EXPERIMENTAL MEDICINE RESEARCH DIVISION

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The Experimental Medicine Research Division is primarily involved in research on hepatic and gastro-intestinal diseases. The division is investigating the prevalence of hepatitis B and hepatitis C infections in different geographical areas and population groups and the associated factors with the aim of determining the burden of hepatitis B and hepatitis C infections and to identify ways of controlling it. The division is also concerned with the diagnosis and management of hepatitis B and hepatitis C carriers who are attending the Hepatitis Carrier Clinic.

RESEARCH PROJECTS

1. COMMUNICABLE DISEASES

1.1. VIRAL HEPATITIS

1.1.1. Hepatitis B infection

1.1.1.1. Detection of hepatitis B virus X and core promoter mutations and gene expression study on Myanmar patients with HBV induced hepatocellular carcinoma

This study was carried out in collaboration with Pusan National University, Republic of Korea. A total of 54 patients with HBs antigen positive hepatocellular carcinoma and 12 chronic hepatitis B and/or cirrhotic patients were recruited for detection of viral DNA and genotyping and detection of gene mutations. Among them, hepatitis B viral DNA was detected in 15 HCC patients' samples. The six HCC patient's samples were sequenced on the whole genome of viral hepatitis B and the sequence data analysis was carried out with the reference sequences. Initial findings showed that the multiple mutations seen on the S gene, polymerase gene, HBx gene and core promoter of HBV. The "a" determinant of HBs antigen protein is regarded as the most important antigenic determinant of HBV vaccine production and HBV diagnostic system. The changes of amino acid, T125K, I126F, P127C at the "a" determinant of HBs antigen were seen on all six HBV infected samples. The position of amino acid changes in this study was critical region of HBs antigen and it plays a role in determination of protein structure of antigenic epitope of HBV S antigen. The initial findings showed that amino acid changes at the "a" determinant of HBs antigen protein and subsequent conformational changes in S protein are seen in this study and the current HBV diagnostic kit might not recognize the newly formed HBV S antigen.

1.1.1.2 Occult hepatitis B infection in chronic hepatitis C patients

Occult hepatitis B infection (OBI) is manifested by presence of very low levels (<200IU/mL) of Hepatitis B viral DNA (HBV DNA) in the blood and the liver while exhibiting undetectable HBV surface antigen (HBsAg). It has been found with a high prevalence in patients with chronic hepatitis C (CHC) because both HBV and HCV share the same parenteral way of transmission. It could be associated with the development of hepatocellular carcinoma and a greater likelihood of progression to cirrhosis and HCC. Regardless of clinical significance of occult hepatitis B, its frequency among CHC patients is unknown yet in Myanmar. This study was carried out in 76 Chronic Hepatitis C patients who were attending the Hepatitis Carrier Clinic, DMR during 2015 for their regular follow up. Among them, 75 patients including 34 males and 41 females were screened as HBsAg negative by using HBV combo test. Six out of 75 patients (8%) showed markers of previous exposure to HBV. HBV DNA was detected in 4 out of 75 patients (5.3%). Among OBI positive patients, 2 (50%) carried serological markers of HBV infection. It would be a preliminary study which can initiate the study of OBI status in Chronic Hepatitis C infection in Myanmar population.

1.1.1.3. Determination of Hepatitis B viral load by using in-house Real-time PCR

Hepatitis B virus (HBV) infection is a major cause of chronic liver diseases worldwide. In addition to genotyping of the virus, quantitative analysis of HBV infection is extensively used for monitoring of disease progression and treatment. Affordable viral load monitoring is desirable in resource limited setting. The aim of study was to develop an in-house real-time PCR based method which is both sensitive and efficient offering an alternative method to conventional PCR for HBV at the Advanced Molecular Research Centre, Department of Medical Research, Myanmar. Plasmid containing sequence of core gene 1.2 mer HBV+ construct was used as HBV-DNA standard. To detect HBV DNA, real-time PCR based on SYBR green chemistry was carried out following proper primer designing and PCR optimization. The interested sequence contained in the plasmid and clinical specimen was quantitatively measured through Real time PCR ABI 3700 (Applied Bio-systems). The detection limit for HBV DNA in this assay was 14 copies per microliter. Linear standard curve was obtained between 10^{-2} and 10^{-7} DNA ng/μL. None of negative samples showed false positive reactions in duplicate. HBV DNA was detected in more than 90 percent of HBsAg positive samples. The coefficient of variation for both intra and inter experimental variability was carried out. The method detecting HBV viral load based on SYBR is reliable, accurate, and reproducible. In addition, the quantification of HBV DNA to monitor the efficacy of HBV therapy is useful in understanding of natural history of HBV in the endemic country like Myanmar.

2. NON-COMMUNICABLE DISEASES

2.1. CANCER

2.1.1. Studies of epigenetic changes in circulating tumor cell in hepatocellular carcinoma

This study was carried out in collaboration with Singapore oncology group. Hepatocellular carcinoma (HCC) is a highly prevalent disease in East Asia, largely due to the prevalence of chronic active hepatitis B and C infection. Despite screening efforts, the majority of HCC patients are diagnosed with advanced disease, leading to high mortality rates. Genetic and epigenetic alterations involved in the pathogenesis of HCC are known.

Detection of these changes in peripheral blood may provide an accurate test for the early detection of cancer. Blood samples were collected from 91 patients with HCC. Patient and control samples were also matched for hepatitis B and C infection status. Twenty eight samples of HCC had sufficient annotation to support a diagnosis of HCC by the American Association for the Study of Liver Diseases (AASLD) criterion or were biopsy proven. Eighteen of HCC samples were included in preliminary analysis in a cohort of 78 HCC patients from Singapore, Japan and Myanmar with 90 control samples matched for age, gender and hepatitis B and C status. Stratifying markers such as ILGF2, MYC, p16 loci and HBVDNA were analyzed. The results were Sensitivity 73.3% (95% CI–48.1% to 89.1%), Specificity 94.4% (95% CI–74.2% to 99%), Positive predictive value (PPV) 91.7% (95% CI–64.6% to 98.5%) and Negative predictive value (NPV) 77.3% (95%CI–56.6 % to 89.9%).

2.2. CIRRHOSIS

2.2.1. Bacteriological profile of ascitic fluid from spontaneous bacterial peritonitis patients in Liver Medical Ward, Yangon Specialist Hospital

A cross sectional hospital and laboratory based descriptive study was done in total 44 cirrhotic patients with ascites from January to October 2015 in Liver Medical Ward, Yangon Specialist Hospital to isolate and identify bacterial pathogens of spontaneous bacterial peritonitis patients, and to determine antibiotic sensitivity pattern. Diagnosis of SBP was done by counting polymorphonuclear leucocyte count (PMN) in ascitic fluid by Neubauer(Bright line) counting chamber and isolating bacteria by blood culture bottle method. Out of 44 cirrhotic patients with ascites, SBP were diagnosed in 24 cases (54.55%) of those patients. Among 24 cases of SBP, 6 cases (25%) were classical SBP, 16 cases (66.67%) were culture-negative neutrocytic ascites (CNNA), 2 cases (8.33%) were monomicrobial nonneutrocytic bacterascites (MNB). Bacteria were isolated in 8 cases (33.33%) of total 24 SBP patients, gram negative bacteria were 5/8 isolates (62.5%) and gram positive bacteria were 3/8 isolates (37.5%). Among these isolates, *Escherichia coli* were 2 in number (25%), *Pseudomonas* species were 2 in number (25%), *Staphylococcus haemolyticus* were 2 in number (25%), *Staphylococcus aureus* were 1 in number (12.5%) and *Acinetobacter baumannii* were 1 in number (12.5%). Most of the pathogenic bacteria were sensitive to amikacin, gentamicin, imipenem and meropenem, but all of Enterobacteriaceae and *Pseudomonas* species were totally resistant to ceftriaxone, cefotaxime, cefepime, and all of Enterobacteriaceae were totally resistant to amoxycillin-clavulanic acid. This study provided the local epidemiological pattern of causal organisms and their antibiotic susceptibility in spontaneous bacterial peritonitis patients.

SERVICES PROVIDED

Hepatitis Carrier Clinic

Viral hepatitis B or C infected patients were given consultation with regular check up at the hepatitis carrier clinic. There were 469 patients with hepatitis B infection and 81 patients with hepatitis C infection attended this year. A total of 2096 patients of old and new hepatitis B or C patients were consulted at the Hepatitis Carrier Clinic.

ACADEMIC

Sr.	Name	Course	Responsibility
1.	Dr. Aye Aye Win	Workshop on Research Methodology (2015) MMedSc (Pathology)	Facilitator Teaching

LABORATORY

Sr.	Laboratory Tests	No. of tests
1.	Anti-HCV	699
2.	Liver function test	23
3.	ALT, AST	894
4.	AFP test	632
5.	HBV markers (Combo test)	456
6.	HBsAg	7