CO-INFECTIONS OF HEPATITIS B PATIENTS WITH OTHER HEPATITIS VIRUSES AND THEIR SEROLOGICAL, BIOLOGICAL AND PROGNOSTIC MARKERS

HUDU ABDULLAHI SHUAIBU

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By

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Hepatitis B patients, who are co-infected with other hepatitis viruses, are associated with more severe infection and higher mortality than in mono-infection therefore, fulminant hepatitis with co-infection is more likely than with infection with HBV alone. Hepatitis B is a non-cytopathic virus therefore; immune response that takes place in the liver against the virus is responsible for the inflammation of the hepatocytes, hence referred to as “hepatitis”. This inflammatory process triggered by the virus is mediated by human cytokines, thereby determining the severity or otherwise of the infection the failure of suppressing hepatitis B virus replication is thought to be as a result of ineffective cytokine response. Therefore, this study aimed to determine the clinical, serological and biochemical and genetic diversity of hepatitis B and co-infection with other hepatitis viruses and propose novel prognostic markers of cirrhosis and acute deterioration of chronic hepatitis B infection.

A total of 82 patients’ blood samples and clinical data were collected from May, 2015 to May, 2016 at the Hepatology Department of Hospital Selayang from chronic hepatitis B patients. All the samples were tested for hepatitis B sero-markers and hepatitis C, D, and E antigens using commercially available ELISA kits according to manufacturer's instruction. Nested PCR was done for HBV and RT-PCR for HCV, and HEV. Sequence and phylogenetic analyses were done using the Molecular Evolutionary Genetic Analyses version7.0 software (MEGA7). HBV DNA was quantified using real time PCR method and human cytokines gene expression was done using RT² PCR array. Plasma cytokines were quantified using the magnetic beads multiplexed Luminex multiplex assay.
Of the 82 patients recruited for this study, 49 (59.8%) were males, 33 (40.2%) females. There were 27 (32.9%) Malay patients, 51 (62.2%) Chinese, 3 (3.7%) Indians while the remaining 1 (1.2%) is orang asli. Majority of the patients, 41 (50%) are within the age of 50 and above, 32 (39%) within 30 to 49 years and the remaining 9 (11%) are below 30 years. The patients recruited for this study were classified as non-cirrhotic 44 (53.7%), cirrhotic 18 (22.0%), acute flare 17 (20.7%) and hepatocellular carcinoma 3 (3.7%). The rate of co-infection was found to be HBV+HEV (10.7%), HBV+HCV (1.3%), and 88% having single infection with HBV. Chemical profile revealed that majority (58%) have high level ALT (High (≥34 IU/L). Physical examination showed jaundice as the most frequent clinical symptoms followed by easy fatigability. A strong positive correlation was found between severity of infection and the level of AST (r=0.693; p<0.01) and jaundice (r=0.714; p<0.01). Similarly, moderate positive correlation was found between severity of infection and ALT (r=0.447; p<0.01) and serum bilirubin level (r=0.543; p<0.01). On the other hand, a negative correlation was found between severity of infection and total protein (r=-0.339; p<0.01) and albumin (r=-0.464; p<0.01). The results also showed that majority (62%) of the patients have high HBV DNA (>2000 IU/mL) and most of the patients with this high viral load were cirrhotic of acute flare. This HBV DNA load was found to correlate positively with hepatitis B e antigen titre (r= 0.950; p<0.01) and hepatitis B surface antigen titer (r= 0.642; p<0.01). This result showed that, HBV load correlated more with HBeAg titer than it does with HBsAg titer. Hence, the significance of HBeAg in monitoring patient progress on therapy in lieu of viral load in a situation where viral load is not available.

Sequence analyses of the HBV shows that 63.9% of the virus belongs to genotype B and 36.1% genotype C. On the other hand, all HEV belong to genotype 4 while HCV belong to genotype 3a. Interestingly, the local HEV isolates UPM14, 23 and 45 demonstrated high sequence identity with a swine isolate, SAAS-FX17 (JF915746), from China (96.7%, 99% and 94.1%, respectively), while isolate UPM75 was found to be 89.3% identical to swine isolate IND-SW-00-01 (AY723745) from India. Cytokines gene expression results showed that of the 84 genes associated with inflammatory pathways, only 7 (8.3%) genes were expressed among non-cirrhotic chronic hepatitis B patients compared with healthy hepatitis negative control group. Some genes were found to be upregulated, with acute flare group having highest number of upregulated genes and are associated with enhancing inflammatory process which might play a significant role in acute flaring of chronic hepatitis B patient. These upregulated genes include mostly pro-inflammatory chemokines such as Chemokine (C-C motif) ligands (CCL) and also interleukin receptor (IL-1R1) while anti-inflammatory cytokines genes such as interleukin 13, 17, as well as interleukin 1 receptor antagonist (IL-1RN) and gamma interferon were down regulated. Circulating IL-8 (χ²=1351.05; DF=1197; p<0.005) and MIP-1beta (χ²=2302.99; DF=2142; p<0.05) were found to be significantly associates with the level of HBeAg. Similarly, hepatitis B viral DNA was also found to be significantly correlated with MIP-1beta (r=0.272; P<0.05).
At the end of this study, an insight into the genetic distributions of hepatitis B, C and E viruses and the rate of HBV co-infection with HCV and HEV among Malaysian chronic hepatitis B patients was highlighted. It also described for the first time, comparative genomic sequence analyses of local HEV isolates suggesting a zoonotic origin with swine and boar HEV. Although, quantitative HBsAg and HBeAg has been identified as an important indicator of therapeutic response, this study revealed HBeAg as the most appropriate marker that correlate well with the HBV DNA. Up-regulation of the IL-3 gene in liver cirrhotic patients indicated poor prognosis and may be associated with the development of HCC. Similarly, this study, also suggested TNFSF-13 as a potential novel molecular prognostic marker in chronic hepatitis B patients. Circulating MIP-1beta was found to be a significant sero-marker in this study, therefore, it can be considered as a novel prognostic marker of liver cirrhosis in chronic hepatitis B patient.
Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

JANGKITAN BERSAMA PESAKIT HEPATITIS B DENGAN VIRUS HEPATITIS YANG LAIN DAN PENANDA SEROLOGI, BIOLOGI DAN PROGNOSIS

Oleh

HUDU ABDULLAHI SHUAIBU

April 2017

Pengerusi : Profesor Zamberi Sekawi, MPath
Fakulti : Perubatan dan Sains Kesihatan


Lapan puluh dua orang pesakit yang terlibat dalam kajian ini, 49 (59.8%) adalah lelaki, 33 (40.2%) perempuan. Terdapat 27 (32.9%) pesakit Melayu, 51 (62.2%) Cina, 3 (3.7%) India manakala baki seorang (1.2%) adalah Orang Asli. Majoriti
pesakit, 41 (50%) yang berada dalam usia 50 tahun ke atas, 32 (39%) dalam tempoh 30 hingga 49 tahun dan selebihnya 9 (11%) adalah di bawah 30 tahun. Pesakit terlibat dalam kajian ini diklasifikasikan sebagai bukan sirotik, 44 (53.7%), sirotik 18 (22.0%), marak akut 17 (20.7%) dan karsinoma hepatoselular 3 (3.7%). Kadar jangkitan bersama didapati HBV + HEV (10.7%), HBV + HCV (1.3%), dan 88% mempunyai jangkitan tunggal dengan HBV. Profil kimia mendedahkan bahawa majoriti (58%) mempunyai tahap tinggi ALT yang tinggi (≥34 IU/L). Pemeriksaan fizikal menunjukkan penyakit kuning sebagai tanda klinik yang paling kerap diiktui oleh mudah lesu. Korelasi positif yang kukuh didapati di antara keterukan jangkitan dan tahap AST (r = 0.693; p <0.01) dan penyakit kuning (r = 0.714; p <0.01). Begitu juga, hubungan yang positif sederhana didapati di antara keterukan jangkitan dan ALT (r = 0.447; p <0.01) dan tahap bilirubin serum (r = 0.543; p <0.01). Sebaliknya, korelasi negatif didapati di antara keterukan jangkitan dan jumlah protein (r = -0.339; p <0.01) dan albumin (r = -0.464; p <0.01). Keputusan juga menunjukkan bahawa majoriti (62%) daripada pesakit mempunyai HBV DNA yang tinggi (> 2000 IU/mL) dan sebahagian besar pesakit dengan beban virus yang tinggi ini adalah sirosis marak akut. Beban HBV DNA ini didapati berkorelasi secara positif dengan titer hepatitis B e antigen (r = 0.950; p <0.01) dan titer hepatitis B antigen permukaan (r = 0.642; p <0.01). Keputusan ini menunjukkan bahawa, beban HBV berkaitan dengan titer HBeAg daripada dengan HBsAg titer. Oleh itu, kepentingan HBeAg dalam memantau kemajuan terapi pesakit sebagai ganti beban virus dalam keadaan di mana ubian beban virus tidak boleh didapati.

Analisis jujukan HBV menunjukkan bahawa 63.9% daripada virus yang tergolong dalam genotip B dan 36.1% genotip C. Sebaliknya, semua HEV adalah genotip 4 manakala HCV milik genotip 3a. Yang menariknya, isolat HEV tempatan UPM14, 23 dan 45 menunjukkan identiti jujukan yang tinggi dengan isolat babi, SAAS-FX17 (JF915746), dari China (96.7%, 99% dan 94.1% masing-masing), manakala isolat UPM75 didapati 89.3% sama dengan isolat babi IND-SW-00-01 (AY723745) dari India. Penyataan gen sitokin menunjukkan daripada 84 gen yang dikenalpasti dengan laluan radang, hanya 7 (8.3%) gen telah dinyatakan di kalangan bukan sirosis pesakit kronik hepatitis B berbanding dengan kumpulan sihat kontrol negatif. Beberapa gen telah didapati upregulated, dengan kumpulan marak akut mempunyai bilangan tertinggi gen upregulated dan berkaitan dengan peningkatan proses radang yang mungkin memainkan peranan yang penting dalam kemarakan akut pesakit hepatitis B kronik. Gen upregulated kebanyakannya kemokin pro-radang seperti kemokin (CC motif) ligan (CCL) dan juga reseptor interleukin (IL-1R1) manakala gen sitokin anti-radang seperti interleukin 13, 17, serta antagonis reseptor interleukin 1 (IL-1RN) dan gamma interferon telah downregulated. IL-8 (χ² = 1351,05; df = 1197; p <0.005) dan MIP-1beta (χ² = 2302,99; df = 2142; p <0.05) didapati berkaitan dengan tahap HBeAg. Begitu juga, hepatitis B DNA virus juga didapati mempunyai hubungan yang signifikan dengan MIP-1beta (r = 0.272; p <0.05).

Pada akhir kajian ini, taburan genetik virus hepatitis B, C dan E dan kadar HBV jangkitan bersama dengan HCV dan HEV di kalangan pesakit hepatitis B kronik di Malaysia dapat diketahui. Bagaimanapun, HDV tidak dapat dikesan. Buat kali pertamanya, jujukan gen isolat HEV tempatan mencadangkan asal usul zoonotik dengan HEV babi. Walaupun, HBsAg kuantitatif dan HBeAg telah dikenalpasti...
sebagai penunjuk penting terapeutik, kajian ini menunjukkan HBeAg sebagai penanda yang paling sesuai yang berhubung kait dengan HBV DNA. Gen IL-3 yang upregulated pada pesakit sirosis hati menunjukkan prognosis tidak baik dan boleh dikaitkan dengan perkembangan karsinoma hepatoselular. Begitu juga, kajian ini juga mencadangkan TNFSF-13 sebagai penanda yang berpotensi dalam pesakit hepatitis B kronik. MIP-1beta pula didapati penanda yang signifikan dalam kajian ini, oleh itu, ia boleh dianggap sebagai penanda novel sirosis hati dalam pesakit hepatitis B kronik.
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I certify that a Thesis Examination Committee has met on 26 April 2017 to conduct the final examination of Hudu Abdullahi Shuaibu on his thesis entitled "Co-Infections of Hepatitis B Patients with Other Hepatitis Viruses and their Serological, Biological and Prognostic Markers" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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<tr>
<td>AIMP1</td>
<td>Aminoacyl tRNA synthetase complex-interacting multifunctional protein 1</td>
</tr>
<tr>
<td>Anti-HBc</td>
<td>Hepatitis B core Antibodies</td>
</tr>
<tr>
<td>Anti-HBe</td>
<td>Hepatitis B e antigen</td>
</tr>
<tr>
<td>Anti-HBs</td>
<td>Hepatitis B Surface Antibodies</td>
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<tr>
<td>APASL</td>
<td>Asian Pacific Association for the Study of the Liver</td>
</tr>
<tr>
<td>APRIL</td>
<td>Proliferation inducing ligand</td>
</tr>
<tr>
<td>AST</td>
<td>Aspartate transferase</td>
</tr>
<tr>
<td>BMP2</td>
<td>Bone morphogenetic protein 2</td>
</tr>
<tr>
<td>C5</td>
<td>Complement component 5</td>
</tr>
<tr>
<td>CCL</td>
<td>Chemokine (C-C motif) ligand</td>
</tr>
<tr>
<td>CCR1</td>
<td>Chemokine (C-C motif) receptor</td>
</tr>
<tr>
<td>CD4+</td>
<td>Cluster of differentiation 4</td>
</tr>
<tr>
<td>CD40LG</td>
<td>CD40 ligand</td>
</tr>
<tr>
<td>CD8+</td>
<td>Cluster of differentiation 8</td>
</tr>
<tr>
<td>COV</td>
<td>Cut off value</td>
</tr>
<tr>
<td>CSF1</td>
<td>Colony stimulating factor 1 (macrophage)</td>
</tr>
<tr>
<td>CSF2</td>
<td>Colony stimulating factor 2 (granulocyte-macrophage)</td>
</tr>
<tr>
<td>CSF3</td>
<td>Colony stimulating factor 3 (granulocyte)</td>
</tr>
<tr>
<td>Ct</td>
<td>Cycle threshold</td>
</tr>
<tr>
<td>CX3CL1</td>
<td>Chemokine (C-X3-C motif) ligand 1</td>
</tr>
<tr>
<td>CX3CR1</td>
<td>Chemokine (C-X3-C motif) receptor 1</td>
</tr>
<tr>
<td>CXCL</td>
<td>Chemokine (C-X-C motif) ligand</td>
</tr>
<tr>
<td>CXCR</td>
<td>Chemokine (C-X-C motif) receptor</td>
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<tr>
<td>DAAs</td>
<td>Direct acting antiviral</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylene diamine tetra acetic acid</td>
</tr>
<tr>
<td>ENA-78</td>
<td>Epithelial-derived neutrophil-activating peptide 78</td>
</tr>
<tr>
<td>FASLG</td>
<td>Fas ligand (TNF superfamily, member 6)</td>
</tr>
<tr>
<td>G-CSF</td>
<td>Granulocyte colony stimulating factor</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>Granulocyte macrophage colony stimulating factor</td>
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<tr>
<td>HBsAg</td>
<td>Hepatitis B Surface Antigen</td>
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<tr>
<td>HLA</td>
<td>Human Leukocyte Antigen</td>
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<tr>
<td>HRP</td>
<td>Anti-Horseradish Peroxidase</td>
</tr>
<tr>
<td>IFNA2</td>
<td>Interferon, alpha 2</td>
</tr>
<tr>
<td>IFNG</td>
<td>Interferon, gamma</td>
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<tr>
<td>IL10RA</td>
<td>Interleukin 10 receptor, alpha</td>
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<tr>
<td>IL10RB</td>
<td>Interleukin 10 receptor, beta</td>
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<tr>
<td>IL1A</td>
<td>Interleukin 1, alpha</td>
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<tr>
<td>IL-1alpha</td>
<td>Interleukin 1, alpha</td>
</tr>
<tr>
<td>IL1B</td>
<td>Interleukin 1, beta</td>
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<tr>
<td>IL1R1</td>
<td>Interleukin 1 receptor, type I</td>
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<tr>
<td>IL-1ra</td>
<td>Interleukin 1 receptor alpha</td>
</tr>
<tr>
<td>IL1RN</td>
<td>Interleukin 1 receptor antagonist</td>
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<tr>
<td>IL27</td>
<td>Interleukin 27</td>
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<tr>
<td>IL5RA</td>
<td>Interleukin 5 receptor, alpha</td>
</tr>
<tr>
<td>IL9R</td>
<td>Interleukin 9 receptor</td>
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<tr>
<td>IMR</td>
<td>Institute for Medical Research</td>
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Kb Kilo base
LTA Lymphotoxin alpha (TNF superfamily, member 1)
LTB Lymphotoxin beta (TNF superfamily, member 3)
MCP-1 Monocyte chemoattractant protein 1
MELD Model for end stage liver disease
MIF Macrophage migration inhibitory factor (glycosylation-inhibiting factor)
MIP-1alpha Macrophage inflammatory protein 1-alpha
MIP-1beta Macrophage inflammatory protein 1-beta
ML Maximum Likelihood
NAMPT Nicotinamide phosphoribosyl transferase
NAT Nucleic Acid Amplification Test
NCBI National Centre for Biotechnology Information
NTC No Template Control
OD Optical Density
OR Odd ratio
ORF Open Reading Frame
OSM Oncostatin M
PC Positive Control
PE Phycoerythrin
 pegINF PEGylated Interferon
PI Protease inhibitors
qHBeAg Quantitative hepatitis e antigen
qHBsAg Quantitative hepatitis B surface antigen
RT Room Temperature
SD Standard Deviation
SPP1 Secreted phosphoprotein 1
STIs Sexual Transmitted Infections
SVR Sustained virological response
TAE Tris-Aacetate EDTA
TE Transient elastography
TNF Tumor necrosis factor
TNFRSF Tumor necrosis factor receptor superfamily
TNF-α Tumor necrotic factor alfa
VEGFA Vascular endothelial growth factor A
CHAPTER 1

INTRODUCTION

Hepatitis B virus co-infection with other viral hepatitis is associated with an increased risk of liver cirrhosis, hepatic decompensation and plays a significant role in the pathogenesis of hepatitis B acute flare. Hepatitis B genome diversity is globally influenced by both phenotypic and genotypic variability. Phenotype variability often developed in the present of selective pressure which is mounted by the immune system of the host during vaccination or therapy. While the genotypic variability often evolves in the absent of such selective pressure. Hepatitis C virus (HCV) is an important underlying factor in the development of liver diseases given rise to 25% of liver cancer aetiology globally (Ferlay et al., 2010). Hepatitis C virus prevalence in Asia has been reported to be 2% with high genetic variability (Lavanchy, 2011; Wasitthankasem et al., 2015). The predominant genotype in Southeast Asia is genotype 6 while genotypes 1-3 are distributed globally (Hajarizadeh et al., 2013; Li et al., 2009; Magiorkinis et al., 2009).

Hepatitis D virus (HDV) on the other hand, is an unusual single stranded RNA virus that is devoid of envelope protein and depends on HBV for its enveloped protein (Flores et al., 2015). The predominant HDV genotype in Asia is genotype 2 most especially in the East Asia, while genotype 1 is of global distribution and the remaining six genotypes are distributed to other geographical areas (Noureddin and Gish, 2014). Hepatitis D virus has also been reported as an important cause of acute and chronic liver diseases in several region of the world (Sureau and Negro, 2016).

According to the Southeast Asia Regional Office of the World Trade Organization (WTO), Asia hosts 6.5 million symptomatic HEV infections with 160,000 annual mortalities and 2,700 stillbirths due to HEV infection in pregnancy. As such, more than 50% of the global HEV infection mortality occurs in Asia (Griffiths et al., 2014). Hepatitis E virus is an enterically transmitted hepatitis virus that causes a self-limiting acute hepatitis infection with no chronic stage. However, in some cases fulminant hepatic failure can result, leading to increases in morbidity and mortality, especially among high risk groups like the elderly, pregnant women, the immunocompromised and patients with pre-existing liver diseases such as with chronic hepatitis B or C infections. Studies have shown that some animal species have serum antibodies to HEV, thereby suggesting HEV to be a zoonotic disease (Meng, 2000). For instance, some HEV cases in Japan were found to be directly linked to consumption of raw deer meat (Tei et al., 2003), while several cases of HEV were epidemiologically related to eating undercooked pork or wild boar meat (Matsuda et al., 2003; Yazaki et al., 2003).

Zoonotically acquired HEV has been described as the most successful zoonotic viral infection in human history (Dalton and Saunders, 2015), as it causes acute infection as well as aggravates any existing chronic hepatitis condition.
The HBV genotype variation is considered important in determining the clinical relevance in disease progression, infection outcome, response to antiviral treatment, and disease prognosis (Sunbul, 2014). For example, variations in clinical outcome as well as response to interferon have been found to be HBV genotype dependent with genotypes C and D HBV infected patients having more severe outcomes than genotype A and B infected patients (Balish et al., 2013). Likewise, hepatocellular carcinoma and liver cirrhosis are commonly diagnosed in patients infected with HBV genotype C and D more than those with genotype A and B infection (VanDersarl et al., 2011). Some studies have shown that, patients with HBV genotype A and B infection are likely to respond to interferon treatment than patient infected with genotypes C and D (Koo et al., 2012).

Hepatitis B surface antigen is usually secreted by infected hepatocytes in the form of subviral particles rather than infectious virons, hence serving as a means of evading immune response by the host (Nguyen et al., 2008; Op den Brouw et al., 2009). These subviral particles contains no HBV genome but are secreted in high amounts that exceed that of mature virons. Serum HBsAg is not only produced by the translation of messenger ribonucleic acid (mRNA) of covalently close circular deoxyribonucleic acid (cccDNA), but also synthesised by viral sequences that are integrated into the host genome. Hepatitis B e antigen (HBeAg) originates from the core gene and is modified and secreted by hepatocytes into the circulation and function as a marker of active viral replication. HBeAg loss in chronic HBV patients is associated with long-term improvements in clinical outcome and is defined by clinical practice guidelines as the main goal of antiviral treatment.

Similarly, the seroconversion of HBeAg concomitant with sustained HBV DNA reduction leads to the development of HBV inactive carrier status, which enables immunological control of the infection, enhancing long-term favourable outcomes with a minimal risk of developing cirrhosis and hepatocellular carcinoma (Liver, 2009). A decrease in HBV DNA is an excellent efficacy measure of anti-viral therapy. However, this decrease alone is transient, especially when the anti-viral agents are discontinued (Leung, 2002; Zhou and Littler, 2006). On the other hand, HBeAg seroconversion is another important surrogate marker of response to therapy and clinical outcome in patients with active chronic hepatitis B infection (Fattovich et al., 1986; Niederau et al., 1996).

HBV replication is strongly associated with the development of liver cirrhosis, HCC and related hepatic mortality, considering the fact that HBeAg is a marker of active viral replication in hepatocytes. Quantitative HBeAg has become an emerging useful tool for predicting viral load and virological response, as well as serological response to therapy. Cytokines, as a family, are composed of several subfamilies, such as the interleukins (ILs), tumour necrotic factors (TNFs), interferons (IFNs), chemokines, and interleukin 6-type cytokines (Richter and Solez, 2013). Increasing evidence supporting the major role of several inflammatory cytokines in liver disease progression and tissue repair are becoming available, including the current study. The cytokine families that are considered the key factors in various stages of liver diseases include: the pro-inflammatory molecule TNF-α, the anti-inflammatory...
cytokine IL-10 and the adipokine adiponectin; this also correlates with biomarkers of autoimmunity (Liu et al., 2015).

Severity of a disease is defined as the extent of organ or system damage or its physiological decompensation of the patient’s condition (Boundless, 2016). Therefore, in this study severity referred to chronic hepatitis B patient progressing to cirrhosis, acute flare and HCC as such severity in this study is classified based on non-cirrhotic, cirrhotic and acute flare, based on Asian-Pacific clinical practice guidelines on the management of hepatitis B: a 2015 update (Sarin et al., 2016) all of which referred to the extent damage to the hepatocytes and patients clinical conditions.

Problem statement

The prognosis of acute deterioration of chronic hepatitis B is extremely poor, with 3-month mortality rates without liver transplantation reported to be more than 50% (García-Martínez and Cordoba, 2011; Sun et al., 2009). Hepatitis B patients, who are co-infected with other viral hepatitis, are associated with more severe infection and higher mortality than those with HBV mono-infection as such, fulminant hepatitis is more likely in co-infection than mono-infection (Govindarajan et al., 1984). Chronic HBV-co-infection is associated with a higher risk of cirrhosis and liver decompensation and the reactivation of HBV infection plays an important role in the progression of acute flare in the Asian region (Sarin et al., 2009).

Similarly, hepatitis B is a non-cytopathic virus which means the virus itself is not capable of causing injury to the hepatocytes but rather the immune response that takes place in the liver against the virus is responsible for the inflammation of the hepatocytes, hence referred to as “hepatitis”. This inflammatory process triggered by the virus is mediated by human cytokines, thereby determining the severity or otherwise of the infection the failure of suppressing HBV replication is thought to be as a result of ineffective cytokine response (Song et al., 2003). Previous studies suggest some metabolic biomarkers such as low level lysophosphatidylcholines (LPCs) (Hao et al., 2011), arterial blood lactate (Bernal et al., 2002), and serum phosphate (Schmidt and Dalhoff 2002) as potential prognostic factors.

There has been much debate surrounding the efficacy of these models (Macquillan et al., 2005; Zaman et al., 2006; Dhiman et al., 2007; Yantorno et al., 2007; Bernal, 2010; Schmidt and Larsen 2010) and none of the studies consider co-infection, genetic diversity of the viruses, clinical presentation and inflammatory cytokines, leading to poor prediction, for instance, in the case of model for the end stage liver disease (MELD) which show an overlap between recovery and non-recovery group (Hoa et al., 2011).

It is, therefore, important to study the genetic diversity of these hepatitis viruses in mono or co-infection, which is responsible for triggering the inflammatory process as
well as the different cytokines (pro and anti-inflammatory) that play a role in the immunological response and severity of the infection. It is also of benefit to study the clinical parameters associated with the pathological process as well as the biochemical parameters associated with the organ involved. Therefore, this study will provide novel prognostic factors that might be responsible for the severity of the infection and hence, help to improve on the clinical evaluation and management of the patients as well as a potential immunomodulatory cytokine that can be used as an add-on therapy and in therapeutic vaccine.

**Research questions**

1. What is the proportion and predominance of hepatitis B co-infection with other hepatitis viruses among chronic hepatitis B patients?
2. What hepatitis virus genotypes are predominantly co-infected with which genotype of hepatitis B virus?
3. What are the inflammatory cytokine genes that are up or down regulated among non-cirrhotic, cirrhotic and acute flare in chronic hepatitis B infected patients?
4. What are the markers that can serve as prognostic factors in identifying chronic hepatitis B patients with potential of cirrhosis and acute deterioration?

**General Objective**

To determine the clinical, serological and biochemical and genetic diversity of hepatitis B and co-infection with other hepatitis viruses and propose a novel prognostic marker of cirrhosis and acute deterioration of chronic hepatitis B infection.

**Specific Objectives**

1. To determine the proportion of hepatitis B co-infection with other hepatitis viruses among chronic HBV patients.
2. To investigate the clinical (signs and symptoms), and laboratory parameters association with cirrhotic and non-cirrhotic and acute flare in patients with chronic hepatitis B infection.
3. To determine the genetic diversity of hepatitis B and its co-infected viruses as it relates to acute deterioration of chronic hepatitis B infection and liver cirrhosis.
4. To determine the correlation between HBV viral load and HBeAg and HBsAg concentrations.
5. To investigate the human cytokine and chemokines genes expression and circulating cytokines associated with non-cirrhotic, cirrhotic and acute flare hepatitis B infection.
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