A cross-sectional study was done on naïve CHB patients from September 2009 to June 2011. Quantitative serum HBsAg measurement was performed using the automated chemiluminescent microparticle immunoassay (Architect HBsAg QT assay, Abbott Laboratories, IL, USA). Serum qHBsAg was measured using the automated chemiluminescent microparticle immunoassay (Architect HBsAg QT assay, Abbott Laboratories, IL, USA). Intrahepatic cccDNA was measured quantitatively from biopsy specimen (QiAmp DNA Mini Kit, Qiagen, Germany). Values were log-transformed before being analyzed. Biopsy specimens should include at least 5 portal systems and 1.5cm length to be eligible for evaluation using the METAVIR score.

Results: 103 patients enrolled; 53(51.9%) of them were men. Mean age was 42±11.6 (range 19-70) years old. There were 60(58.3%) patients with HBeAg negative. Mean of log serum quantitative HBsAg was 2.54 for F0-F1 patients vs. 3.48 for F2-F4 patients (p<0.001, student t test). The mean log serum HBV-DNA was 4.65 for F0-F1 vs. 6.69 for F2-F4 patients (p<0.001, student t test). Intrahepatic rcDNA levels was higher in F2-F4 (median 14.37; range 0-3258.4 copies/GEq) than F0-F1 patients (median 0.50; range 0-514.4 copies/GEq); p=0.001 (Mann-Whitney U test). In contrast, virion productivity was not differed statistically between the two groups (p=0.096).

Conclusion: Quantitative serum HBsAg, HBV-DNA serum, and intrahepatic cccDNA are associated with fibrosis stage in chronic hepatitis B patients. HBV seromarkers might be used to predict disease severity.

Background

Quantitative HBsAg has been widely used as a predictor for successful antiviral therapy in chronic hepatitis B (CHB) patients despite HBV DNA serum levels. Intrahepatic HBV-DNA levels have also been tested to know their relevance with clinical manifestation. However, their associations with liver fibrosis are still debatable.

Objective: This study was aimed to evaluate the association between serum qHBsAg, serum HBV-DNA, intrahepatic HBV markers and hepatic fibrosis in CHB patients.

Method: A cross-sectional study was done on naïve CHB patients from September 2009 to June 2011. Quantitative serum HBsAg measurement was performed using the automated chemiluminescent microparticle immunoassay (Architect HBsAg QT assay, Abbott Laboratories, IL, USA). Serum qHBsAg was measured using the automated chemiluminescent microparticle immunoassay (Architect HBsAg QT assay, Abbott Laboratories, IL, USA). Intrahepatic cccDNA was measured quantitatively from biopsy specimen (QiAmp DNA Mini Kit, Qiagen, Germany). Values were log-transformed before being analyzed. Biopsy specimens should include at least 5 portal systems and 1.5cm length to be eligible for evaluation using the METAVIR score.

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The association among serum quantitative hepatitis b surface antigen (qhbsag), intrahepatic HBV markers and liver fibrosis in chronic hepatitis b (chb) patients.

Quantitative HBsAg was measured using chemiluminescent micro particle immunoassay (CMIA) method (ARCHITECT® HBsAg Reagent Kit, Abbott Diagnostics, Abbott Park, Illinois, USA). The results were expressed as signal sample/ cutoff ratios (S/CO). The values were then calibrated (ARCHITECT® HBsAg Calibrators) and verified (ARCHITECT® HBsAg Controls) to obtain quantitative levels in IU/mL.

Serum HBV-DNA levels was measured with COBAS TaqMan® HBV Test (Roche Diagnostics, Manheim, Germany). The detection range was ~30U/mL to 1x10^8U/mL (or 1.7x10^6 to 8.5 x 10^12 copies/mL). Samples were diluted and re-analyzed if the titer was above the upper limit of quantification.

Genotype measurement

Hepatitis B virus genotyping was measured using line probe assay (INNO-LiPA HBV, Innogenetics, Belgium).

Liver biopsy

Liver biopsy was done to all patients to obtain liver tissue specimen and histopathological assessment. It is performed using a 16-gauge Menghini needle (Hepafix®, B Braun Melsungen AG, Melsungen, Germany) under local anesthesia and ultrasound guided. Tissue specimens were first prepared for histopathology examination and the rest was stored in -80°C for genomic analysis. The examination was performed by experienced liver pathologist who blinded to clinical data. Fibrosis stage was assessed using the METAVIR scoring system.

Intrahepatic total and cccDNA quantification

Intrahepatic HBV quantitative was measured at the Victorian Infectious Diseases Reference Laboratory, North Melbourne, Australia. The method has been previously established and reported. In brief, DNA extraction was performed using the tissue lysis protocol with the QIAamp DNA Mini Kit® (QIAGEN, Germany). Determination of HBV cccDNA levels was carried out by real-time PCR using the LightCycler® instrument (Roche Diagnostics, Manheim, Germany). Reaction volumes of 20ul consisted of 2ul of extracted DNA, 5mM MgCl2, 0.5uM of primer mix and 0.2uM of hybridization probes. Selective HBV cccDNA primers consisted of two upstream primers, CC1 5'-GGGGGCTCTCCCGTGTGCGC-3' and CC2 5'-GTCGTTCCCTCTCAATCTGC-3' and the downstream primer, CC3 5'-GTCCATGCCCCAAAGCCACC-3'. FRET hybridization probes were 5'-GGTGTGGTGATGCTCCATATGCCACGT-3' and 5'-CCCTCCATGGCCCAAAAGCCACC-3'. Quantification standards were derived by dilution of a linearized plasmid containing a greater than full length HBV genome. This had been previously titrated against the WHO international HBV reference standard to correlate quantification values. Variation in the amounts of liver tissue was normalized by quantifying β-globin in each sample with the Roche DNA control kit (Roche Diagnostics). The relaxed circular HBV DNA was calculated as total HBV-DNA minus cccDNA.

Statistical analysis

Characteristics of the study subjects were presented descriptively as proportion or median. Mean difference was tested using the student t test for normally distributed data. Median difference was tested on skewed data using the Mann-Whitney U test. A p value of less than 0.05 was considered significant. Analyses were done using the statistical software SPSS version 15 for Windows PC (SPSS Inc., Chicago, Illinois, USA).

Results

There were 103 cases enrolled during the study period. Mean age was 42±11.6 years old, ranging from 19 to 70 years old. Sixty (58.3%) cases were HBeAg-negative (Table 1). Patients with significant fibrosis had higher serum quantitative HBsAg and HBV-DNA levels (Figure 1) (Figure 2).

Citation: Lesmana CRA, Jackson K, Hammond R, et al. The association among serum quantitative hepatitis b surface antigen (qhbsag), intrahepatic HBV markers and liver fibrosis in chronic hepatitis b (chb) patients. J Liver Res Disord Ther. 2015;1(2):32-35. DOI: 10.15406/jlrdt.2015.01.00007
The association among serum quantitative hepatitis b surface antigen (qhbsag), intrahepatic HBV markers and liver fibrosis in chronic hepatitis b (chb) patients

Figure 3 Total intrahepatic HBV-DNA levels was higher in F2-F4 (median 14.65; range 0.08-3284.35 copies/GEq) than F0-F1 patients (median 0.66; range 0.14-516.72 copies/GEq); p < 0.001 (Mann-Whitney U test).

Figure 4 Intrahepatic rcDNA levels was higher in F2-F4 (median 14.37; range 0-3258.4 copies/GEq) than F0-F1 patients (median 0.50; range 0-514.4 copies/GEq); p < 0.001 (Mann-Whitney U test).

Figure 5 Intrahepatic cccDNA levels was higher in F2-F4 (median 0.9; range <0.002-49.227 copies/GEq) than F0-F1 patients (median 0.111; range <0.002-8.956); p < 0.001 (Mann-Whitney U test).

Figure 6 Virion productivity was not statistically significant between F2-F4 (median 54.7; range 0 – 1045.5 rcDNA/cccDNA) and F0-F1 patients (median 12.2; range 0 – 437.5 rcDNA/cccDNA); p = 0.096 (Mann-Whitney U test).

Table 1 Characteristics of the study subjects (N=103)

| Variable      | Hbeag-positive | Hbeag-negative | P value |
|---------------|----------------|----------------|---------|
| Sex           |                |                |         |
| Male          | 18 (41.9%)     | 35 (58.3%)     | 0.099   |
| Female        | 25 (58.1%)     | 25 (41.7%)     |         |
| Age group     |                |                |         |
| <40 years     | 33 (76.7%)     | 15 (25.0%)     | <0.001  |
| >40 years     | 10 (23.3%)     | 45 (75.0%)     |         |
| Serum ALT levels |         |                |         |
| Normal        | 23 (53.5%)     | 44 (73.3%)     |         |
| 1-2x ULN      | 7 (16.3%)      | 12 (20.0%)     | 0.006   |
| >2x ULN       | 13 (30.2%)     | 4 (6.7%)       |         |
| Genotype(n=97)|                |                |         |
| Genotype B    | 31 (77.5%)     | 43 (75.4%)     | 0.814   |
| Genotype C    | 9 (22.5%)      | 14 (24.6%)     |         |

Discussion

Our study has shown that Intrahepatic and serum viral markers level has been associated with the of liver disease progression. To our knowledge, this is the first study in Asia with large number of CHB patients who predominantly genotype B which showed the comprehensive association between liver histology, Intrahepatic HBV markers and HBV sero markers.

Based on natural history of CHB infection, young patients with HBV DNA levels and normal ALT (NALT) levels are usually well-known as immuno tolerance phase group; however, in this study most of the patients have NALT levels. We have shown in our previous publication that most of our young CHB patients even with NALT already had advanced liver fibrosis. To differentiate between immuno tolerance phase and immuno active or immuno clearance phase might not be easy since the ALT level can be fluctuated for many years and the immuno clearance phase itself can be more than 10 years.9,14,15

Based on APASL HBV guideline, liver histology assessment is needed for CHB patients with NALT before treatment decision.16

This study result has shown that quantitative HBsAg and HBV DNA serum levels could predict the severity of liver fibrosis. There have been many studies about non-invasive tools that can be used for liver fibrosis assessment in CHB patients,17-19 however, looking at the Intrahepatic viral activity which also been shown in this study, the use of non-invasive assessment might not be the right tools to start antiviral therapy since it has been reported that there was a grey area which is liver biopsy is still needed to confirm the disease activity or progression. These findings have given a new insight to assess our CHB patients more comprehensively by putting all together between Intra hepatic viral activity and disease activity. However, based on our study results, quantitative HBsAg and HBV-DNA serum levels might be used for starting antiviral treatment without any liver histology assessment. A recent study has shown that there was declining of Intrahepatic cccDNA along with liver histology improvement after oral antiviral therapy.20

Interestingly, virion productivity was not differed between mild fibrosis group and significant fibrosis group suggesting that HBV sero
markers are very important markers for CHB patients monitoring either with or without antiviral therapy. This study also has limitations. First, this was a cross sectional study where liver biopsy was taken in one time only. However, it would be difficult to perform serial liver biopsy in clinical practice. Second, we did not differentiate in each CHB phase of infection, but based on our study subjects, it was not easy to differentiate the CHB phase of infection and it is more important to decide when the treatment should be started to prevent liver cancer development. Further study is needed to find out the accurate cut off from HBV sero markers for antiviral treatment decision.

Conclusion

Quantitative serum HBsAg and intrahepatic cccDNA are associated with fibrosis stage in chronic hepatitis B patients. Higher quantitative serum HBsAg and intrahepatic cccDNA levels could reflect more progressive liver disease. HBV sero markers might be used to start antiviral treatment in CHB patients.

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None.

Conflict of interest

Author declares that there is no conflict of interest.

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