The Analysis of Mutation Profile on Pre-S1 and Pre-S2 Region of Hepatitis B Virus in Chronic Liver Disease

by Gwenny Ichsan Prabowo

Submission date: 25-Jul-2019 01:10PM (UTC+0800)
Submission ID: 1154828416
File name: Pre-S2_Region_of_Hepatitis_B_Virus_in_Chronic_Liver_Disease.pdf (331.81K)
Word count: 6586
Character count: 33596

THE ANALYSIS OF MUTATION PROFILE ON PRE-S1 AND PRE-S2 REGION OF HEPATITIS B VIRUS IN CHRONIC LIVER DISEASE

Article *in* Malaysian Journal of Biochemistry and Molecular Biology · December 2018

CITATIONS
0

READS
38

7 authors, including:

Citrawati Dyah Kencono Wungu
Airlangga University
11 PUBLICATIONS **0** CITATIONS
SEE PROFILE

Mochamad Amin
Airlangga University
40 PUBLICATIONS **196** CITATIONS
SEE PROFILE

Ulfa Kholili
Airlangga University
21 PUBLICATIONS **0** CITATIONS
SEE PROFILE

Gwenny Lestari Prabowo
Airlangga University
14 PUBLICATIONS **0** CITATIONS
SEE PROFILE

MJBMB, 2018, 3, 85 - 92

MALAYSIAN JOURNAL OF BIOCHEMISTRY & MOLECULAR BIOLOGY

The Official Publication of The Malaysian Society For Biochemistry & Molecular Biology (MSBMB)
http://mjbmb.org

THE ANALYSIS OF MUTATION PROFILE ON PRE-S1 AND PRE-S2 REGION OF HEPATITIS B VIRUS IN CHRONIC LIVER DISEASE

Citrawati Dyah Kencono Wungu[1], Mochamad Amin[2], Ulfa Kholili[3], Gwenny Ichsan Prabowo[1], Poernomo Boedi Setiawan[3], Soetjipto[1,2], Retno Handajani[1,2]

[1]*Department of Medical Biochemistry, Faculty of Medicine, Universitas Airlangga, Surabaya*
[2]*Institute of Tropical Disease, Universitas Airlangga, Surabaya*
[3]*Departement of Internal Medicine, Faculty of Medicine, Universitas Airlangga, Dr. Soetomo Hospital, Surabaya*

*Corresponding author: cicit.biokimia@gmail.com

SHORT COMMUNICATIONS

History
Received: 12th September 2018
Accepted: 4th November 2018

Keywords:

Hepatitis B virus, Chronic Liver Disease, mutation, pre-S1, pre-S2

Abstract

Objective
The purpose of this study was to complete the data frequency and mutation profile of Hepatitis B Virus (HBV) pre-S1 and pre-S2 in Indonesia.

Methods
This cross-sectional study was used 32 blood serum samples of Chronic Liver Disease (CLD) patients with Hepatitis B surface antigen (HbsAg) at Endoscopy Outpatient Clinic, RSUD Dr. Soetomo Hospital, Surabaya. Polymerase Chain Reaction (PCR) of HBV DNA was performed on the samples based on pre-S1 and pre-S2 region. Then, electrophoresis was performed on the PCR product and followed by sequencing on samples with positive electrophoresis result. The sequencing results were analyzed by comparing them with the published sequences of HBV nucleotide.

Results
The amplification results of nested PCR DNA HBV with primers based on HBV pre-S1 and pre-S2 region were positive at 21 serums. In patients with CLD in this study, pre-S1 and / or pre-S2 HBV mutations were found in 11 (84.62%) chronically infected HBV patients, 4 (100%) patients with liver cirrhosis, and 4 (100%) HCC patients. Dominant mutations were L101V (16.57%), M120I / T or pre-S2 start codon (10.82%), and F141L (10.81%). M120 and F141L mutations have been previously reported to be associated with CLD, while the dominant L101V mutation in this study as well as several other mutations has not been reported in previous studies.

Conclusions
Mutations of pre-S1 and pre-S2 HBV regions were obtained in 90.48% of CLD patients in the form of substitution and deletion of amino acids.

INTRODUCTION

Hepatitis B virus infection (HBV) is a serious infectious disease that becomes a worldwide concern. According to World Health Organization (WHO) data, it is estimated that more than two billion people worldwide have been infected by HBV. HBV is transmitted through blood, genital secretions, or other body fluids from patients infected with HBV. HBV infections could be acute or chronic. It is estimated that about 780,000 people die annually of chronic liver disease (CLD) due to HBV infection, mainly by cirrhosis of the liver and Hepatocellular Carcinoma (HCC) (1). In CLD, chronic liver damage occurs due to inflammation, necrosis, fibrosis, and it may progress to carcinogenesis. Clinically called CLD includes chronic hepatitis B, liver cirrhosis, and HCC (2).

According to the WHO classification, Southeast Asia including Indonesia belongs to areas with high HBV endemicity, where in this region an estimated 100 million people live with chronic HBV infection and 70% -90% of HBV-infected populations occur before age 40 (3). The estimation of HBV infected patients in Indonesia are to be 18 million, 10 percent of whom will become liver fibrosis which could then develop into cirrhosis of the liver as well as HCC (4).

Although the pathogenesis of CLD is not fully understood, there are several factors suspected to affect the progression of HBV infection to CLD, one of which is a mutation in the pre-S HBV region (5). In previous studies, there has been a reported association between mutations in the pre-S1 and pre-S2 HBV regions with CLD occurrence (6–9). HBV pre-S1 and pre-S2 regions encode three HBV surface proteins: *Small Hepatitis* B *surface protein* (S-HBs), *Middle Hepatitis B surface protein* (M-HBs), and *Large Hepatitis B surface protein* (L-HBs); or S (*small)*, M (*middle*), dan L (*large*) proteins (10). Pre-S1 and pre-S2 regions are highly immunogenic regions containing many B and T lymphocyte epitopes (11). Besides containing B and T epitopes, pre-S1 and pre-S2 areas also have other functional sites that play important roles in the attachment, replication and progression of viral infections (5).

Mutations in the pre-S1 and pre-S2 regions could lead to the immune escape of the virus as both regions have many epitopes of both B and lymphocytes. Immune escape from virus causes persistence and chronicity of HBV infection. Mutations in the pre-S1 and pre-S2 regions could lead to an imbalance between three HBV surface proteins, Small (S), Middle (M), and Large (L) proteins. The portion of the L protein facing the cytoplasm serves to form the nucleocapsid, while the one facing the ER lumen plays a role in the attachment of HBV with hepatocytes (12). Of the three surface proteins, L protein is only expressed as much as 5-15%, M protein as much as 1-2%, while the rest is S protein (13). Excessive retention of L protein in the endoplasmic reticulum (ER) in the absence of S and M proteins that assist its secretion will cause ER stress and lead to oxidative stress that could trigger liver damage and fibrosis (14,15). ER stress could also activate hepatic carcinogenesis pathways (12).

Data on pre-S1 and pre-S2 HBV mutations in CLD patients in Indonesia is still very limited, thus this research was aimed to analyze pre-S1 and pre-S2 HBV mutation profile in CLD patients in Indonesia.

MATERIALS AND METHODS

Sampling

This study was a cross-sectional study that used a thirty-two (32) blood serum samples obtained from chronically infected HBV patients with a history of positive HBsAg for more than 6 months. Samples were taken at Endoscopy Outpatient Clinic, RSUD Dr. Soetomo Hospital, Surabaya in November, 2015. This study had received approval from the Ethics Committee of RSUD Dr. Soetomo, Surabaya. Informed consent was explained and voluntarily signed by participants before blood sampling. Blood was collected into Venoject tube without anticoagulant. The blood serum was separated, inserted into 3 Eppendorf tubes of 1.5 mL and stored at minus 80ºC at the Hepatitis Institute of Tropical Disease (ITD) until being used. Serum HBsAg and ALT were re-examined. Positive samples of HBsAg will be performed PCR on HBV DNA and positive PCR results will be sequenced for analysis of mutations in pre-S1 and pre-S2 regions.

Examination of HBsAg and ALT

ALT was checked with ALT Activity Kit with Ref No.981769 Lot.KB33 from Thermo, Finland by colorimatric enzyme activity assay method, while HBsAg was checked using HBsAg ELISA Kit with Ref No.1231-1021b010763-01 from Acon, USA by ELISA method.

Viral DNA Extraction and PCR

HBV DNA extraction was performed using QiaAMP DNA Blood Mini Kit with Cat.No.51104 from Qiagen, Germany. The amplification reaction was based on the pre-S1 and pre-S2 HBV regions. Nested PCR DNA HBV in pre-S1 and pre-S2 based gene samples was carried out using a PCR Invitrogen kit with Ref No.25027 from Intron. In the first round reaction PCR used PS-1 (5'-GGGTCACCATATTCTTGGGA-3') (2814-2839) and PS-2 (5'- CCCCGCCTGTAACACGAGCA-3') (208-189) primer pair, which, if a negative result obtained, was followed by second-round PCR HBV using PS-3 (5'-TTGGGAACAAGAGACACC-3') (2828-2847) and PS-4 (5'-CACGAGCAGGGGTCCTAGGA-3') (196-177) primer pair (16). PCR was done by using a PCR Thermal Cycler machine Applied Biosystem Verity 96 Well. The PCR procedure began with the initial denaturation at 94ºC for 2 min, followed by PCR cycle with the following steps of 36 cycles: denaturation at 94ºC for 60 seconds, annealing at 58ºC for 30 seconds and elongation at 72ºC for 60 seconds. After the 36th cycle a final elongation with temperature 72ºC for 7 minutes was performed.

This study also confirmed the presence of positive HBV DNA in samples with HBsAg positive, but still negative with PS-1, PS-2, PS-3, and PS-4 primers. In a sample with a negative HBV DNA, PCR was performed using surface and core regions. The primers used to detect HBV DNA surface region in this study were P7 (256-278): 5'-GTGGTGGACTTCTCTCAATTTTC-3 'and P8 (796-776): 5'-CGGTAWAAAGGGACTCAMGAT-3' for first round PCR and HBS1 (455 -474): 5'-CAAGGTATGTTGCCCGTTTG-3 'and HBS2 (713-694): 5'-AAAGCCCTGCGAACCACTGA-3' for the second round PCR (17). Primers 12F (3075-3094): 5'-GTGGAGCCCTCAGGCTCAGG-3 'and 12R (256-237): 5'-CGAGTCTAGACTCTGTGGTA-3' were also used for amplification of pre-S2 to surface area (18). The primers of core region used to detect the HBV DNA in this study were HBC1 (1650-1669) 5'-TTACATAAGAGGACTCTTGG-3 'and HBC2 (2494-2475) 5'-TAAAGCCCAGTAAAGTTTCC-3' for first round PCR and HBC3 (1744-1761) 5'-GGGAGGAGATTAGGTTAA-3 'and HBC4 (2476-2457) 5'-CCCACCTTATGAGTCCAAGG-3' for second round PCR (19).

Detection of PCR products

Electrophoresis was performed using 2% agarose gel run at 100 volts, and then viewed under ultraviolet light and documented using a digital camera. In samples with positive PCR HBV DNA results, purification, labelling, and pro sequencing PCR were performed.

Sequencing

Purification of PCR products was carried out with QiaQuick Gel Extraction Kit Qiagen Cat. #2870. PCR labelling was done with Sequencing Reaction Kit (ABI Prism Big Dye Terminator vl.l Ready Reaction Cycle Sequencing Kit, Applied Biosystems). DNA Sequencing was performed using ABI Prism 310 Genetic Analyzer (Perkin Elmer) after previously prepared with a given

Big Dye Terminator and SAM solution, heated, incubated in ice, and transferred into a sterile microtube.

Analysis of mutations in the pre-S1 and pre-S2 regions

Genotype and homology analysis with the nuclear BLAST nucleotide program (NCBI) was performed on the nucleotides of this study and the nucleotides used as a comparison. HBV nucleotide sequences from samples were compared with the sequence of HBV nucleotides obtained from international DNA data banks (DDBJ, GenBank, EMBL) according to the genotype result from BLAST. The sequence results were analyzed in the Clone Manager 9 computer software to determine the mutations in the pre-S1 and pre-S2 regions.

RESULTS AND DISCUSSION

A total of 32 serum samples of CLD patients from Endoscopy Outpatient Clinic, Dr. Soetomo Hospital, Surabaya had been collected in this study. All the patients showed a history of positive HBsAg examination in the past for more than six months and until the time of blood sampling for this study, HBsAg was still positive.

This study showed that CLD patients in Endoscopy Outpatient Clinic, Dr. Soetomo Hospital, Surabaya has the youngest age 14 years old and the oldest age 65 years old, with an average age of 46.06 years. Male CLD patients 71.88% (23/32 people) were more than women 28.12% (9/32 people). The mean age of CLD patients in male patients was 46.43 years with the age range of 21 to 65 years, whereas the mean age in women was 45.11 years with the age range of 14 to 65 years. This supports data from previous studies suggesting that HBV-infected men are more likely to be CLD than women (20–23).

In this study, the majority of CLD patients were found to be chronic HBV infection (65.62%), followed by liver cirrhosis (21.88%) and HCC (12.5%). In each CLD group, the male patients outnumbered the female patients. This was in accordance with WHO (2015) data suggesting the incidence of liver cirrhosis and/or HCC in patients with chronic HBV infection by 20-30%. HCC patients were found in small numbers because HCC patients have a low survival rate, which was about 5 months if without getting adequate therapy (24).

Mean age of chronic HBV infected patients was 40.52 years with age range 14 to 65 years, mean age in patients with liver cirrhosis was 55 years with age range 40 to 65 years, mean age in HCC patients was 59,5 years old with age range 49 to 65 years. The results of this study indicated that chronic HBV infected patients had younger mean and range of age than patients with cirrhosis of the liver and HCC. The age difference of chronic HBV infected patients, cirrhosis of the liver, and HCC was due to the progression of hepatitis B disease which takes time to become liver cirrhosis and HCC. Weledji et al (2014) suggests that it generally takes about ten years for HBV infection to develop into chronic, 20 years to become liver cirrhosis, more than 20 years to become HCC, and also it was suggested that patients with cirrhosis of the liver and HCC were commonly found in the range age 50 to 70 years (25).

There were 15.625% of CLD patients who have an ALT increment exceeding 2 times the normal value limit, 9.375% of CLD patients who have an ALT increase of 1 to 2 times the normal value, and as many as 75% of CLD patients have normal ALT. In chronically infected HBV patients, only 5 of 21 individuals had ALT elevations. In patients with cirrhosis of the liver, none had increased ALT. In HCC patients, 3 out of 5 people had ALT elevation. Patients with normal ALT might be in the immune tolerance or immune control phase of the course of HBV infection (26) or in the immune clearance and the immune escape phase where ALT is fluctuating and not always elevated (27).

In the electrophoresis of PCR results of this study, of 32 serum specimens, HBV DNA in PCR product was detected in 21 (65,63%) serum specimens. Nineteen serum specimens gave positive results with PS-1 and PS-2 primers (a 595 bp HBV DNA band), 2 specimens gave positive results with PS-3 and PS-4 primers (569 bp DNA band HBV) whereas 11 specimens gave negative results with both primer pairs, although it had been repeated several times either by using the same primary pair or by pairing PS-1 with PS-4 and PS-3 primers with PS-2. Some electrophoresis results showed a positive result, but dubious HBV DNA band, as it did not match the expected bond length. The sequencing results from some of these doubtful samples proved to be incompatible with the expected sequence. This could be due to a HBV DNA titer that was too low because it was possible that the patient has taken anti-viral drugs that suppressed previous HBV DNA replication. The use of anti-viral drugs as a treatment for HBV infection, either interferon injection or the use of oral nucleoside analogs could suppress HBV DNA replication so that undetectable DNA HBV titer could be considered a marker of treatment efficacy even if HBsAg remains positive (28).

In this study, from samples with negative HBV DNA using PS-1 and PS-2 primers and PS-3 and PS-4, 3 samples with positive HBV DNA were obtained using other primer pairs. One sample was positive with P7-P8 primer pair, another one sample was positive with HBS1-HBS2 primer pair, and one sample was positive by using 12F-12R primer pair. A total of eight samples still gave negative results with the use of 3 sets of primer pairs above.

In samples that gave positive DNA HBV amplification results, sequencing was performed. There was one sample that had not been successfully sequenced with good results although the result of HBV DNA through positive electrophoresis showed the expected band and several repetitions of sequencing had been performed. Mutations were determined by comparing the nucleotide sequences of the pre-S1 and pre-S2 genes obtained in this study with the nucleotides obtained by other researchers and published. Genotype and homology analysis with the nuclear BLAST nucleotide program (NCBI) was performed on the nucleotides of this study and the nucleotides used as a comparison.

Based on the analysis with the BLAST program, all samples had a high homology (99%) with the B3 genotype so that 3 sequences of nucleotide HBV with B3 genotype which had the highest homology with samples to be processed with sample sequence, ie sequence with accession number EU92618 (Indonesia, homology 99 %), GQ924675 (Malaysia, homology 99%), AB219430 (Philippines, 98% homology). In a genotype analysis to determine the standard sequences used by the BLASTN program, it was found that all samples in this study had high homology with HBV genotype B3. Based on the results of other previous researchers, mutations in the form of pre-S1 and pre-S2 deletions were more common in other HBV genotypes (A, C, and D) compared with HBV genotype B (29).

Based on the results of multiple alignments with Clone Manager 9 program (**Figure 1**), from 20 sequenced samples, mutations in pre-S1 and / or pre-S2 regions were obtained in 18

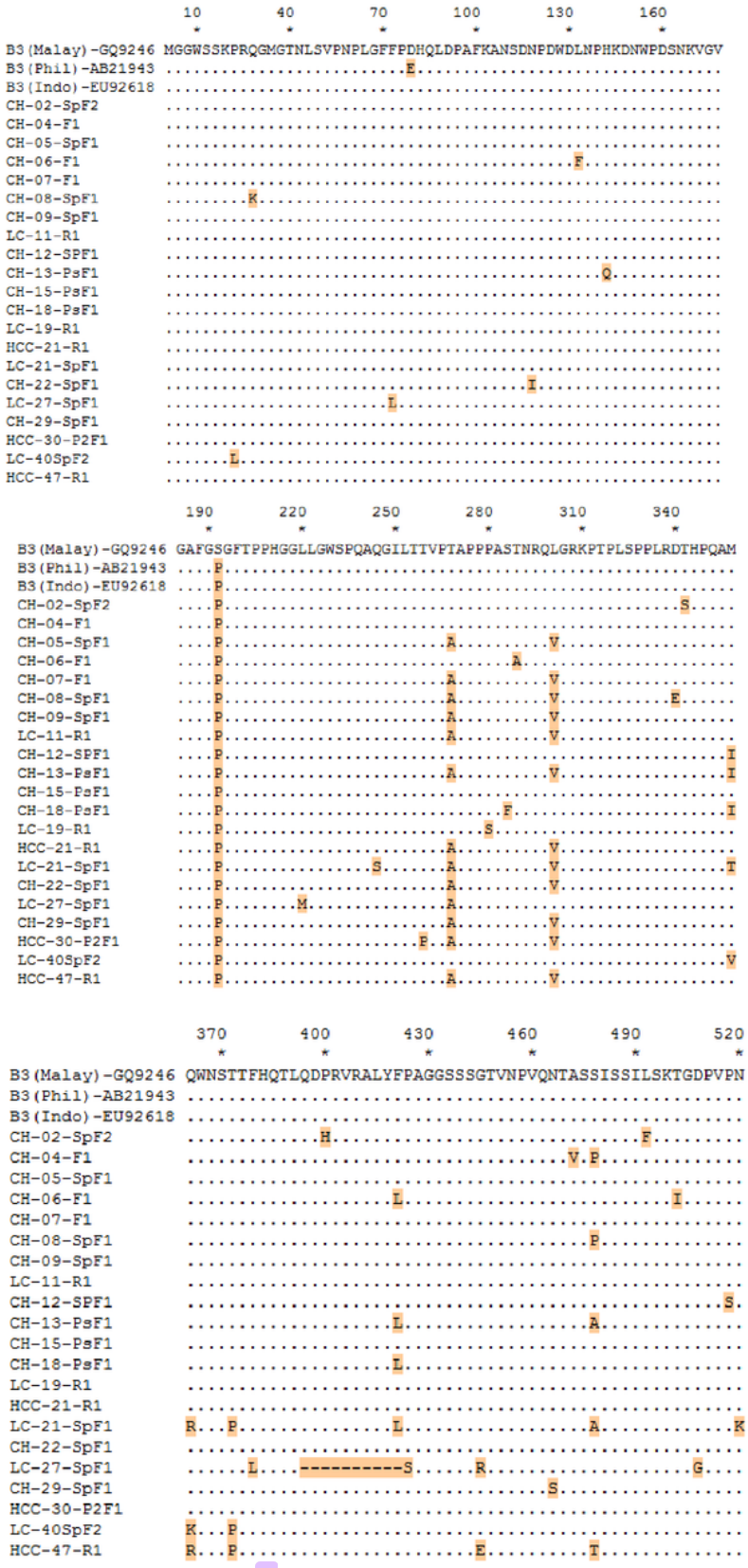

Figure 1. Multiple alignment of HBV pre-S1 and pre-S2 regions using Clone Manager 9 software

samples (90%), primarily in the form of one or two nucleotide substitutions which resulted in amino acid changes. Most of the samples (55.55%) had mutation only in the pre-S1 area, followed by mutations in pre-S1 and pre-S2 (33.33%), and few had mutations in pre-S2 regions only (11. 11%). One sample had a mutation of 30 nucleotides (10 amino acids) deletions in the middle of the pre-S2 region. From samples with HBV DNA of positive PCR products and from sequencing results, pre-S1 and / or pre-S2 HBV region mutations were obtained in 84.62% of chronic hepatitis B patients, 100% of liver cirrhosis patients, and 100% of HCC patients. CLD patients with the highest number of mutations were two patients with liver cirrhosis who each had 6 different mutations. HCC patients had 1 to 2 types of mutations in which all three HCC samples in this study all had L101V mutations. In contrast to previous studies (5,16,30), HCC patients in this study had no mutations in the form of deletions, however a mutation of L101V was obtained.

Mapping of mutations in this study showed that all successful sequencing samples had mutations in the epitope of B lymphocytes. Many mutations occurred in epitopes of T lymphocytes and pre-S1 functional areas and / or pre-S2 HBV. Only one mutation (Q82S) occurred in the pre-S functional area without concerning the epitope areas of B and or T lymphocytes. In this study, the affected pre-S functional areas include the Hsc70, CAD, nucleocapsid binding regions, the binding region of the hepatocytes, transactivator domain, S-promoter, pre-S2 start codon, as well as pHSA binding region. This is consistent with previous studies showing that pre-S1 and HBV pre-S2 mutations generally include epitopes against B and T lymphocytes and then trigger mutations in pre-S functional areas (14,31).

The dominant type of mutation in this study was the substitution of Leusin amino acid at position 101 to Valin (L101V) found in 13 of 21 samples of CLD patients (61.9%), followed by substitution of pre-S2 start codons (amino acid 120 position) to threonin or isoleucine (M120I / T) found in 5 of 21 CLD patients (23.8%), and substitution of Amino acid Fenilalanin at 141 to leucine (F141L) found in 4 of 21 CLD patients (19.04 %).

Previous studies have shown that mutations in the pre-S1 and pre-S2 HBV regions are found in many CLD patients (30,32,33). The frequency of pre-S1 and pre-S2 HBV mutations in CLD patients is high in hepatitis B endemic countries (34). Other results suggest that the HBV mutation in the form of amino acid substitution at various nucleotide sites is found up to 75.7% in CLD patients (5). Based on the mapping done by Zhang et al (2015), mutations in the pre-S1 and pre-S2 HBV regions may interfere with the recognition of immune epitopes and cause functional impairment of HBV surface protein when they occur in epitopes against B cells (aa 12-53 , aa 72-78, aa 94-117, aa 120-143, and aa 157-167), epitopes against T cells (aa 21-30, aa 52-67, aa 120-145, and aa 163-172), pre-S1 start codons (aa 1), S promoter (nt 3045-3180), CCAAT box binding factor binding site (nt 3137-3141), topological domains covering Hsc70 (aa 74-118) and CAD (aa 81-105) binding sites, nucleocapsid binding sites (aa 103-127), pre-S2 start codons (aa 129), and pHSA (aa 122-135).

Mutations in the pre-S region are found to be deletions, although there are a few researches that suggest mutations in the form of amino acid substitutions at different sites. In a study conducted by Zhang et al (2015) on CLD patients, there were many point mutations in the pre-S1 and / or pre-S2 regions in the form of amino acid substitutions in some codons, ie W4, D27G, N51Q, D54A, V60, A62S , Q100, T125S, R137, S166, and K167R (5). Mutation at codon 125 in the study was also obtained in this study. A study conducted by Chien et al (2008) mentions a mutation of amino acid substitution at codons 4, 7, and 81 of the pre-S1 region and the pre-S2 start codon in HCC patients (35). In another study conducted by Yin et al (2010) mentioned the substitution of codons 4 and 60 of pre-S1 in CLD patients (36).

In this study, the dominant type of mutation was the substitution of Leusin amino acid at position 101 to Valin (L101V), followed by substitution of pre-S2 start codon (M120I / T), and substitution of amino acid F141L. This type of L101V mutation has not been reported in previous studies. In this study, 100% (3 of 3) patients with HCC had L101V mutations.

In this study, there was only one mutation in the form of pre-S2 deletion of 30 nucleotides in the middle of the pre-S2 region in patients with cirrhosis of the liver. This is consistent with Chen et al's (2006) study showing that in HBV with genotype B, fewer deletion mutations were obtained than genotype C (6). The study conducted by Utama et al (2011) also reported the frequency of pre-S1 deletions and/or pre-S2 was low in CLD patients in Indonesia (30). Based on research conducted by Kim (2014), deletions in the pre-S2 region of HBV are known to play a role in the transition of chronic HBV infection to liver cirrhosis (37). Another study by Su et al (2014) reported that on the dissection of hepatic liver cirrhosis, there was a deletion in the dominant pre-S2 region in the nucleotide region of the 2-57 pre-S2 regions, with or without the M120 mutation. Deletions in the nucleotide region 2-57 pre-S2 region cause decreased synthesis of S and M proteins as well as an increase in L protein causing the formation of Ground Glass Hepatocyte (GGH) in hepatocytes and causing liver fibrosis (38).

In a study conducted by Mun et al (2011), a mutation of F141L was associated with CLD risk and was significantly associated with M120 mutations in the pathogenesis of CLD. F141L and M120 double mutations can enhance the pathogenic ability of HBV and help HBV maintain its viral life cycle. The combination of F141L and M120 has a higher cell proliferation capacity than a single mutation. It is said that F141L mutations did not occur simultaneously with pre-S2 deletions (9). This was in line with the results of this study wherein a sample of liver cirrhosis having a pre-S2 deletion no F141L mutation was found.

The M120 mutation located in the pre-S2 start codon was a frequent mutation in previous studies (12,29,34). Mutations in the pre-S2 start codon may interfere with M protein synthesis, thus impairing the balance of the S, M, and L protein ratios. The decreased M protein leads to increased and retention of the L protein until ER stress arises. This leads to mutations in the pre-S2 start codons reported to be associated with CLD, especially in patients with cirrhosis of the liver (29,39).

Other mutations found in this study were amino acid substitutions in codons 25, 40, 74, 82, 94, 96, 114, 115, 121, 125, 127, 134, 142, and 149. Based on a study conducted by Lin et al (2013), amino acid substitution occurring in the pre-S region might cause changes in the immune target region when it concerns the epitope of lymphocytes B and or T resulting in immune-escape conditions, disturbance of introduction by class II MHC-dependent helper T cells or reduce the affinity of the class I MHC binding on the hepatocyte surface (40). Grottola et al (2002) showed that the number of pre-S2 mutations was associated with recurrence of HBV infection after liver transplantation independent of immunoglobulin prophylaxis (41). Variations of mutations in the pre-S1 and pre-S2 regions might alter the overall conformation of the HBV surface protein, altering the antigenic

properties of HBV against anti-HBs and causing the progression and chronicity of HBV infection (40). Other than that, studies in molecular field show that pre-S mutant interacts with many signalling proteins in cell cycle, cell survival, apoptosis resistance, genetic instability, and cell proliferation (42).

In HBV infection, in the event of mutations that may cause epitope functional disruption of B cells and T lymphocytes, immune escape from the virus might result in HBV replication and further liver damage. Mutations in the epitope region against B and T lymphocytes might also cause further mutations in other regions of pre-S1 and pre-S2 (14,35).

Mutations in Hsc70 or CAD causes L proteins unable to maintain their topological structure, as both Hsc70 and CAD are important determinants for maintaining the double topological structure of protein L (43). This will lead to the retention of L proteins and subvirus particles in the ER due to non-secretion (44).

Disorders in S promoter could suppress S protein production and cause a disruption of the balance ratio between protein S and protein L. Normally, L protein requires S protein to be secreted out of ER. When S protein production decreases, there could be retention of L protein in ER that can cause stress of ER (45,10). It was reported in a previous study by Zhang et al (2015) that point mutations in the S promoter region might cause a reduction in HBV surface protein that leads to intracellular retention of viral envelope proteins and causes ER stress (5).

Nucleocapsid binding regions were important for viral maturation so that virus particles and components could be secreted out of the ER without causing ER stress (16). Mutations in pHSA could cause M-protein synthesis disturbances thereby altering the balance of S, M, and L protein ratios to also cause ER stress (31).

Studies on the incidence of pre-S1 and pre-S2 mutations of HBV in CLD patients in Indonesia were previously performed by Utama et al in 2011. In the study, it was reported that mutations in the form of deletions and insertions in pre-S1 and pre-S2 HBV were only found in 12.1% of CLD patients. The incidence of pre-S1 and pre-S2 HBV mutations in the study was said to be very low when being compared with other Asian countries (30). It was consistent with this study, in which a pre-S2 deletion mutation was found in only 5% of CLD patients. In a study also conducted by Utama et al in 2012, it was reported that the frequency of pre-S2 start codon mutation (M120) was quite high (22%), especially in patients with liver cirrhosis and HCC. In this study, M120I / T mutations were quite frequent (20%) but not found in HCC. Other mutations reported in Utama et al (2012) were T130 (26.1%), Q132 (28.7%), and A138 (29.5%) (46).

There were several factors that affect the progression of HBV to CLD, including host factors, viral factors, and other factors. Host factors include age, sex, and immune status. Viral factors include HBV genotype, viral load, pre-S mutation, and T1762 / A1764 mutations. Other factors include co-infection, aflatoxin, cigarette smoke, and alcohol. Virus factors could mutually influence each other so that there could be different mutation patterns between genotypes or double mutation which have the different influence to the incidence of CLD (47).

So far, studies on pre-S1 and pre-S2 mutations of HBV in CLD patients in Indonesia were still limited and no research had been found in other regions in Indonesia, so there was still possibility of genotypic distribution in Indonesia causing various frequency and types of different pre-S1 and pre-S2 HBV mutations in different regions.

ACKNOWLEDGEMENT

Our thanks go to Universitas Airlangga as a research funder through Medical Faculty of Universitas Airlangga. Also, we thank CLD patients who have contributed to give blood in this study. Thank you also to the staffs at Internal Medicine Department in Dr.Soetomo Hospital Surabaya and Institute of Tropical Disease (ITD) which has helped the course of research, and various parties involved in this research that we could do not mention one by one. The authors state that there is no conflict of interest in this study.

REFERENCES

1. WHO. Hepatitis B Fact Sheet [Internet]. 2016 [cited 2016 Dec 27]. Available from: http://www.who.int/mediacentre/factsheets/fs204/en/

2. Kotsafti A, Farinati F, Cardin R, Cillo U, Nitti D, Bortolami M. Autophagy and apoptosis-related genes in chronic liver disease and hepatocellular carcinoma. BMC Gastroenterol [Internet]. 2012;12(1):118. Available from: http://bmcgastroenterol.biomedcentral.com/articles/10.1186/1471-230X-12-118

3. Hollinger F, Liang T. Hepatitis B virus. In: Knipe DN, Howley PM, editors. Fields Virology. 4th editio. Philadelphia: Lippincott Williams & Wilkins; 2001. p. 2071–3036.

4. Departemen Kesehatan Republik Indonesia. Sebagian Besar Kematian Akibat Hepatitis Virus Berhubungan dengan Hepatitis B dan C Kronis [Internet]. 2016. Available from: http://www.depkes.go.id/article/view/16042700001/sebagian-besar-kematian-akibat-hepatitis-virus-berhubungan-dengan-hepatitis-b-dan-c-kronis.html

5. Zhang X. Key role of hepatitis B virus mutation in chronic hepatitis B development to hepatocellular carcinoma. World J Hepatol. 2015;7(9):1282.

6. Chen BF, Liu CJ, Jow GM, Chen PJ, Kao JH, Chen DS. High Prevalence and Mapping of Pre-S Deletion in Hepatitis B Virus Carriers With Progressive Liver Diseases. Gastroenterology. 2006;130(4):1153–68.

7. Gao ZY, Li T, Wang J, Du JM, Li YJ, Li J, et al. Mutations in preS genes of genotype C hepatitis B virus in patients with chronic hepatitis B and hepatocellular carcinoma. J Gastroenterol. 2007;42(9):761–8.

8. Lee SA, Kim KJ, Kim DW, Kim BJ. Male-specific W4P/R mutation in the pre-s1 region of hepatitis b virus, increasing the risk of progression of liver diseases in chronic patients. J Clin Microbiol. 2013;51(12):3928–36.

9. Mun H-S, Lee S-A, Kim H, Hwang E-S, Kook Y-H, Kim B-J. Novel F141L Pre-S2 Mutation in Hepatitis B Virus Increases the Risk of Hepatocellular Carcinoma in Patients with Chronic Genotype C Infections. J Virol [Internet]. 2011;85(1):123–32. Available from: http://jvi.asm.org/cgi/doi/10.1128/JVI.01524-10

10. Lin C-M, Wang G-M, Jow G-M, Chen B-F. Functional analysis of hepatitis B virus pre-s deletion variants associated with hepatocellular carcinoma. J Biomed Sci [Internet]. 2012;19(1):17. Available from: http://www.jbiomedsci.com/content/19/1/17

11. Shouval D, Roggendorf H, Roggendorf M. Enhanced immune response to hepatitis B vaccination through immunization with a Pre-S1/Pre-S2/S Vaccine. Med Microbiol Immunol. 2015;204(1):57–68.

12. Pollicino T, Cacciola I, Saffioti F, Raimondo G. Hepatitis B virus PreS/S gene variants: Pathobiology and clinical implications. J Hepatol [Internet]. 2014;61(2):408–17. Available from: http://dx.doi.org/10.1016/j.jhep.2014.04.041

13. Yokosuka O, Arai M. Molecular biology of hepatitis B virus: Effect of nucleotide substitutions on the clinical features of chronic hepatitis B. Med Mol Morphol. 2006;39(3):113–20.

14. Chen. Clinical Significance of the Hepatitis B Virus Pre-S Deletion. FJJM. 2010;85–95.

15. Churin Y, Roderfeld M, Roeb E. Hepatitis B virus large surface protein: function and fame. Hepatobiliary Surg Nutr. 2015;4(1):1–10.

16. Huang H-P, Hsu H-Y, Chen C-L, Ni Y-H, Wang H-Y, Tsuei D-J, et al. Pre-S2 deletions of hepatitis B virus and hepatocellular carcinoma in children. Pediatr Res [Internet]. 2010;67(1):90–4. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19816238

17. Lindh M, Andersson a S, Gusdal a. Genotypes, nt 1858 variants, and geographic origin of hepatitis B virus--large-scale analysis using a new genotyping method. J Infect Dis. 1997;175(6):1285–93.

18. Sugauchi F, Mizokami M, Orito E, Ohno T, Kato H, Suzuki S, et al. A novel variant genotype C of hepatitis B virus identified in isolates from Australian Aborigines: Complete genome sequence and phylogenetic relatedness. J Gen Virol. 2001;82(4):883–92.

19. Lusida MI, Nugrahaputra VE, Soetjipto, Handajani R, Nagano-Fujii M, Sasayama M, et al. Novel subgenotypes of hepatitis B virus genotypes C and D in Papua, Indonesia. J Clin Microbiol. 2008;46(7):2160–6.

20. Baig S. Gender disparity in infections of hepatitis B virus. J Coll Physicians Surg Pakistan. 2009;19(9):598–600.

21. Durazzo M, Belci P, Collo A, Prandi V, Pistone E, Martorana M, et al. Gender specific medicine in liver diseases: A point of view. Vol. 20, World Journal of Gastroenterology. 2014. p. 2127–35.

22. Montella M, D'Arena G, Crispo A, Capunzo M, Nocerino F, Grimaldi M, et al. Role of Sex Hormones in the Development and Progression of Hepatitis B Virus-Associated Hepatocellular Carcinoma. Vol. 2015, International Journal of Endocrinology. 2015.

23. Wang SH, Chen PJ, Yeh SH. Gender disparity in chronic hepatitis B: Mechanisms of sex hormones. J Gastroenterol Hepatol. 2015;30(8):1237–45.

24. Nguyen V, Law M, Dore G. Hepatitis B - related Hepatocellular Carcinoma : Epidemiological Characteristics and Disease Burden. J Viral Hepat. 2016;16(7):453–63.

25. Weledji EP, Enow Orock G, Ngowe MN, Nsagha DS. How grim is hepatocellular carcinoma? Ann Med Surg [Internet]. 2014;3(3):71–6. Available from: http://dx.doi.org/10.1016/j.amsu.2014.06.006

26. Pungpapong S, Kim WR, Poterucha JJ. Natural history of hepatitis B virus infection: an update for clinicians. Mayo Clin Proc. 2007;82(August):967–75.

27. Sarin SK, Kumar M. Should chronic HBV infected patients with normal ALT treated: Debate. Vol. 2, Hepatology International. 2008. p. 179–84.

28. Perhimpunan Peneliti Hati Indonesia. Konsensus Nasional Pelaksanaan Hepatitis B. In 2012. p. 5–25.

29. Biswas A, Panigrahi R, Banerjee A, Pal M, De BK, Chakrabarti S, et al. Differential pattern of pre-S mutations/deletions and its association with hepatitis B virus genotypes in Eastern India. Infect Genet Evol [Internet]. 2012;12(2):384–91. Available from: http://dx.doi.org/10.1016/j.meegid.2012.01.007

30. Utama A, Siburian MD, Fanany I, Intan MDB, Dhenni R, Kurniasih TS, et al. Low Prevalence of Hepatitis B Virus Pre-S Deletion Mutation in Indonesia. J Med Virol. 2011;83:1717–1726.

31. Chen C-J, Yang H-I, Su J, Jen C-L, You S-L, Lu S-N, et al. Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. JAMA [Internet]. 2006;295(1):65–73. Available from: http://jama.jamanetwork.com/article.aspx?doi=10.1001/jama.295.1.65%5Cnhttp://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=16391218&retmode=ref&cmd=prlinks%5Cnpapers3://publication/doi/10.1001/jama.295.1.65

32. Fan YF, Lu CC, Chen WC, Yao WJ, Wang HC, Chang TT, et al. Prevalence and significance of hepatitis B virus (HBV) pre-S mutants in serum and liver at different replicative stages of chronic HBV infection. Hepatology. 2001;33(1):277–86.

33. Hsieh Y-H, Su I-J, Wang H-C, Chang W-W, Lei H-Y, Lai M-D, et al. Pre-S mutant surface antigens in chronic hepatitis B virus infection induce oxidative stress and DNA damage. Carcinogenesis [Internet]. 2004;25(10):2023–32. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15180947

34. Huy TT, Ushijima H, Win KM, Luengrojanakul P, Shrestha PK, Zhong Z, et al. High Prevalence of Hepatitis B Virus Pre-S Mutant in Countries Where It Is Endemic and Its Relationship with Genotype and Chronicity High Prevalence of Hepatitis B Virus Pre-S Mutant in Countries Where It Is Endemic and Its Relationship with Genotype and . 2003;41(12):5449–55.

35. 35. Chen C-H, Changchien C-S, Lee C-M, Hung C-H, Hu T-H, Wang J-H, et al. Combined mutations in pre-s/surface and core promoter/precore regions of hepatitis B virus increase the risk of hepatocellular carcinoma: a case-control study. J Infect Dis. 2008;198(11):1634–42.

36. Yin J, Xie J, Zhang H, Shen Q, Han L, Lu W, et al. Significant association of different preS mutations with hepatitis B-related cirrhosis or hepatocellular carcinoma. J Gastroenterol. 2010;45(10):1063–71.

37. Kim B-J. Hepatitis B virus mutations related to liver disease progression of Korean patients. World J Gastroenterol [Internet]. 2014;20(2):460–7. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3923020&tool=pmcentrez&rendertype=abstract

38. Su I-J, Wang LH-C, Hsieh W-C, Wu H-C, Teng C-F, Tsai H-W, et al. The emerging role of hepatitis B virus Pre-S2 deletion mutant proteins in HBV tumorigenesis. J Biomed Sci [Internet]. 2014;21(1):98. Available from:

http://jbiomedsci.biomedcentral.com/articles/10.1186/s12929-014-0098-7

39. 39. Choi MS, Kim DY, Lee DH, Lee JH, Koh KC, Paik SW, et al. Clinical significance of pre-S mutations in patients with genotype C hepatitis B virus infection. J Viral Hepat. 2007;14(3):161–8.

40. Lin YM, Jow GM, Mu SC, Chen BF. Naturally occurring hepatitis b virus b-cell and t-cell epitope mutants in hepatitis b vaccinated children. Sci World J. 2013;2013.

41. Grottola A, Buttafoco P, Del Buono MG, Cremonini C, Colantoni A, Gelmini R, et al. Pretransplantation pre-S2 and S protein heterogeneity predisposes to hepatitis B virus recurrence after liver transplantation. Liver Transplant. 2002;8(5):443–8.

42. Levrero M, Zucman-Rossi J. Mechanisms of HBV-induced hepatocellular carcinoma. Vol. 64, Journal of Hepatology. 2016. p. S84–101.

43. Lambert C, Mann S, Prange R. Assessment of determinants affecting the dual topology of hepadnaviral large envelope proteins. J Gen Virol. 2004;85(5):1221–5.

44. Gerner P, Schäfer HMC, Prange R, Pravitt D, Wirth S. Functional analysis of a rare HBV deletion mutant in chronically infected children. Pediatr Res. 2003;53(6):891–7.

45. Goodarzi Z, Jazayeri SM. Hepatitis B virus preS variant and hepatocellular carcinoma. Vol. 8, Hepatitis Monthly. 2008. p. 129–33.

46. Utama A, Siburian MD, Fanany I, Intan MDB, Dhenni R, Kurniasih TS, et al. Hepatitis B virus pre-S2 start codon mutations in Indonesian liver disease patients. World J Gastroenterol. 2012;18(38):5418–26.

47. Liaw YF, Sollano JD. Factors influencing liver disease progression in chronic hepatitis B. Liver Int. 2006;26(SUPPL. 2):23–9.

The Analysis of Mutation Profile on Pre-S1 and Pre-S2 Region of Hepatitis B Virus in Chronic Liver Disease

ORIGINALITY REPORT

24%	15%	21%	0%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	www.wjgnet.com Internet Source	2%
2	hepatmon.com Internet Source	1%
3	Se Eun Kim, Yeong Hong Park, Woo Yeong Chung. "Study on hepatitis B virus pre-S/S gene mutations of renal tissues in children with hepatitis B virus-associated membranous nephropathy", Pediatric Nephrology, 2006 Publication	1%
4	Viral Hepatitis and Liver Disease, 1994. Publication	1%
5	doaj.org Internet Source	1%
6	T. T.-T. Huy. "High Prevalence of Hepatitis B Virus Pre-S Mutant in Countries Where It Is Endemic and Its Relationship with Genotype and Chronicity", Journal of Clinical Microbiology,	1%

12/01/2003
Publication

7	Virine, Boris, Carla Osiowy, Shan Gao, Tong Wang, Eliana Castillo, Steven R. Martin, Samuel S. Lee, Kimberley Simmonds, Guido van Marle, and Carla S. Coffin. "Hepatitis B Virus (HBV) Variants in Untreated and Tenofovir Treated Chronic Hepatitis B (CHB) Patients during Pregnancy and Post-Partum Follow-Up", PLoS ONE, 2015. Publication	1%
8	KaiYu Zhang. "Analysis of the complete hepatitis B virus genome in patients with genotype C chronic hepatitis in relation to HBeAg and anti-HBe", Journal of Medical Virology, 06/2007 Publication	<1%
9	www.scribd.com Internet Source	<1%
10	journals.plos.org Internet Source	<1%
11	Yuan Yanag, Dan Du, Li Jin, Zhen Tian et al. "A molecular epidemiology study investigating familial clustering of hepatitis B virus infection in families with unfavorable prognoses in Northwest China", Journal of Medical Virology, 2017	<1%

	Publication	
12	expert.taylors.edu.my Internet Source	<1%
13	hal.archives-ouvertes.fr Internet Source	<1%
14	Andi Utama. "Low prevalence of hepatitis B virus pre-s deletion mutation in Indonesia", Journal of Medical Virology, 10/2011 Publication	<1%
15	www.ncbi.nlm.nih.gov Internet Source	<1%
16	Huang, Xiangyan, Yanghua Qin, Wei Li, Qingfen Shi, Yan Xue, Jie Li, Chao Liu, F. Blaine Hollinger, and Qian Shen. "Molecular analysis of the hepatitis B virus presurface and surface gene in patients from eastern China with occult hepatitis B", Journal of Medical Virology, 2013. Publication	<1%
17	HSIANG-PO HUANG. "Pre-S2 Deletions of Hepatitis B Virus and Hepatocellular Carcinoma in Children :", Pediatric Research, 01/2010 Publication	<1%
18	Qu, Li-Shuai, Jin-Xia Liu, Tao-Tao Liu, Xi-Zhong Shen, Tao-Yang Chen, Zheng-Pin Ni, and Cui-Hua Lu. "Association of Hepatitis B Virus Pre-S Deletions with the Development of	<1%

Hepatocellular Carcinoma in Qidong, China", PLoS ONE, 2014.
Publication

19	etd.uovs.ac.za Internet Source	<1%
20	Yu-Min Lin, Guey-Mei Jow, Shu-Chi Mu, Bing-Fang Chen. "Naturally Occurring Hepatitis B Virus B-Cell and T-Cell Epitope Mutants in Hepatitis B Vaccinated Children", The Scientific World Journal, 2013 Publication	<1%
21	pure.uva.nl Internet Source	<1%
22	www.spandidos-publications.com Internet Source	<1%
23	repository.bilkent.edu.tr Internet Source	<1%
24	"Abstract of Papers Submitted to the American Association of the Study of Liver Disease", Hepatology, 10/1995 Publication	<1%
25	www.science.gov Internet Source	<1%
26	academic.oup.com Internet Source	<1%

27	link.springer.com Internet Source	<1%
28	"Abstracts 1 to 257", Hepatology, 10/2005 Publication	<1%
29	Utama, Andi. "Hepatitis B virus pre-S2 start codon mutations in Indonesian liver disease patients", World Journal of Gastroenterology, 2012. Publication	<1%
30	www.thaiscience.info Internet Source	<1%
31	pdfs.semanticscholar.org Internet Source	<1%
32	Chenggang Shi, Jiyi Huang, Xun Liu, Xiaolin Zeng, Cailian Cheng, Qiongli Yin, Mei Li, Guifeng Cheng. "Diagnostic significance of hepatitis B viral antigens in patients with glomerulonephritis-associated hepatitis B virus infection", Diagnostic Microbiology and Infectious Disease, 2012 Publication	<1%
33	eprints.soton.ac.uk Internet Source	<1%
34	www.zjzfg.com Internet Source	<1%

35	You, San-Lin, Hwai-I Yang, and Chien-Jen Chen. "Seropositivity of hepatitis B e antigen and hepatocellular carcinoma", Annals of Medicine, 2004. Publication	<1%
36	Pok Yeung, Danny Ka-Ho Wong, Ching-Lung Lai, James Fung, Wai-Kay Seto, Man-Fung Yuen. "Association of Hepatitis B Virus Pre-S Deletions with the Development of Hepatocellular Carcinoma in Chronic Hepatitis B", The Journal of Infectious Diseases, 2011 Publication	<1%
37	Svetlana A. Konnova, Oleg E. Makarov, Igor M. Skvortsov, Vladimir V. Ignatov. " Isolation, fractionation and some properties of polysaccharides produced in a bound form by and their possible involvement in -wheat root interactions ", FEMS Microbiology Letters, 1994 Publication	<1%
38	Hantz, O.. "P.028 No amplification of the hepatitis B virus covalently closed circular DNA after in vitro infection of the human hepatoma HepaRG cells", Journal of Clinical Virology, 2006 Publication	<1%
39	Sonali Sengupta. "Role of surface promoter mutations in hepatitis B surface antigen	<1%

production and secretion in occult hepatitis B virus infection", Journal of Medical Virology, 03/2007
Publication

40	jnci.oxfordjournals.org Internet Source	<1%
41	Agata Budkowska. "A biphasic pattern of anti-pre-s responses in acute hepatitis B virus infection", Hepatology, 12/1990 Publication	<1%
42	www.biomedexperts.com Internet Source	<1%
43	Lee, S.-A., K.-J. Kim, D.-W. Kim, and B.-J. Kim. "Male specific W4P/R preS1 mutation of Hepatitis B virus increasing the risk for the progression of liver diseases of chronic patients", Journal of Clinical Microbiology, 2013. Publication	<1%
44	Chen, C.. "Pre-S Deletion and Complex Mutations of Hepatitis B Virus Related to Advanced Liver Disease in HBeAg-Negative Patients", Gastroenterology, 200711 Publication	<1%
45	www.frontiersin.org Internet Source	<1%
46	Mukesh Kumar Krishanani, Waris Qidwai, Badar	

	Sabir Ali, Ali Khan Khuwaja. "Educational Intervention among Barbers about Liver Cancer-Inducing Viruses: A Pilot Study from a Developing Country", Journal of Cancer Education, 2010 Publication	<1%
47	www1.universalrewind.com Internet Source	<1%
48	"Abstracts 1279 to 1393", Hepatology, 10/2005 Publication	<1%
49	Pisit Tangkijvanich, Pattaratida Sa-nguanmoo, Varocha Mahachai, Apiradee Theamboonlers, Yong Poovorawan. "A case–control study on sequence variations in the enhancer II/core promoter/precore and X genes of hepatitis B virus in patients with hepatocellular carcinoma", Hepatology International, 2010 Publication	<1%
50	www.impactjournals.com Internet Source	<1%
51	www.jove.com Internet Source	<1%
52	vir.sgmjournals.org Internet Source	<1%
53	Michael Y. Shapira, Evelyn Zeira, Ruth Adler, Daniel Shouval. "Rapid seroprotection against	<1%

hepatitis B following the first dose of a Pre-S1/Pre-S2/S vaccine", Journal of Hepatology, 2001
Publication

54	Kamol Suwannakarn. "Molecular epidemiological study of hepatitis B virus in Thailand based on the analysis of pre-S and S genes", Hepatology Research, 8/16/2007 Publication	<1%
55	Jeong Han Kim. "Hepatitis B Viral Surface Mutations in Patients with Adefovir Resistant Chronic Hepatitis B with A181T/V Polymerase Mutations", Journal of Korean Medical Science, 2010 Publication	<1%
56	f1000.com Internet Source	<1%
57	Nobukazu Yuki. "Quantitative analysis of pre-S1 and pre-S2 in relation to HBsAg expression", Hepatology, 01/1990 Publication	<1%
58	uad.portalgaruda.org Internet Source	<1%
59	Yang, Hung-Chih, Shiou-Hwei Yeh, Pei-Jer Chen, and Ding-Shinn Chen. "Hepatitis B Virus: Pathogenesis and Host Immune Response", Viruses and Human Cancer, 2014.	<1%

Publication

60	air.unimi.it Internet Source	<1%
61	Kitab, Bouchra, Abdellah Essaid El Feydi, Rajaa Afifi, Christian Trepo, Mustapha Benazzouz, Wafaa Essamri, Fabien Zoulim, Isabelle Chemin, Hanane Salih Alj, Sayeh Ezzikouri, and Soumaya Benjelloun. "Variability in the Precore and Core Promoter Regions of HBV Strains in Morocco: Characterization and Impact on Liver Disease Progression", PLoS ONE, 2012. Publication	<1%
62	icmr.nic.in Internet Source	<1%
63	eprints.mums.ac.ir Internet Source	<1%
64	Hsu, J.-L., W.-J. Chuang, I.-J. Su, W.-J. Gui, Y.-Y. Chang, Y.-P. Lee, Y.-L. Ai, D. T. Chuang, and W. Huang. "Zinc-Dependent Interaction between JAB1 and Pre-S2 Mutant Large Surface Antigen of Hepatitis B Virus and Its Implications for Viral Hepatocarcinogenesis", Journal of Virology, 2013. Publication	<1%
65	jbiomedsci.biomedcentral.com Internet Source	<1%

66	Molecular and Translational Medicine, 2016. Publication	<1%
67	Chao-Hung Hung. "Anemia associated with antiviral therapy in chronic hepatitis C: incidence, risk factors, and impact on treatment response", Liver International, 11/2006 Publication	<1%
68	Niveen Saudy. "Genotypes and phylogenetic characterization of hepatitis B and delta viruses in Egypt", Journal of Medical Virology, 08/2003 Publication	<1%
69	carcin.oupjournals.org Internet Source	<1%
70	Datta, Somenath, Alip Ghosh, Debanjali Dasgupta, Amit Ghosh, Shrabasti Roychoudhury, Gaurav Roy, Soumyojit Das, Kausik Das, Subash Gupta, Keya Basu, Analabha Basu, Simanti Datta, Abhijit Chowdhury, and Soma Banerjee. "Novel Point and Combo-Mutations in the Genome of Hepatitis B Virus-Genotype D: Characterization and Impact on Liver Disease Progression to Hepatocellular Carcinoma", PLoS ONE, 2014. Publication	<1%
71	onlinelibrary.wiley.com Internet Source	<1%

72	www.cdc.gov Internet Source	<1%
73	Haeok Lee. "Public Health Policy for Management of Hepatitis B Virus Infection: Historical Review of Recommendations for Immunization", Public Health Nursing, 03/2010 Publication	<1%
74	www.chinasciencejournal.com Internet Source	<1%
75	jcm.highwire.org Internet Source	<1%
76	Efrat Ziv, David Attia, Gleb Vasilyev, Orit Mendelson, Eyal Zussman, Rachel Yerushalmi-Rozen. "The role of polymer–solvent interactions in polyvinyl-alcohol dispersions of multi-wall carbon nanotubes: from coagulant to dispersant", Soft Matter, 2018 Publication	<1%
77	medcraveonline.com Internet Source	<1%
78	H.-S. Mun. "Novel F141L Pre-S2 Mutation in Hepatitis B Virus Increases the Risk of Hepatocellular Carcinoma in Patients with Chronic Genotype C Infections", Journal of Virology, 01/01/2011 Publication	<1%

79	Nematollah Jonaidi Jafari, Abbas Hosseinpour Azari, Masood Beykpoor, Morteza Izadi et al. "Short-term Atorvastatin Administration and Efficacy of Hepatitis B Vaccination: A Randomized, Double-blind, Placebo-Controlled Clinical Trial", Hepatitis Monthly, 2018 Publication	<1%
80	G. Cotrozzi, P. Relli, G. Strazzulla, M. Rossi, R. Cersosimo, A. Brocchi, G. Buzzelli. "Thyroxine-binding globulin in elderly patients with hepatocellular carcinoma", Archives of Gerontology and Geriatrics, 1996 Publication	<1%
81	Brichler, S., G. Lagathu, M. A. Chekaraou, F. Le Gal, A. Edouard, P. Deny, R. Cesaire, and E. Gordien. "African, Amerindian and European hepatitis B virus strains circulate on the Caribbean Island of Martinique", Journal of General Virology, 2013. Publication	<1%
82	Yun-Fan Liaw. "Natural history of chronic hepatitis B virus infection and long-term outcome under treatment", Liver International, 01/2009 Publication	<1%
83	Sueki, Ryota, Shinya Maekawa, Mika Miura, Makoto Kadokura, Kazuki Komase, Hiroko	<1%

Shindo, Asuka Kanayama, Takako Ohmori, Kuniaki Shindo, Fumitake Amemiya, Yasuhiro Nakayama, Tomoyoshi Uetake, Taisuke Inoue, Minoru Sakamoto, and Nobuyuki Enomoto. "Correlation between pretreatment viral sequences and the emergence of lamivudine resistance in hepatitis B virus infection", Journal of Medical Virology, 2012.
Publication

---

84 Zhen-Hua Zhang. "Genetic variation of hepatitis B virus and its significance for pathogenesis", World Journal of Gastroenterology, 2016 <1%
Publication

Exclude quotes	On	Exclude matches	Off
Exclude bibliography	On		

The Analysis of Mutation Profile on Pre-S1 and Pre-S2 Region of Hepatitis B Virus in Chronic Liver Disease

GRADEMARK REPORT

FINAL GRADE

/100

GENERAL COMMENTS

Instructor

PAGE 1

PAGE 2

PAGE 3

PAGE 4

PAGE 5

PAGE 6

PAGE 7

PAGE 8

PAGE 9