial origin and serial measurements can be useful for monitoring disease activity towards antibiotic therapy. In our study involving 75 samples from 39 neonates admitted to the Special Care Nursery for various reasons including suspected sepsis, we are able to demonstrate the usefulness of serial measurement of this acute phase protein. Twenty five of these neonates have 2 specimens, 2 had 3 specimens and 1 each with 4 and 5 specimens examined. The other 10 have unfortunately only have one specimen sent. The CRP level and its trend were compared with their clinical progress and other laboratory investigations as well as final patient outcome. The level returned to normal in patients with good recovery and those discharge well and in few others, the level remained high or even higher in patients who had poor outcome.

##### 2. Radioimmunoassay of acetylcholine receptor antibodies in healthy blood donors

*Fariza MY, Azizah MR, Zaiton Y, Shahnaz M, Abdul Karim T, and Nasuruddin BA*

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Myasthenia gravis (MG) is a disorder of neuromuscular function caused by an autoimmune disorder against the acetylcholine receptor (AChR) at the postsynaptic region of the neuromuscular junction. The assay to detect antibodies against AChR is an important tool in the diagnosis of the disease. Using a commercial radioimmunoassay (RIA) kit we measured antibodies to AChRs in a group of 91 healthy blood donors in our population to establish its normal reference interval. They included 54 (59.3%) Malays and 37 (40.7%) Chinese with a mean age of 33.2 years (+/-SD 8.9). Seventy-five (82.4%) were males and 16 (17.6%) were females. The result of antibody titres showed a non-Gaussian distribution. Thus, a non-parametric method of analysis was used. Taking the 95th percentile as the cut-off point, antibody titres of above 0.38 nmol/l are considered to be abnormally raised. Four individuals (4.4%) had levels ranging from 0.4 - 0.96 nmol/l. We did not find any association between anti-AChR antibody titre and female or male gender and race. We are now providing this assay routinely for serological diagnosis of this malady.

##### 3. A hypothesis: Low antischizont IgG in HLA-B\*1513 positive Malaysian Aborigines [Orang Asli (OAs)] – The result of efficient elimination of the antigen by T lymphocytes?

*Nasuruddin BA, Lokman Hakim S, \*Hirayama K, Mak JW*

*Institute for Medical Research, Kuala Lumpur, Malaysia and \*Medical Faculty, Saitama University, Saitama, Japan*

For 30 years, HLA associations with certain diseases have provided some important clues to their pathogenesis. In 1991, Hill *et. al.* described in Nature the strong, protective association between HLA-Bw53 and resistance to severe malaria among the Gambians in West Africa.

In 1994, we observed a high prevalence of 33% of HLA-B\*1513 (HLA-B\*1513) among the Orang

Aslis (OAs) in Kampong Gapoi in Pahang (compared with prevalences of 2.5%, 0.7% and 0.4% among the Zairen, Javanese and Thais, respectively). Only one of the OAs tested had HLA-Bw53. **Very interestingly**, on subsequent sequencing of the amino acids, we found that the sequence from the 62nd to the 88th amino acids of HLA-B\*1513 is identical to the corresponding segment of HLA-Bw53.

Of interest, too, is the fact that the antischizont IgG levels of those OAs with HLA-B\*1513 when compared with that of those without HLA-B\*1513, are significantly lower; be they in adults or among children.

This could therefore be explained thus: the T lymphocytes when presented with the malarial antigens in the groove of the beta-pleated sheet of the HLA-B\*1513, become so activated that they subsequently are able to eliminate the malaria antigens very efficiently, even before B lymphocytes are able to produce the specific immunoglobulins in significant amounts.

#### 4. Blood group serology and bone marrow transplantation

*Menaka N and Kua GE*

*University Hospital Kuala Lumpur*

Blood group antigens and bone marrow transplantation are intimately linked in more ways than one. The most important role is to see whether a major ABO incompatibility exists between the recipient and the donor since this may lead to a delay in marrow engraftment. Serial erythrocyte genotyping and monitoring isoagglutinin titre levels may provide valuable information with regards to time of engraftment and impending graft failure or rejection. However pitfalls do exist. These will be discussed by ways of case studies.

#### 5. Mucinous carcinoma of breast - profile of 8 cases

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Mucinous carcinoma of the breast is a distinctive well differentiated type of invasive adenocarcinoma, constituting 2-3% of breast cancers. It has been reported to have a more favourable prognosis than well differentiated adenocarcinoma of breast, a lower frequency of axillary lymph node metastases and a better survival. Over a 2½ year period from January 1994 to June 1996 we encountered 8 cases of mucinous carcinoma of breast and we had the opportunity to do FNA cytologic study in 7 of these cases. Cytologic appearance in all cases was similar consisting of a monotonous population of tumor cells floating in variable amounts of mucin. Cells occurred singly and in clusters and were small and uniform looking. A positive cytologic identification of mucinous carcinoma could be made prospectively in 7/8 cases and retrospectively in all 8 cases. Histopathologic correlation confirmed all 8 to be pure mucinous carcinomas. Knowledge of the distinctive cytologic appearance of mucinous carcinoma would enable correct identification of these innocuous looking cells as malignant and prompt treatment which could further enhance the survival of these prognostically favourable breast cancers.

#### 6. Limited placental examinations

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*Department of Pathology, Universiti Kebangsaan Malaysia*

144 placentas from all cases of stillbirth weighing 500 g and above were sampled over a period of thirteen months between 1994 and 1995 in the Maternity Hospital, UKM unit, Kuala Lumpur. Sampling was limited to 1 - 3 blocks per placenta for morphological study. Placental abnormalities were found in 121 placentas (85%), out of which 78 placentas had definite lesions while the remainder showed lesions suggestive of an underlying disease. 15% of the 144 placentas were normal. This is a review of the usefulness of placental examination given that there are limited samples only.

## 7. Pap smear study: The sequalae. Is there a need to change the sampling tool?

**Nor Hayati Othman, and \*Mukarramah Che Ayob**

*Hospital Universiti Sains Malaysia and \*Hospital Kota Bharu, Kelantan*

This is a follow-up study on pap smears in which a total of 2289 consecutive samples were analyzed in 2 institutions, Hospital Universiti Sains Malaysia (HUSM) 596 cases and Hospital Kota Bharu (HKB) 1693 cases. Transformation zone cells were included in 832 smears (36.7%) while 1356 smears (59.2%) did not contain these cells. 4.1% of these samples were technically non interpretable.

Of these satisfactory smears, **68/832** (8.2%) had **CIN** changes and 1.1% showed frank cancer. The rest of the satisfactory samples showed inflammatory features. The causes of the inflammation were noted to be Human Papilloma virus in 21.1%, **candida albicans** 7.8%, trichomonas 3.2% while in the majority the organisms were not identified.

The samples which did not contain transformation zone cells were seen in all age groups; in descending order, 31 to 40 years **40.8%**, 21 to 30 years **37.6%**, 41 to 50 years 13.8%. 17 to 20 years 4.1%. 61 to 70 years 0.6% and above 71 years 0.5%.

The conventional method of taking pap smears in Malaysia is by using the wooden spatula. Several studies have indicated that this mode of sampling does not sample the transformation zone area satisfactorily. Endocervical brush have been used on selected cases in university or private institutions. We propose its use for all cases.

## 8. Proliferating cell nuclear antigen (PCNA) index in thyroid follicular neoplasms.

**Thuaibah H., and Sharifah N.A.**

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The purpose of this study is to determine the proliferating cell nuclear antigen (PCNA) index in normal tissue, follicular adenoma and follicular carcinoma of the thyroid and to correlate PCNA value with histological diagnosis of malignancy. A total of 87 cases comprising 21 follicular carcinomas, 58 follicular adenomas and 8 normal thyroid tissues were investigated by immunohistochemistry using monoclonal antibody against PCNA. A minimum number of five hundred cells were manually counted for nuclear positivity for PCNA. It was found that PCNA index ranges from 0.2-1% in normal thyroid, 0.4-23% in follicular adenomas and 1-30% in follicular carcinomas. PCNA index tend to be higher in follicular carcinomas (mean = 12.87%) compared to follicular adenomas (mean = 6.69%) however there is no statistically **significant** difference between the two groups (**p=0.21**). Histology still remains the most reliable method of differentiating follicular carcinoma from follicular adenoma.

## 9. Lamina propria lymphocytes in ulcerative colitis: Immunohistochemical study

**NS Nik Malihan and P. Jayalakshmi**

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Ulcerative **colitis** is an uncommon disease in Malaysia. In the last **15** years, there have been an abundance of research into the nature of lymphoid cells that characteristically infiltrate the colonic **mucosa** in inflammatory bowel disease. The purpose of the present study is to assess the distribution of T & B **lymphocytes in ulcerative colitis** (UC). Rectal biopsies from 20 patients with UC were selected. Another 20 rectal biopsies with normal histology were used as controls. Formalin-fixed, paraffin embedded sections were stained for T and B cells using the indirect **immunoperoxidase** method. T and B lymphocytes in the lamina propria were counted using a light microscope (Olympus) equipped with a grid at X400 magnification. Statistical analysis was done using a non-parametric method. In this study we noted, that there was a significant increase in both T and B lymphocytes in the lamina propria in cases of UC compared to controls ( $p < 0.001$ ). The ratio of T and B lymphocytes in UC is **2:1** compared to **10:1** in controls ( $p < 0.001$ ).

## Poster presentations:

**P1. Cag A gene of *Helicobacter Pylori* - Is it a marker of ulcerogenic strains?**

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The **cag A** gene of *Helicobacter pylori* (**HP**) has been described to be present more frequently in strains associated with peptic ulcers than those associated with gastritis. This study was carried out to determine if such a relationship existed in HP strains isolated from a local population.

Thirty-eight HP strains isolated from gastric biopsy samples taken from the antrum and body of 30 patients with either duodenal ulcer, non-ulcer dyspepsia or who were asymptomatic, were analysed for the presence of **cag A** gene.

Amplification of **cag A** gene fragments of HP were carried out using the polymerase chain reaction (PCR). Template DNA from the clinical isolates were prepared by vortex mixing 10  $\mu$ l loopful of colonies in 200  $\mu$ l of sterile distilled water and boiling the solution for 15 minutes. The samples were then centrifuged at 12,000 g for 4 min, and the supernatants stored in sterile vials at **-70°C** until used. PCR primers based on the published sequences of HP **cag A** genes were used. Amplification of HP template DNA was carried out in a total volume of 50  $\mu$ l containing PCR buffer, 1.5 mM **MgCl<sub>2</sub>**, 200  $\mu$ l of each deoxynucleotides, 0.4  $\mu$ M of each primer, 5 U of **Taq** polymerase (Betasda Research Laboratories) and 2  $\mu$ l of boiled HP supernatant. The reaction included an initial denaturation of target DNA at **94°C** for 5 min, and then 38 cycles each at **94°C** for 1 min, **60°C** for 1 min and **72°C** for 1 min, followed by 1 cycle at **94°C** for 1 min, **60°C** for 1 min and **72°C** for 5 min. The amplified products obtained by PCR were observed on agarose gel (1.2%, wt./vol.) electrophoresis and ethidium bromide staining.

24 strains from 18 patients with duodenal ulcers, 10 strains from 8 patients with non-ulcer dyspepsia and 4 strains from 4 asymptomatic patients were analysed for the presence of **cag A** gene. Cag A gene was detected in all the strains studied.

This study suggests that in the local population, the presence of **cag A** gene in HP strains is not a discriminatory marker for ulcerogenic strains.

**P2. Addisonian crisis with hypercalcemia in tuberculous adrenalitis: A case report**

*M. Swaminathan and Rozanah Mohd Yusof*

*Department of Pathology, Universiti Kebangsaan Malaysia.*

Although tuberculosis is common in this country, tuberculous adrenalitis is infrequently encountered. In this report, we discuss the case of a 62-year-old Chinese man with pulmonary tuberculosis, bilateral massive destruction of adrenals and hypercalcemia.

On admission he was found to be in a state of shock (B.P. 70/40 mmHg), dehydration and cachexia. Chest X-Ray showed a fairly homogenous opacification of right upper lobe and patchy opacities with cavitation at left mid-zone and lower zone. A liver profile showed elevated serum calcium, inorganic phosphate, alkaline phosphatase and SGOT. Renal profile indicated renal dysfunction. No acid fast bacilli were seen in gastric washings. In view of hypercalcemia, renal dysfunction and X-ray changes, a clinical diagnosis of carcinoma of lung with secondaries to bone was made. The patient **died** on the sixth day. Post-mortem examination was done. Both lungs showed extensive focal caseous necrosis, bronchopneumonic consolidation and early cavitation. Both adrenals were grossly enlarged and had multiple coalescent caseous foci. The right adrenal weighed 64 gm and the left adrenal weighed 50 gm. No gross abnormalities were seen in the liver, spleen, both kidneys, prostate and testis.

This case illustrates an unusual acute presentation of tuberculosis.

**P3. Ovarian mucinous cystadenoma with osteoid and chondroid component in giant cell stromal nodule: a case report**

**M. Swaminathan, S. Subathra, \*Harold Fox and \*\*Ashar Abdullah**

*Departments of Pathology and \*\*Obstetrics and Gynaecology, Universiti Kebangsaan Malaysia and \*Department of Pathological Services, University of Manchester, United Kingdom.*

Ovarian mucinous tumours are common. Exceptionally, ovarian mucinous tumours have been found to contain foci of sarcoma-like nodules, with a configuration and cytologic composition similar to those of giant cell tumour of soft parts. This report is on a case of borderline papillary mucinous cystadenocarcinoma with the presence of benign osteoid and chondroid components in addition to a giant cell tumour in the stromal nodule. The patient was a 33-year-old Malay housewife who presented with abdominal distension. She had a left ovarian tumour measuring 22 x 20 x 13 cm. Cut surface showed multiloculated cystic spaces and a solid stromal nodule measuring 10 cm diameter. The patient had an uneventful post-operative recovery.

To our knowledge, a stromal nodule containing an osteoid and chondroid component has not been reported before.

**P4. Retrospective study of smooth muscle tumours of the gastrointestinal tract**

**M. Swaminathan**

*Department of Pathology, Universiti Kebangsaan Malaysia.*

Gastrointestinal tract smooth muscle tumours are not seen as frequently as epithelial tumours. This report describes thirty-one cases of smooth muscle tumours of the gastrointestinal tract and retroperitoneum seen at the Medical Faculty, UKM, from 1978 to 1995. The incidence of both leiomyomas and leiomyosarcomas were highest in the 50-59 years age group. The stomach was the most common site of occurrence (30% of all cases) with 45% of these being **benign**. There was a female preponderance; 1:2.5 for leiomyomas and 1:2.7 for leiomyosarcomas. **Leiomyosarcomas** were commonest among the Malays; leiomyomas were commonest among the Chinese. Two cases of leiomyoma and a single case of leiomyosarcoma showed **hyalinization**. Calcification was seen in one case of leiomyosarcoma. Ulceration or **umbilication** was seen in three out of thirteen cases of leiomyoma and four out of eighteen cases of leiomyosarcoma. Cystic degeneration or cavitation was seen in three out of thirteen cases of leiomyomas and six out of eighteen cases of leiomyosarcomas.

These tumours presented in a wide variety of ways from an acute abdomen to long standing abdominal distension. The correct **preoperative** diagnosis was made in 13 out of 31 cases.

**PS. Adenocarcinoma of cervix - a report of 16 cases**

**S I Abdul Wahab, Siti Aishah Md. Ali and \*Nik Mohd Nasri Ismail**

*Department of Pathology and \*Obstetrics and Gynaecology, Universiti Kebangsaan Malaysia.*

From 1983-1987, 16 cases of adenocarcinoma of the cervix were reported in the Department of Pathology, UKM. The age of presentation ranges from 28 to 75 year old, with the mean age of presentation of 42.4 years. 37.5% are under the age of 40 years, and 62.5% above 40. 68.8% (11/16) are Malay, and the rest (5/16) of Chinese ethnic group. At presentation, 6/16 were stage I, 4/16 were stage II, and 4/16 were stage III-IV. Histologically, 50% were graded as well differentiated, 12.5% moderately differentiated, and 12.5% poorly differentiated, and no grading given for 4 cases. No adenocarcinoma *in situ* cases reported. 8 cases were reviewed **histologically** (due to availability of material.) 6 cases are mucinous adenocarcinoma, 2 cases are endometrioid adenocarcinoma. The follow-up in 3 cases show 2 cases (both stage I b) surviving well 5 years after **Wertheim** hysterectomy and radiation therapy, and another surviving well 2 years (stage II b) after radiation therapy. The higher occurrence of mucinous adenocarcinoma is compatible with other studies.

**P6. DNA ploidy analysis in colorectal carcinomas****Zubaidah Z, \*Tong S, Gregory ARA and Boon CS***Institute for Medical Research and \*Department of Surgery, UKM*

Colorectal cancer is one of the commonest cancers worldwide and is the second most prevalent cancer in the western world. In Malaysia colorectal cancer ranks one of the ten most important cancers with an increasing trend in the number cases being diagnosed each year. During the last decade DNA ploidy analysis of human solid tumours using flowcytometer has been investigated extensively and it appears to be a promising new tool in the prognostic evaluation of several common tumours. Several published reports suggest that tumour DNA content is an important prognostic indicator and the presence of DNA aneuploidy has been used as an adverse prognostic indicator in relation to survival. A similar study was conducted to **determine** the ploidy status in colorectal cancers among Malaysian patients and then correlated with the clinical progression of the disease. Tissue samples sent were **first** confirmed cytologically by touch imprint and then processed for DNA ploidy studies using flowcytometry. The majority of cases studied showed hyperdiploidy correlating with the clinical staging and progression of the disease. This was a **significant** finding which corresponded to other studies worldwide.

**W. Hemangiopericytoma of the middle ear presenting as a "Glomus tumour": a case report****M. Swaminathan, Wan Anna Mohd Amin, \*Balwant Singh Gendeh and \*\*B.W. Scheithauer***Department of Pathology and \*Otolaryngology, Universiti Kebangsaan Malaysia, and \*\*Division of Anatomic Pathology, Mayo Clinic, Rochester, United States of America.*

Hemangiopericytomas are usually seen in somatic soft tissues and bone. This report describes an unusual presentation and **immunostaining** reaction of a hemangiopericytoma. The patient was a 51-year-old Chinese man who was referred as a case of glomus jugulare with the complaint of progressive left ear obstruction and deafness of four months duration. Examination of the left ear showed a 2x2 cm swelling in the auditory canal. **Angiogram** showed the lesion to be vascular. MRI showed an enhancing tumour of the left temporal bone extending **intracranially**. The intraoperative findings were an expansive **filling** of the mastoid and middle-ear cleft with erosion of part of the temporal bone by the tumour. Extracranial part of the tumour was removed. Microscopic examination showed a cellular spindle cell lesion with prominent vascular spaces. Immunocytochemistry was positive for S-100 and negative for vimentin. Despite immunoreactivities being somewhat strange (S-100 positive) a diagnosis of hemangiopericytoma was favoured. The pattern of reticulin staining was strongly intercellular.

The patient is currently being followed up at the ENT clinic.

**PS. A case of unsuspected endometrial stromal sarcoma - a case report.****Noor Kaslina Mohd Kornain, Siti Aishah Md Ali and \*Nik Mohd Nasri Ismail***Departments of Pathology and \*Obstetric and Gynaecology, Faculty of Medicine, Universiti Kebangsaan Malaysia, 50300 Kuala Lumpur.*

A **56-year-old** female **underwent** TAHBSO for uterine fibroid, responsible for post-menopausal bleeding. The operative finding was an enlarged uterus, about 24 weeks sized with a large right posterior uterine mass. Both ovaries were atrophic. Fallopian tubes and pouch of Douglas were unremarkable. Pathological examination of the resected specimen showed a huge mass situated at the right posterior uterine wall, measuring 15 x 15 x 10 cm. At the **center**, foci of necrotic tissue were noted. Histopathological sections from the lesion showed sheets of the neoplastic cells with round to oval nuclei, dispersed chromatin and small nucleoli. There was marked nuclear pleomorphism with ill defined cytoplasmic outline. Mitotic figures were frequent and infiltration of tumour into the smooth muscle and vascular spaces were noted. Therefore a diagnosis of endometrial stromal sarcoma, high grade was made. This is a rare tumour and furthermore it is difficult to diagnose **pre-operatively**.

### **P9. P53 overexpression in breast disease (preliminary results)**

**Wan Muhaizan WM and Sharifah NA**

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The expression of p53 protein in benign breast disease and malignant breast lesions were studied and its expression in malignant disease were also correlated to lymph node metastasis, age, menopausal status, tumour grade and size by an immunohistochemical technique using monoclonal anti-human p53 (**Dako-Do7**) on paraffin-embedded, formalin-fixed tissue following antigen retrieval by microwave exposure. Positive cases were identified by positive nuclear staining. 54 cases of benign breast disease of different types and 58 cases of malignant breast lesions which are mainly **infiltrating** ductal carcinoma were selected. 1/54 benign lesion (1.8%) and 13/58 malignant lesion (22.4%) were positive. There is significant difference in the expression of p53 between benign and malignant breast disease ( $p < 0.01$ ). However there is no correlation between p53 expression with age, menopausal status, lymph node metastasis, size and grade. The result suggest that p53 status can be use as an indicator of malignant potential but not as a prognostic marker.

### **P10. Postpartum associated hemolytic uremic syndrome: a case report**

**M Swaminathan, Soo Peng Yen and \*Lau Siew Gek**

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Hemolytic uremic syndrome (HUS) is commonly seen in children. It can occur in adults. This report illustrates an uncommon but well documented association of HUS with a **postpartum** state. A 32-year-old Chinese lady, para 3, abortion 1, had a preterm delivery at 28 weeks gestation at a private hospital. On the 18th post-partum day she was referred by a GP for being 'unwell' with hypertension (BP 140/100 mmHg), severe anemia (Hb 6.3%) and proteinuria (urine albumin 2+). Urine output was low (500 ml/day) and blood investigation indicated acute renal failure (Blood urea = 56.6; Na = 128; K=5). Urine analysis revealed proteinuria 3+ and **hematuria**. The peripheral blood film showed anisopoikilocytosis, schistocytes and acanthocytes. The reticulocyte count was 2.6%. She was given **antihypertensives** and was on **hemodialysis** on alternate days. A renal biopsy showed **microangiopathic** changes with marked **intimal** thickening and occlusion of blood vessels.

HUS in a post-partum patient must be suspected when hemolytic anemia and acute renal failure is seen.

### **P11. Testicular feminisation with seminoma in the left gonad: a case report**

**M. Swaminathan and Wan Muhaizan Wan Mustafa**

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Testicular feminisation is a type of male pseudohermaphroditism with presence of vagina, no uterus, and bilateral cryptorchid testes. The syndrome is of clinical importance because of the eventual occurrence of malignant tumours in the cryptorchid testes of about 9% of these patients. This report highlights the importance of early diagnosis and removal of the cryptorchid testis. A 34-year-old Malay lady, Para 0, married for 9 years, was referred from a district hospital with the complaint of left iliac fossa pain for 3 months. Physical examination revealed a suprapubic abdominal mass of about 14-16 weeks gestation size uterus. Pelvic examination: vulva, vagina normal except for blind vault, absence of cervix and a mass felt in the pelvic cavity. Investigations: FBP, urinalysis, IVP and renal profile were normal. Buccal smear: sex chromatin less than 1%. Plasma estradiol (E2) = 51 pg/ml; FSH: >40.0 IU/L; LH: >50.0 IU/L;  $\beta$ HCG = 2.7 IU/L; S. testosterone = 0.8 pgm/ml.

She underwent a laparotomy. There was absence of uterus and tubes. The left gonadal tumour was removed. Histologic sections of this showed seminoma. Histologic sections of the right gonad showed testicular tissue. Recovery was uneventful. The patient was given radiotherapy.

## **P12. Histomorphological parameters in glomerulonephritis assessed by computerised image analysis**

**Looi LM, Cheah PL and Wong TK**

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In the histopathological interpretation of the renal biopsy, a subjective assessment of the histomorphometric parameters of glomeruli, such as size and shape, is often made and incorporated into the diagnostic interpretation. We have attempted to make this assessment more objective by undertaking a computerised morphometric analysis of glomerular parameters. PAS-methanamine-silver stained histological sections of renal biopsies from 10 cases of minimal change nephropathy (MCGN), 12 idiopathic membranous GN (MGN), 12 diffuse mesangioproliferative GN (MPGN), 11 diffuse proliferative lupus nephritis WHO Class IV (DPLN), 9 diffuse membranous lupus nephritis WHO Class V (MLN) and 3 acute diffuse proliferative GN (ADPGN) were subjected to morphometric analysis using a Leica DMRB light microscope (set at 10X eye-piece and 20X objective) linked to a Leica Quantimet 500+ CPU. All non-sclerotic and complete glomeruli in the biopsies were analysed. The parameters measured were glomerular area, maximum glomerular diameter (length), minimum glomerular diameter (breadth), perimeter and roundness. The number of glomeruli studied were 78 MCGN, 87 MGN, 87 MPGN, 88 DPLN, 68 MLN and 31 ADPGN. Length, breadth and roundness measurements indicated that there was no significant difference in distortion of glomerular anatomy in the various glomerulopathies due to processing of the biopsies. Area and perimeter measurements indicated that the glomerulopathies could be ranked by glomerular size as: membranous GNs (largest), proliferative GNs and minimal change nephritis (smallest). These findings may provide insight into the pathophysiology of these disorders.

## **P13. Eosinophilic abscess of the spleen in Schistosomiasis: a case report**

**M. Swaminathan, Suryati MD, Jusoh, \*Ishak MD, Sood and \*\*Pakeer Oothman**

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Schistosomiasis is an infection caused by flukes (trematodes) of any species of the genus *Schistosoma*. Schistosomiasis in humans was first reported in Malaysia in 1973 from an autopsy (Murugasu & Dissanaik, 1973; Murugasu & Por, 1973). A number of cases of schistosomiasis have now been reported in Malaysia. This report illustrates a case of this uncommon infestation with abscess formation in the spleen. The patient was a 37-year-old Malay lady who presented with high fever and left shoulder tip pain of two months duration. On examination she had tenderness on deep palpation over the left hypochondrium. Blood examination revealed eosinophilia. Splenectomy was performed.

The spleen measured 9.5 x 6 x 3 cm. Multiple areas of haemorrhagic necrosis were seen. Microscopic examination showed collections of eosinophils and intense eosinophil infiltrate with reactive hyperplasia of the white pulp. Non-caseating granulomatous reaction, multinucleate giant cells and *Schistosoma* ova of the japonicum type were seen. No adult worm was seen.

With chemotherapy, the fever and blood eosinophilia subsided.

## **P14. Ovarian granulocytic sarcoma: a case report**

**O Hanita and M Lily**

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SR, a 32-year-old Malay housewife, a case of acute myeloid leukaemia FAB M4 presented with progressive abdominal swelling. Total abdominal hysterectomy with bilateral salphingo-oophorectomy was performed. Bilateral ovarian tumours which were well-encapsulated and composed of friable yellowish tissue were received. Histologically the tumours were composed of neoplastic cells exhibiting pleomorphic vesicular nuclei with coarse chromatin. Some of these cells have plasmacytoid appearance. Immunohistochemical stain showed them to be positive for Naphtol-AS-D-chloracetate

esterase (**Leder's** stain). The diagnosis of ovarian granulocytic sarcoma was made.

Ovarian granulocytic sarcoma is a rare form of extramedullary leukaemia. It occurs in 3% of adult **AML** and 4.7% of childhood **AML**. This is the first case reported in this center. The prognosis of this form of leukaemia is poor.

#### **P15. Malignant rhabdoid tumour of the central nervous system: a case report**

**M Swaminathan, Julia Munchar, \*Thomas Krausz and \*\*Benedict Marius Selladurai**

*Departments of Pathology and \*\*Surgery, Universiti Kebangsaan Malaysia, and \*Department of Pathology, Hammersmith Hospital, London.*

The term malignant rhabdoid tumour (MR) was introduced in 1981 to specify a highly aggressive neoplasm having distinctive morphologic features by light microscopy. Although MRTs were originally and most frequently reported as arising in the **kidney**, similar neoplasms have been seen in the thymus, liver, oral cavity and various soft tissue sites. The patient was a three-year-old Chinese boy who presented with a three week history of features of raised **intracranial** pressure and left sided weakness. CT scan showed a large enhancing lesion deep in the right hemisphere. Microscopically, the neoplasm was composed of a trabecular arrangement of polygonal cells in a myxoid background. The cells had a eosinophilic cytoplasm and a vesicular nucleus with single nucleolus. Many cells contained a single well-demarcated hyaline globular inclusion adjacent to the nucleus. **Immunocytochemical** staining was negative for myoglobin, **desmin**, cytokeratin and glial **fibrillary** acidic protein. The globular inclusions were positive for vimentin.

Malignant rhabdoid tumour of the central nervous system is a rare entity (5 cases in the world literature). **MRTs** should be distinguished from other childhood brain tumours because of its aggressive biologic potential.

#### **P16. Vascular cell adhesion molecule-1 (VCAM-1) expression in lupus nephritis**

**PL Cheah, LM Looi, SF Yap, CS Tan\*, T Lau\* and CT Chua\***

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Vascular cell adhesion molecule-1 (VCAM-1) is a 90 **kDa** cell surface glycoprotein transiently expressed on vascular endothelial cells in response to cytokine activation and subsequently released into the circulation during inflammation. A study was initiated at the University of Malaya Medical Centre to look into the possibility of using serological assessment of VCAM-1 as an index of "activity" in lupus nephritis. 20 cases of known lupus nephritis clinically suspected to have active disease were subjected to renal biopsy. At the same time, a sample of blood was taken for serological assay of VCAM-1 using a commercial (R+D Systems) **ELISA kit**. Histologically, all cases exhibited "activity" with scores ranging from 5 to 15, using a standard scoring system (Austin *et al*, 1983). 17 cases were categorised as diffuse proliferative (WHO IV) and 3 as diffuse membranous (WHO V) lupus nephritis. Serum VCAM-1 was raised beyond the upper limit of the expected normal range (395-714 **ng/mL**, mean 553 **ng/mL**) in 19 (95%) cases and measured 679 **ng/ml** in one. It appears that serum VCAM-1 estimation correlates well with the presence of histological "activity" in lupus nephritis and may be a feasible and less traumatic substitute for renal biopsy in the follow-up assessment of lupus "activity".

#### **P17. The pattern of Ki-67 and bcl-2 expression in lymphoid malignancies**

**SC Peh and \*PL Wang**

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The International Working Formulation divides non-Hodgkin's lymphoma (**NHL**) into three grades: low, intermediate and high. This grading system implies rate of tumour growth and hence may be related to prognosis. Ki-67 antigen is a proliferation related nuclear antigen and bcl-2 oncogene

product is known to inhibit apoptosis. This study aimed to determine the pattern of expression of Ki-67 antigen and bcl-2 oncoprotein in various grades of NHL. Paraffin-embedded tissues of 42 cases of NHL (9 low, 14 intermediate, 19 high grade) were **retrieved** from The Department of Pathology, University of Malaya, Kuala Lumpur. Ki-67 antigen and bcl-2 oncoprotein were stained using immunohistochemical technique. The percentage of positively stained neoplastic cells was determined by semiquantitative estimation method and then given score points from 0 to 6. Partition chi squared test demonstrated the association of Ki-67 antigen expression and histological grade ( $p = 0.002$ ). Further analysis indicated no significant difference in Ki-67 antigen expression between intermediate and high grade ( $p = 0.002$ ), whereas significant between low and **intermediate/high** grade ( $p < 0.001$ ). Bcl-2 oncoprotein expression in the neoplastic cells varied widely within the three histological grades. Statistical analysis showed no association between the expression of bcl-2 oncoprotein and histology ( $p = 0.31$ ). Ki-67 **immunostaining** is therefore a useful adjunct to histological grading of NHL.

#### **P18. Acute lead poisoning in a young, male adult**

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Lead poisoning is uncommon in adults. It is acquired by **inhalation** or ingestion of lead. Absorption from the intestine is enhanced in iron deficiency state and decreases in the presence of food. We report a case of lead poisoning in a 24-year-old man from Bangladesh who presented with a chronic, colicky abdominal pain, of 1 month duration. The pain was associated with loss of appetite, nausea and vomiting. It was not related to meals and not associated with **hematuria** or **dysuria**. Four months prior to admission, he had been employed in a factory making batteries. On examination he was pale, lethargic and the **fingers** were clubbed. There were no other significant physical findings. Preliminary blood investigations showed a hypochromic, microcytic anaemia with basophilic stippling. Serum amylase and urine microscopy were normal. The free erythrocytes protoporphyrin was 310 **umol/mmol heme** zinc protoporphyrin (reference range 30 - 70 **umol/mmol heme ZPP**). The urine for **porphyrin** was strongly positive. The blood lead level was 3.38 **umol/l** (reference range  $< 1.93$  **umol/l**). The usefulness of various laboratory indices in the diagnosis of lead poisoning will be presented.

#### **P19. Serum calcium and magnesium in chronic renal failure patients**

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Abnormalities of calcium and magnesium metabolism are common in end-stage renal disease. A retrospective study of serum calcium (Ca) and magnesium (Mg) levels in chronic renal failure patients treated at Hospital Kuala Lumpur over a six-month period was carried out. All patients were undergoing either haemodialysis or continuous ambulatory peritoneal dialysis. A total of 291 chronic renal failure patients had both serum calcium and magnesium tested. Ninety-two percent (268) of the patients had **elevated** serum magnesium (greater than 1.10 **mmol/l**) of which 17.2, 62.2 and 20.6 per cent had accompanying, low ( $< 2.14$  **mmol/L**), normal and high ( $> 2.65$  **mmol/L**) serum calcium respectively. Hypomagnesemia (less than 0.7 **mmol/L**) was seen in 2 patients (0.7%). Hypermagnesemia was the commonest **abnormality followed** by hypercalcemia. Hypermagnesemia and hypercalcemia co-existed in 18.9% of the test population (55 patients), whereas 16.2% showed both **hypomagnesemia** and hypocalcemia. **Only 15/291** (5.1%) show normal serum calcium and magnesium. The high prevalence of calcium and magnesium abnormalities in chronic renal failure patients in the hospital and related issues are addressed.

**P20. The relationship between plasma potassium levels and induced haemolysis.**

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Haemolysis is an important consideration in rejecting samples for potassium measurement. It is a cause for artefactual rise in plasma potassium. However, the magnitude of increase in plasma potassium associated with varying degrees of haemolysis is unknown. A study was carried out to determine their relationship. Ten mls. of venous blood were collected in **heparinized** tubes from 14 patients attending the University Physicians' Clinic. Whole blood hemoglobin (Hb) and plasma potassium were determined by the Coulter Counter and atomic absorption spectrophotometry (AAS) respectively. Dilutions of whole blood were prepared to conform to a **10, 20, 30, 40** and 50 percent solutions. Plasma were separated and measured for potassium, Hb and percentage hemolysis were calculated. Plasma Hb were measured by a manual cyanide - femcyanide method using **Drabkin** solution. The results indicate that (1) non-visible hemolysis is associated with mean plasma Hb of 1.4 g/L (range **0.3g - 3.4g/L**), (2) visible severe hemolysis is associated with a mean plasma Hb level of 5.8 g/L (range 1.2 - **12.4g/L**) and (3) increased osmotic stress on erythrocytes induced a greater degree of haemolysis and higher potassium leakage with mean potassium levels of about 27.4 **mmol/l** at 80-90% hemolysis.

**P21. An evaluation of the serum lithium method on Kodak Ektachem analyser.**

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Lithium is a common drug used in the treatment of manic-depressive disorders. Due to its low therapeutic ratio, serum lithium concentration is monitored to ensure patient's compliance and to avoid toxic effects. The common methods of lithium measurement include flame photometry and atomic absorption spectrophotometry (AAS). A more recent method using dry chemistry is available on the Kodak Ektachem Analyser. A study was conducted to evaluate its performance. The technique was compared with that of the AAS. Sera with lithium levels ranging from 0.3 **mmol/L** to 3.91 **mmol/L**, prepared by spiking known quantities of lithium standard to reconstituted sera were used. The lowest detection limit is 0.3 **mmol/L**. The method is shown to be linear in the range of 0.3-3.9 **mmol/l**. **Intrassay** imprecision varies from 0.6% (3.3 **mmol/L**) to 3.6% (**1.2mmol/L**). The interassay imprecision varies from 0.8% (2.40 **mmol/L**) to 3.9% (0.6 **mmol/L**). The recovery studies indicate an overestimation ranging from 108% (2.54 **mmol/L**) to 167% (0.3 **mmol/L**). The method showed a positive bias to that of the AAS with a good correlation,  $r = 0.95$ ,  $p < 0.001$ .

**P22. Migraine and tension-type headache amongst medical students**

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Headache is a common complaint among females and males of all ages. Using the International Headache Society (**IHS**) criteria, we conducted a study to assess the prevalence of migraine and tension-type headache amongst local university students. This study also looked at the trigger factors, disability and other factors associated with headache. A total of 533 students participated in the study. The prevalence of migraine was 13.7%. Male to female ratio was **1:3**. The prevalence of tension-type headache was 79% and the male to female ratio was **1:1**. Common trigger factors for migraine were stress (**86.3%**), **heat/sunlight (82.2%)**, physical activity (42.5%), hunger (26.0%) and certain types of food (24.7%). 19% had severe pain and daily activities were suspended. 78% described moderate pain which affected but not prevented daily activities. 20.5% reported absenteeism in the past year due to migraine. Students with migraine were more likely to have a history of allergy when compared with students who do not suffer from migraine ( $p=0.018$ ). History of food allergy was also more common in students with migraine ( $p = 0.005$ ). It appears that there could possibly be a link between migraine and allergy, in particular, food allergy.

## P23. Anticardiolipin antibody isotype profile and its clinical significance in lupus nephritis

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Thirty-six patients with lupus nephritis (LN) attending the Nephrology Clinic, Hospital Kuala Lumpur were studied for the prevalence of **anticardiolipin** antibody (ACA) **isotypes (IgG and IgM)** and other associated antibodies (ANA, anti-ds DNA) and to determine the possible association between serological and clinical parameters. The study population consisted of 20 (55.6%) Malays, 15 (41.7%) Chinese and 3 (2.8%) Indians with a mean age of 31.4 years (SD 11.3, age range 14-60 years). The female to male ratio was 11:1. The average time between diagnosis and blood sampling was 4.4 years (range: 0.25-15 years). The ACA was measured using the ELISA while the other autoantibodies were detected by **immunofluorescence**. Overall, increased ACA levels were found in 20 (55.6%) patients where raised **IgG** ACA and **IgM** ACA were observed in 20 (55.6%) and 2 (5.6%) cases respectively. **ANA** and anti-ds DNA antibodies were detected in 22 (61.1%) and 4 (11.1%) individuals respectively, with the majority (82%) showing a speckled pattern of nuclear staining. We observed that an increased level of **IgG** ACA correlated significantly with an increased **IgM** ACA. However, neither the **IgM** ACA nor **IgG** ACA showed any significant association with thrombosis or any other clinical parameters. Our preliminary study indicates that ACA is a frequent finding in LN and that the **IgG** isotype is more predominant. The **finding** here that ACA is not significantly associated with thrombotic events points to the fact that it may not be a serological marker but only a part of other immune reaction process.

## P24. Surfactant protein A (SP-A) in neonatal respiratory distress syndrome

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Respiratory distress syndrome (RDS) remains as one of the major causes of respiratory distress and neonatal mortality. SP-A is one of the proteins required to maintain surface tension in the alveolus at the air-liquid interface. This study was carried out in order to evaluate the level of SP-A in 104 tracheal aspirate specimens collected from neonates with and without respiratory distress syndrome. They were obtained from Special Care Nursery, Institute of Pediatrics, Hospital Kuala Lumpur. Measurement was done using an ELISA method developed and optimised in-house. Comparison as done between the mean levels of SP-A in tracheal aspirates in RDS and non-RDS neonates and it was found that the mean concentration of SP-A in RDS neonates was significantly lower ( $15.08 \pm 9.93 \mu\text{g/ml}$ ) than non-RDS neonates ( $114.51 \pm 68.78 \mu\text{g/ml}$ ) at  $\alpha = 0.05$ . Further study was performed to evaluate the mean level of SP-A in the non-RDS neonates. It was found that the mean concentration of SP-A in RDS neonates was significantly lower ( $15.08 \pm 9.93 \mu\text{g/ml}$ ) than that of neonates with asphyxia ( $152.24 \pm 40.04 \mu\text{g/ml}$ ) and pneumothorax ( $93.71 \pm 29.88 \mu\text{g/ml}$ ), but it was not significantly lower than that of neonates with **meconium** aspiratory syndrome (**MAS**) ( $51.91 \pm 25.52 \mu\text{g/ml}$ ) at  $\alpha = 0.05$ . The latter could be due to secondary SP-A deficiency associated with MAS. Measurement of SP-A in tracheal aspirates has the potential as a parameter to guide **surfactant** treatment as surfactant deficiency occurs in RDS and MAS as shown in our study.

## **P25. DNA extraction from fine needle aspiration (FNA) samples**

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Techniques in molecular biology have become useful tools for the detection of inherited abnormalities and the diagnosis of neoplasms. The isolation of high molecular weight DNA is normally carried out from blood, bone marrow, tissue or cultured mononuclear cells. We have experimented with extracting DNA from fine needle aspiration (**FNA**) samples. This is less demanding technologically than extracting DNA from surgical biopsy and does not require complicated equipment or extensive training. DNA extraction by **urea/SDS** and phenolchloroform method were performed on 34 FNA samples from lymph node, breast and thyroid, obtained from our FNA clinic. DNA was successfully extracted from 19 (55.8%) of the 34 samples. **Hypercellular FNA** samples gave good DNA yield with DNA concentration varying from 5  $\mu\text{g/ml}$  - 560  $\mu\text{g/ml}$ . Poor DNA yield was obtained from **hypocellular** samples. DNA of superior purity defined by the value of optical density ratio at 260 nm and 280 nm between 1.73 - 1.84 were obtained from samples with good DNA yield. The quality of the DNA samples were checked by **performing** polymerase chain reaction for amplification of the **exon 2** of the human **G6PD** gene. **All** the 19 samples with good DNA yield produced satisfactory amplification, showing the 241 bp DNA fragment on **agarose** electrophoresis. No amplification was seen in any of the samples with poor DNA yield.

## **P26. Trends of haemoglobinopathies diagnosed in IMR (1986-1995)**

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This study was carried out to evaluate the status of haemoglobinopathies in Malaysia from the years 1986-1995. 30,000 blood samples were received and analysed for haemoglobinopathies. Haemoglobin analysis was done by using alkaline denaturation for Haemoglobin F, Haemoglobin A2 **quantitation** and electrophoresis of haemoglobin on starch gel. We then evaluated the results of 6592 patients with haemoglobinopathies in relation to types of haemoglobin, ethnic groups and geographic areas. From the results obtained,  **$\beta$ -thalassemia** trait was the commonest haemoglobinopathy constituting 41.9% of all haemoglobinopathies followed by Hb E trait (17.8%) and  **$\beta$ -thalassemias** (16.2%).  **$\beta$ -thalassemia** trait also found highest in Perak followed by **Sarawak** and Penang. **Our** study showed that 9.1% of the total blood samples received were diagnosed with  **$\beta$ -thalassemia** trait. This suggests there is a high chance of marriage between persons of  **$\beta$ -thalassemia** trait which increases the **risk** of having offsprings with  **$\beta$ -thalassemia** major. Overall, Perak has the highest number of haemoglobinopathies followed by Kedah and Penang. The Malays constitute the highest number of patients with haemoglobinopathies 72.396, followed by Chinese **21.7%**, Indians 1.6% and **Orang Asli** 1.53%.  **$\beta$ -thalassemia** trait was the commonest Hb type found in both Malays and Chinese. Among the  **$\alpha$ -thalassemia** group, **HbH** is the haemoglobinopathy most commonly seen. Sickle cell disease is found more commonly in Indians (**HbS** trait 57.3% and **HbS** homozygous 38.8%). The commonest haemoglobinopathy in Orang Asli is Hb E (56.4%) trait followed by Hb E homozygous and  **$\beta$ -thalassemia** trait. As haemoglobinopathies form a major health concern in this country, newer and improved diagnostic techniques are important for early management and for the formulation of more effective thalassemia control programmes. Future research in thalassemia particularly gene therapy should be undertaken.

**P27. Spectrum of beta-thalassaemia mutations in Kelantan**

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Beta-thalassaemia mutations in 83 alleles of 61 unrelated individuals from the Haematology Unit, Hospital Universiti Sains Malaysia (USM) and the Paediatric Department of Kota Bharu Hospital were studied. The characterisation was done by reverse dot blot hybridization of polymerase chain reaction-amplified DNA with allele-specific oligonucleotide probes. The aim of this study is characterise the thalassaemia mutations in this area of Malaysia. Seventy-six (91.6%) of the alleles were characterised. Nine different known mutations were detected, at different frequencies. The mutations were nonsense RNA translation at **codon 17** (AAG-TAG), coding region substitution at **codon 19 (AAC-AGC)** and **codon 26** (GAG-AAG), splice junction site at **IVS1** position 1 (G-T), consensus site at **IVS1** position 5 (G-C), **frameshift** mutations at **codon 41** (-C), codons 41/42 (-TTCT) and codons **71/72** (+A) and internal **IVS** changes at **IVS2** position 654 (C-T). The nonsense mutants at **codon 26** (GAG-TAG) and **codon 35** (TAC-TAA), the two original Thai mutations, previously described among the Thais were not detected in this study despite the close proximity of Kelantan and South Thailand. Kelantan has a population of about 1.5 millions of which 92.8% are Malays, 5.4% Chinese, 0.8% Indians and 1.0% others respectively. The commonest mutations among the Malays is **IVS1** position 5 (G-C), a common mutation seen among the Asian Indians, **Melanesians**, Chinese, and also among the Thais in South Thailand. Hemoglobin E, a beta-globin structural variant resulting from a mutation at **codon 26** (Glu-Lys), is also the most common beta-hemoglobinopathies among the Malays in Kelantan. The frequency is accounted for 47.0% of the alleles studied. Similar findings have been reported among the Malays in other parts of Malaysia. The frequencies of both Hemoglobin Malay, also a beta-globin structural variant resulting from a mutation at **codon 19** (Asn-Ser) and 4 bp deletion at codons 41/42, which is expected to be high in this population were found to be about 6.0%. It is interesting to note that 4bp deletion at codons **41/42**, the commonest mutations seen among the Malaysian Chinese is also common among the Kelantanese Malays and is reported to occur at high frequency among the Thais in Thailand.

**P28. A sickle cell trait presenting with a sigmoid megacolon - a case report**

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The  $\beta^s$  gene occurs widely throughout Africa (with the prevalence as high as 25 - 30% in Western Africa), parts of Asia, the Arabian Peninsula and parts of Southern Europe. However, the condition is not common in South East Asia (prevalence: 7.61%). Sickle cell disorder is caused by a single base mutation which results in a substitution of valine for glutamic acid at the sixth **codon** of the  **$\beta$  globin** chain. **HbS** will crystallise at low oxygen tension where the red cells assume a sickle-like appearance. Typically sickle cell disease manifests clinically with chronic haemolytic anemia **punctuated** by painful episodes caused by veno-occlusion, intercurrent infections and sickle cell crises. Sickle cell trait is **usually** asymptomatic. We present a rare case of a sickle cell trait, in a patient who presented with an acute abdomen. A laparotomy was done and showed that he had a sigmoid megacolon which was removed. Histopathological examination revealed multiple microscopic benign ulcers. He was also anaemic and the **full** blood picture showed severe **hypochromic** microcytic anaemia (**Hb - 6.0g/dl**, **MCH - 13.8 pg**, **MCV - 55.4 fl**, **MCHC - 25 g/dl**, **RDW - 21%**). Red cells also showed marked anisopoikilocytosis. There was also **thrombocytosis** (platelets - **700,000/mm<sup>3</sup>**). The serum **iron/TIBC** results confirmed iron deficiency. However, a coincidental finding of sickle cell trait was made on haemoglobin analysis with the finding of **HbS** - band on the electrophoresis. **Sickling** test was also positive. Although sickle cell was present on the peripheral smear, it was masked by the marked anisopoikilocytosis secondary to iron deficiency due to inadequate diet and gastrointestinal haemorrhage.

**P29. Expression of myeloid-associated antigen of childhood acute lymphoblastic leukaemia is not a predictor of relapse**

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Myeloid-associated antigen expression has been reported in up to 24% of childhood acute lymphoblastic leukaemia (ALL) cases. The prognostic significance of myeloid-associated antigen expression in childhood ALL remains uncertain. Some studies have shown that myeloid-associated antigen expression is a predictor of poor outcome while others failed to find any prognostic effect. In our Haematology Unit, we have studied the immunophenotypic features of 189 cases of childhood ALL and 20 cases of relapsed childhood ALL from 1993 to July 1996. The aim of the study was to ascertain whether myeloid-associated antigen expression is a predictor of relapse. 125 (66%) out of 189 cases of childhood ALL and 11 (55%) out of 20 cases that relapsed have myeloid associated antigen expression ( $X^2 = 1.24$ ,  $p = 0.27$ ). It appears that myeloid-associated antigen expression is not a predictor of relapse in childhood ALL.

**P30. Morphology and surface markers in acute myeloid leukaemia**

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Seventy-nine patients were included in this study. Fifty-four percent of the patients belonged to children aged less than 12 years old. Morphological classification was based on the FAB classification (French, American and British group). Surface marker expression was determined by flowcytometry using **FACScan** (Becton Dickinson). Surface marker studies included - CD3, **CD19**, **CD10**, **CD33**, **CD4**, CD8, CD15 + 56, CD7, **CD13**, **CD34**, HLA-DR, CD2, CD5, **CD11c**, **CD61**, **CD71**, CD3, CD22 surf/cyto K/L, surface **IgM** and surface/cyto **IgG**.

Seventy-eight cases (98%) were expressing myeloid marker of CD33 and or **CD13**, both are good marker for myeloid blast. One case which does not have myeloid marker was M2 case. Thirty seven cases (46%) were expressing CD34 and mainly from M0, M2 and M4 cases. HLA-DR were expressed in 61 cases (77%) and fourteen cases HLA-DR negative and 6% of these HLA-DR negative belonged to M3 blast. **CD11c** were expressed in 30 cases (26%) of AML. 70% of M4 and 80% of M5 cases were positive for this marker. Lymphoid marker either T or B, **CD19**, **CD10**, CD7, CD5, CD3, CD4 were detected in 42 cases (53%). CD **16+56** marker was detected in 13 cases (16%) of acute myeloid leukaemia.

**P31. Gene rearrangement in B-lymphoproliferative disease by polymerase chain reaction (PCR) – a preliminary report.**

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Study of gene rearrangement in lymphoproliferative diseases helps to distinguish neoplastic from reactive changes and assign lineage. It can be carried out by Southern Blotting which is a complex and time consuming procedure. Recently PCR-based assay for assessment of **immunoglobulin heavy (IgH)** gene has been reported. We studied the gene rearrangement of 2 cases of chronic lymphocytic leukaemia (CLL), using the PCR method. The primers used for amplification of the **IgH** gene were: **FR3A-5'**- ACACGGC(C/T)(G/C)TGTATTACTGT3' for the 3' end of the V region; and **LJH-5'**TGAGGAGACGGTGACC3' and **VLJH-5'**GTGACCAGGGT(A/G/C/T)CCTTGGCCCCAG3'- for the 3' end of the J region. For the amplification of the **IgH** genes, a semi-nested PCR was performed: a first round of 30 cycles with primers **LJH** and **FR3A**, followed by 1:2,500 dilution, and then a second round of 20 cycles with primers **VLJH** and **FR3A**. Rearranged bands were seen in both cases

**and** was negative for the normal control. Parallel Southern Blott study was also carried out using **IgH** probes and confirmed the findings. The procedure was less than 12 hours as compared to 7-8 days using the Southern Blott technique. **PCR** based method provides a rapid alternative approach in the study of gene rearrangement in B lymphoproliferative diseases.

**P32. Detection of molecular abnormality: BCR-ABL gene in chronic myeloid leukaemia, by RNA analysis using reverse-transcriptase polymerase chain reaction (RT-PCR).**

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The chromosomal translocation **t(9;22)**, is present in more than 95% cases of chronic myeloid leukaemia (CML). This translocation fuses the ABL protooncogene on chromosome 9 to the BCR gene on chromosome 22. The fusion results in the expression of the leukaemia specific chimeric BCR-ABL messenger RNA. Detection of these unique m-RNA transcripts is useful in the diagnosis of **CML** and also in following the course of the disease with bone marrow transplant or interferon therapy. The RT-PCR method for detection of the fusion gene is well established and involves the use of RNA as the starting template. The complementary DNA strand (**cdNA**) for the BCR-ABL is initially produced from the RNA, using oligo-primers in the presence of reverse transcriptase (RT). This is followed by the E R amplification of the chimeric **cdNA** sequences. We performed this **RT-PCR** technique on RNA extracted from the peripheral blood **and** the bone marrow specimens of 10 cases of CML. The oligo-nucleotides used for amplification and detection of BCR-ABL **m-RNAs** were 5'-GGAGCTGCAGATATGCTGACCAAC-3', 5'-TCAGACCCTGAGGCTCAAAGTC3', 5'-GCTGAAGGGATTGAACTCTGGCTT-A3' **and** 5'-GCTGAAGGGCTTCTTCTTATTGATG-3'. Analysis of the PCR amplified products was done using 3% agarose gel electrophoresis in **Tris-Borate-EDTA** buffer. Amplification was seen in all 10 cases of CML, with 5 cases showing **125bp** fragment and 5 cases showing **200bp** fragment. Analysis of the normal controls was negative in all cases. We have successfully demonstrated that the method RT-PCR can be used to detect BCR-ABL gene. Since the process takes less than 24 hours to perform, it may be the preferred method for confirmation of diagnosis and follow up in patients with CML.

**P33. Precore mutants in chronic hepatitis B carriers negative for Hepatitis B e-antigen**

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The presence of Hepatitis B virus (HBV) variants containing a defective precore region in chronic hepatitis B patients has been reported by several workers. The most commonly encountered defect in the region is a point mutation at nucleotide 1896 (**codon** 83) resulting in the introduction of an in-frame stop **codon**. This variant form of the HBV is characterised by the inability to express HBeAg **and** is associated with continued viral replication despite seroconversion from HBeAg to anti-HBe antibody. Clinically, patients carrying the mutant virus tend to experience rapid progression of the chronic disease. This observation prompted the development of rapid assays for the detection of the mutant HBV. We report the establishment of a ER-based hybridisation assay for the detection of the mutant virus and the results of analysis of 19 cases of chronic HBV carriers who are HBeAg negative. All 19 patients **were** HBV DNA positive on standard hybridisation analysis.

Overall, 9 cases were reactive for the wild **type** virus, 2 cases were reactive for the precore mutant, 3 cases were positive for both the wild **type** and the mutant virus. The remaining 5 cases (including 2 anti-HBe positive and 3 anti-HBe negative) were not reactive for either the wild **type** or the mutant **virus**. The latter observation could be due to the presence of mutations not detectable by the probe used in this assay. In summary, 26% of chronic hepatitis B patients negative for HBeAg carry mutant hepatitis B virus.