PATHOLOGY RESEARCH DIVISION

Deputy Director & Head	… Dr. Kyaw Soe MBBS (IM1), PhD (Medical Science) (Nagasaki)
Research Scientist	… Dr. Min Min Win MBBS (UM1), MMedSc (Pathology) (UM1)
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Research Assistant (4)	… Daw Khin Zar Chi Aung
Laboratory Attendant	… Daw Nilar Tun

The Pathology Research Division is actively engaged in research on chromosomal disorders and association of aflatoxin B1with liver disease. Moreover, research projects on common cancers, particularly carcinogenic, cellular kinetic changes and epigenetic alterations of liver, gastrointestinal and nasopharyngeal cancer were studied by immunohistochemistry (IHC) and in-situ hybridization (ISH) are being conducted in collaborating with other organizations and departments. The division also performs ultrastructural analysis of infection carrying vector by electron microscopy.

RESEARCH PROJECTS

1. COMMUNICABLE DISEASES

1.1. Differentiation of *Aedes aegypti* and *Aedes albopictus* eggs using scanning electron microscope

Aedes aegypti and *Aedes albopictus* are common arboviral mosquitoes causing the fatal diseases globally. However these two vectors are bionomically similar, the differences in morphology of egg between these two mosquitoes are very limited in Myanmar. This study was performed collaboratively with Medical Entomology Research Division, Department of Medical Research. In this study, the morphology of *Ae.aegypti* and *Ae.albopictus* eggs were described using scanning electron microscopy (SEM). *Ae.albopictus* eggs were smaller and taper at the posterior end. But the micropylar disc of *Ae. Aegypti* was wider and had incomplete circular sectors. *Ae.albopictus* had a narrower circular disc without sectors. Outer chorionic cells enclosing both large central tubercle and peripheral tubercles were also different between these two species. Furthermore, the exchorionic networks in *Ae. albopictus* were narrow, prominent, solid wall like whereas they were interwoven, reticulated and extremely wide in *Ae.aegypti*. The morphological analysis of the eggs attributes of *Ae. aegypti* and *Ae. albopictus* using SEM enables the differentiation of the species and may be helpful in understanding the egg biology.

2. NON-COMMUNICABLE DISEASES

2.1 Establishment of in-house production of Phytohaemagglutinin (PHA) reagents for detection of chromosal disorders

Phytohaemagglutinin (PHA), the lectin extract from the red kidney bean (*Phaseolusvulgaris*)(ဧမြဇောက်ပဲ), contains potent, cell agglutinating and mitogenic activities. It possesses the ability to stimulate lymphocytes to undergo mitosis and can be used for karyotyping to analyse human chromosome. The aim of this study was to establish in-house phytohaemagglutinin (PHA) reagents extracted from red kidney beans for karyotyping of chromosomal diseases and assess the quality of the extracted in-house PHA from red kidney beans for application in a laboratory based study. PHA was extracted from red kidney beans by using normal saline (0.9% NaCl) and fractionally precipitated with ammonium sulphate at 40%, 50%, 60% and 70% saturation. The extracted in-house PHA reagent was haemagglutinated with 2% chicken red blood by Haemagglutinating Activity Assay. The comparison between in-house PHA and imported commercial PHA were cultured with same concentration in blood samples of healthy individual donor for mitotic stimulation of human lymphocytes. Mitogenic activity of the in-house PHA reagent was good reasonably compared with commercial PHA in karyotyping. The protein concentration of the extracted PHA reagent from red kidney beans was 18.6 mg/ml for karyotyping. The commercial PHA contained 5-10 mg/ml of protein. A sodium dodecyl sulfate polyacrylamidegel electrophoresis (SDS-PAGE) was performed with the method of Laemmli. The fresh preparation of indigenous PHA mitogen is useful as commercial products, low cost and easily accessible.

2.2 Genotypic analysis of Epstein–Barr virus (EBV) in nasopharyngeal carcinoma patients in Myanmar

Nasopharyngeal carcinoma (NPC) is endemic in certain populations and contributes for 0.6% of all cancers in the world. It occurs at high incidence in South East Asia, Southern China and North Africa. In Myanmar, the prevalence of NPC is gradually rising yearly and it is the most commonest head and neck cancer in Yangon General Hospital (YGH). Epstein Barr virus (EBV) or Lymphocryptovirus is a member of the Herpesviridae family. It is a well known causative agent in NPC and mainly infects lymphocytes and epithelial cells. The polymerase chain reaction (PCR) was used to study DNA extracted from the blood samples of 35 histologically confirmed NPC patients. The commonest age group is 51-60 years in both gender and more common in males than females (1.5:1). The most common histological type was poorly differentiated squamous cell carcinoma (SCC) (45.7%) and other histological types were non-keratinized carcinoma (20%), undifferentiated anaplastic carcinoma (17.2%), moderately differentiated SCC (11.4%) and well differentiated SCC (5.7%) according to World Health Organization (WHO) classification. Primers were directed to conserved regions of the EBV genome encoding Epstein Barr nuclear antigen 1(EBV-NA1) region. Specific EBV amplification (262 bp fragment) was found in 5 samples of NPC patients (14.3%). The purified PCR products were sequenced by using ABI 3500 XL genetic analyzer. These isolates were found to be EB virus (Human Herpesvirus genotype 4) and this study is the first detection in blood samples of nasopharyngeal carcinoma patients in Myanmar. A phylogenetic tree was generated and the new Myanmar EBV sequences were analyzed with a group of 14 previously published EBV strain sequences including 8 from China, 5 from Australia and one from Japan within years 2006-2016. The new Myanmar EBV strains were recorded that was different from other isolates of these countries. Cancer treatment for early stage of NPC has a good response but 70-80% of NPC is found in advanced or metastatic

state. EBV DNA may be currently related biomarker in NPC which is one of the indicators for early diagnosis, better prognosis, treatment response and recurrence of disease during cancer therapy.

2.3 Role of epigenetic regulation in hepatocellular carcinoma

Deregulation of upstream epigenetic regulatory proteins promotes epigenetic alterations and contributed to aberrant silencing of tumor suppressor genes in human cancers. Enhancer of zeste homolog 2 (EZH2), the catalytic subunit of Polycomb Repressive Complex 2 (PRC2), is one of the most commonly up-regulated epigenetic regulators in different human cancers. EZH2 is a histone methyl transferase that specifically catalyzes histone H3 lysine 27 tri-methylation (H3K27me3), which in turn acts as a repressive histone modification to epigenetically control gene transcription. EZH2 epigenetically inactivates expressions of multiple tumor and metastasis suppressor microRNAs in human hepatocellular carcinoma (HCC), thereby promotes HCC tumorigenicity and metastasis. Among 24 HCC tissue left over specimens analyzed were19 male and 5 were female and age ranged from 43-82 years (56.96±10.40, Mean±SD). Haematoxylin and eosin (H&E) stain was used for histological grading and immunoexpression of Ki67 for cell proliferating activity and immunoexpression of EZH2 and H3K27 forhistone modification were done. Among the total samples, four were well differentiated (16.67%), 8 were moderately differentiated (33.33%) and 12 were poorly differentiated HCC (50%). Ki67 mean labeling indices were3.8±2.2, 1.8±1.3, 1.5±1.1 in poorly differentiated, moderately differentiated and well differentiated cases, respectively. Mean labeling indices for immunoexpression of EZH2 were 4.5±2.4, 2.3±1.4, 1.9±1.1 in poorly differentiated, moderately differentiated and well differentiated cases, respectively. Also, Labeling indexes for immunoexpression of H3K27 were 4.3±2.1, 2.2±1.3 and 1.6±1.2, in poorly differentiated, moderately differentiated and well differentiated cases, respectively. Therefore, up-regulation of EZH2 and H3K27 possibly plays a crucial role in promoting HCC tumorigenicity and metastasis.

2.4 Profile of liver enzymes and histopathological changes of liver in Aflatoxin B1 induced rat

Aflatoxin B1 (AFB1) is a potent hepatotoxic and hepatocarcinogenic mycotoxin that induces lipid peroxidation in rat liver. AFB is secondary toxic fungal metabolites produced as *Aspergillusflavus* and *A. parasiticus*. It is associated with aflatoxicosis and hepatocellular carcinoma. An experimental study based on laboratory animal especially (36) numbers of three months old male albino rats that separated into 6 groups (6 rats per group) including untreated control group and five study groups. AFB1 was administered through intraperitoneal route by different concentrations viz., 20, 40, 60, 80 and 100 ppm respectively for 8 days. After completion of the treatment, the blood samples were collected from all rats for determination of liver enzymes (AST and ALT). All liver samples were sacrificed to continue tissue processing and staining by haematoxylin and eosin for observation of histological changes of rat liver. In control untreated group, liver enzymes and histological findings are apparently within normal range and normal liver cellular pattern. Liver enzymes (AST and ALT) were higher than normal value in 20 ppm AFB1 treated group (AST > 190 U/L and ALT > 110U/L) and in 40, 60, 80 and 100 ppm AFB1 treated rats (AST < 190 U/L and ALT < 110U/L). In the lowest concentration treated group of 20ppm AFB1, histological findings showed the lowest score (< 3) observed with mild parenchymatous degeneration characterized by granular appearance of hepatocytes cytoplasm. Other four groups of serially higher concentration of AFB1 treated have high score (> 3) characterized by serially significant cytoplasmic visualization with disseminated necrotic cells, central vein dilation,

congestion of sinusoids and haemosiderin pigmentations in hepatocytes and periportal area fibrosis. The biochemical and histological analysis of rat liver showed AFB1 has significant increased in lipid peroxidation during which significant reduction of liver enzymes and mitochondrial dysfunction that indicates impaired liver functions. AFB1 had seriously toxic to the rat liver because of impairment of liver function, denaturation of protein structure and developed to liver cancer. The repeated exposure of AFB1 has disturbed metabolic actions on rat liver and increase the risk of liver cancer.

Liver enzymes changes of control and AFB1 treated rats

Group No.	Amount of AFB1 induced (single dose of intraperitoneally)	mean AST (U/L)	mean ALT (U/L)
1	Normal control	120.10	79.98
2	20 ppm	193.77	112.22
3	40 ppm	132.18	82.68
4	60 ppm	132.40	82.76
5	80 ppm	138.70	85.83
6	100 ppm	168.22	100.22

SERVICES PROVIDED

ACADEMIC

Sr.	Name	Course	Responsibility
1.	Dr. Kyaw Soe	M.Med.Sc (Pathology, Oral Medicine, Medical Jurisprudence) M.Med.Tech.(Med Lab Tech) B.Med.Tech (Med Lab Tech)	Teaching
2.	Dr. Min Min Win	M.Med.Sc (Pathology, Oral Medicine, Medical Jurisprudence) M.Med.Tech.(Med Lab Tech) B.Med.Tech (Med Lab Tech)	Teaching
3.	Daw Myat Mon Oo	M.Med.Sc (Pathology, Oral Medicine, Medical Jurisprudence) M.Med.Tech.(Med Lab Tech) B.Med.Tech (Med Lab Tech)	Teaching
4.	Dr. Khin Kant Kaw Oo	M.Med.Sc (Pathology, Oral Medicine, Medical Jurisprudence) M.Med.Tech.(Med Lab Tech) B.Med.Tech (Med Lab Tech)	Teaching
5.	Daw Than ThanSwe	M.Med.Sc (Pathology, Oral Medicine, Medical Jurisprudence) M.Med.Tech.(Med Lab Tech) B.Med.Tech (Med Lab Tech)	Teaching

PATHOLOGY RESEARCH DIVISION (POL)

Research Scientist & Head	…	Dr. Khin Moe Aung MBBS, MMedSc (Pathology) (UM 2)
Research Officer	…	Dr. Nyein Nyein Thaung MBBS (UMM)
	…	Dr. Wut Hmon Min MBBS (UMM)
	…	Dr. Thae Thae Moe Han MBBS (UMM)
	…	Dr. Ei PhyoWai MBBS(UM1)
	…	Dr. Su Su Lin MBBS (UMM)
	…	Dr. Nandar Ko MBBS (UMM)
	…	Dr. Yadanar Aung Myo Han BVSc (NPW)
	…	Daw Myint Myint Khaing BSc (Chemisty) (UDE)
	…	Daw Tin Moe Khaing BSc (Chemistry) (UDE)
Research Assistant (2)	…	Daw Su Su Myaing BSc (Chemistry) (UDE), EGTI (Civil) Pyin Oo Lwin
	…	Daw Htay HtayKywe BSc (Botany) (UDE)
	…	Daw Nan Phyu Phyu Mar BSc (Mathematics) (MU)
	…	Daw Than Than Maw BSc (Chemistry) (UDE)
	…	Daw May Thin Kyu BMedTech (Medical Laboratory Technology) (UMTM), Diploma in English
	…	Daw Haung Naw BMedTech (Medical Laboratory Technology) (UMTM)
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	…	U Phone Zin Myint BA (English) (UDE)
	…	Daw Ohn Myint Aye BSc (Botany) (Meikhtila University)
Research Assistant (4)	…	DawThandar Win BSc (Chemistry) (UDE)

Pathology research division is primarily involved in research activities of the following diseases such as Anaemia, Iron Deficiency Anaemia, haemoglobinopathies, thyroid disorders, intestinal worm infestation and genetic diseases.

RESEARCH PROJECTS

1. COMMUNICABLE DISEASES

1.1 DENGUE INFECTION

1.1.1 Serum Electrolyte disturbances in children with dengue infection at 500 bedded children Hospital, Mandalay

This study was designed to describe demographic characteristics of children with dengue infection, to determine the severity of dengue infection according to WHO classification (2009) and to determine serum electrolyte levels according to severity of dengue infection. This study was hospital based cross-sectional descriptive study and done in 550 bedded children Hospital Mandalay from 1st January 2015 to 31st December 2015. Seventy seven patients with clinical diagnosis of dengue infection according to WHO criteria (2009) were participated in this study. Serum electrolyte levels were measured before initiating intravenous fluid therapy by using Easylyte plus analyzer using ion selective electrolyte mode. Statistical analysis was done using Chi-squared test with continuity

correction, ANOVA with Post hoc test. Out of the 77 patients with dengue infection, 37(48.1%) were boys and 40(51.9%) were girls. The most affected age group was 1 month-5yr (50.6%). Among 77 patients, 20 patients (26%) developed probable dengue without warning signs. 34 patients (44.2%) had probable dengue with warning signs and 23 patients (29.9%) had severe dengue. The mean serum sodium levels in severe dengue group, probable dengue with warning signs, probable dengue without warning signs were 132.8± 6.25mmol/L, 138.2 ± 4.68mmol/L, 137.5 ± 4.54mml/L and there was a significantly decrease in the mean serum sodium levels in severe dengue patients when compared either to other groups (p=0.001). Among three groups, mean serum potassium levels in probable dengue with warning signs were 4.13 ± 0.53mmol/L which was lowest value. Serum potassium levels in each groups were not significantly different (p=0.42). Mean serum sodium bicarbonate levels were 23.17 ± 3.80mmol/L in dengue without warning signs, 23.96 ± 4.12mmol/L in dengue with warning signs and 20.23 ± 4.11mmol/L in severe dengue. Serum sodium bicarbonate levels were statistically different among dengue groups (p=0.004). This study is hoped to be beneficial to some extent in making early diagnosis of electrolyte disturbances and early intervention can provide in decreasing the mortality of children with dengue infection.

2. NON-COMMUNICABLE DISEASES

2.1 BREAST CANCER

2.1.1 Serum ferritin and lactate dehydrogenase in carcinoma of breast

The purpose of this study was to study serum ferritin and lactate dehydrogenase in patients with carcinoma of the breast. This is a hospital- based cross sectional comparative study involving 60 patients with carcinoma of the breast admitted to surgical units, Mandalay General Hospital (MGH) and 30 healthy women served as controls. Determination of Clinical ferritin and lactate dehydrogenase (LDH) were done at Department of Clinical Pathology, MGH from 1st October 2015 to 30th September 2016. The serum ferritin and LDH levels were found to be increased in patients with carcinoma of the breast compared to healthy controls (p value 0.008 and 0.03 respectively). In patients with axillary lymph node metastasis, serum ferritin and LDH were further elevated and significantly higher compared to patients without metastasis (p value 0.0006 and 0.006 respectively). Serum ferritin and LDH significantly increased in carcinoma of the breast and furthermore increased in lymph node metastasis. These data suggested that elevated serum ferritin and LDH in patients with carcinoma of the breast could be regarded as a predictor of positive lymph node involvement.

2.2 CENTRAL NERVOUS SYSTEM DISEASE

2.2.1 Parental perceptions towards lumbar puncture in children attending at 500 Bedded Children Hospital, Mandalay

Infection of the central nervous system (CNS) is one of the most common diseases in children because it is one of top 25 causes of death in children and one of top 10 causes of long term disability in Myanmar. The diagnosis of CNS infections depends on examination of cerebrospinal fluid (CSF) obtained by lumbar puncture (LP). A hospital based cross-sectional study was done in medical units of 550 Bedded Children Hospital, Mandalay from January 2015 to December 2015. It involved 88 parents whose children need LP during their admission. Data were collected by using questionnaire filled up by the candidate. Face to face interviews were conducted at medical units, 550 Bedded Children Hospital, Mandalay. This

study showed that most of the study population fell between 20-55 years. Only 12.5% had high knowledge. Forty-five point five percent had average knowledge and 42% had low knowledge. Sixty-seven point one percent (67.1%) had positive attitudes. Twenty-two point seven percent (22.7%) had negative attitudes towards LP. Ten point two percent (10.2%) had neutral attitudes. There was a significant association between knowledge and LP acceptance or refusal (*p* value= 0.006). There was also significant association between attitudes and LP acceptance or refusal (*p* value = 0.000). Knowledge and attitudes of the parents or guardians are the important factors for LP acceptance or refusal. Although background knowledge and attitudes of parents or guardians on LP were fairly satisfactory, some caregivers considered LP would result in death or complications. Further researches on parental perception towards LP should be conducted in order to get more accurate and comprehensive findings for designing the related educational programs on LP.

2.3. ANAEMIA

2.3.1. Prevalence of anaemia in reproductive age women (15-45) years in Pyin Oo Lwin Township

Anemia is an important health problem and it is caused by a wide range of causes. Early diagnosis and effective treatment is important because morbidity and mortality of the disease is high.According to community surveys by NNC-DOH, the prevalence of anemia was 45% among non-pregnant women(2001), and 26% among adolescence school girls(2002), 71% among pregnant women(2003), and 75% among under-five children(2005), in Myanmar. The present study was designed to assess the prevalence of anemia, severity of anemia and types of anemia in reproductive age women in PyinOoLwin Township.This study was community and laboratory based descriptive study. The reproductive age women (15-45) years from PyinOoLwin Township were studied for prevalence, severity and types of anemia.The prevalence of anemia among reproductive age women was 27.5%, which included 19% of mild and 8.5% of moderate anemia cases. According to blood film examination, 75.5% were normal blood film and 25.5% showed these results, 13.5% were mild hypochromic microcytic anemia, 3.5% eosinophilia, 2.5% in normochromic normocytic anemia and neutrophil leukocytosis, 1% in hypochromic microcytic anemia with thrombocytosis and mild hypochromic microcytic anemia with eosinophilia respectively.Anemia is one of the most common and intractable nutritional problems in the world today. The main causes of anemia are dietary iron deficiency, infectious diseases such as malaria, hookworm infections and schistosomiasis, deficiencies of other key micronutrients including folate, vitamin B12 and vitamin A of inherited conditions that affect red blood cells (RBCs) such as thalassaemia. This study was preliminary data or baseline data for anemia in reproductive age women in Pyin Oo Lwin Township and further study for causes of anemia should be done in community level for public awareness and health education for anemia.

SERVICES PROVIDED

ACADEMIC

Sr.	Name	Course	Responsibility
1.	Dr. Khin Moe Aung	M.Med.Sc (Pathology) 2nd year M.Med.Sc (Biochemistry) 1st year	Demonstration of gel electrophoresis

LABORATORY

Sr.	Laboratory Test	Tested Samples
1.	Histopathology (H and E stain) - Rat	34 samples
2.	Cervical cancer (Pap smear)	500 samples
3.	Blood for Complete Picture (CP)	200 samples