CORRELATION OF HBsAg TITERS WITH SERUM FIBROTIC MAKER IN PATIENTS WITH CHRONIC HEPATITIS B INFECTION

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Abstract. We evaluated the correlation between hepatitis B surface antigen (HBsAg) and a serum fibrotic marker, the procollagen type III N-terminal peptide (PIIINP), levels, an indicator of synthesis and degradation of type III collagen, among patients with chronic hepatitis B virus (CHB) infection. Eighty-four patients with chronic HBV infection without previous antiviral treatment were recruited into the study. There were 58 males and 26 females and the median age was 40 years old. The patients were divided into 3 groups of 28 patients each by stage of chronic HBV infection. PIIINP levels and HBsAg titers were determined by ELISA for all the subjects. The mean overall HBsAg titer correlated significantly with the mean PIIINP level (r=0.548; p<0.01). The correlation between the mean HBsAg titer and the mean PIIINP level in the low replicative phase group (r=0.808) was significantly greater than in the other 2 groups (p<0.01). The PIIINP level may be a disease activity parameter, especially during the low replicative phase of chronic HBV infection.

Keywords: chronic hepatitis B, procollagen type III N-terminal peptide, PIIINP, HBsAg level, fibrotic marker

INTRODUCTION

Chronic hepatitis B (CHB) infection is a worldwide public health problem. At least 350 million people are chronically infected; 25-30% will develop liver disease and 600,000 people will die from CHB infection per year (Kao and Chen, 2002). Thailand has a high prevalence of CHB causing a public health burden (Theamboonlers *et al*, 1999). Chronic hepatitis B patients with high viral loads are at increased risk of cirrhosis and hepatocellular carcinoma (HCC) (Chen *et al*, 2006). Among patients with CHB infection who are hepatitis B e antigen (HBeAg)-negative and have low viral loads [low replicative phase (LR)] there is still a higher risk of HCC among those with high hepatitis B surface antigen (HBsAg) levels (Tseng *et al*, 2012). The risk of a flare-up of hepatitis disease activity and cirrhosis is higher

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among LR patients with high HBsAg levels (Tseng *et al*, 2012). Procollagen type III N-terminal peptide (PIIINP) is a maker of fibrosis marker and an indicator of the synthesis and degradation of type III collagen. PIIINP is a degradation product of type IIIpN collagen. PIIINP contains three distinct domains: a triple-helical Col 3-domain in the middle of the molecule, the Col 1-domain at the aminoterminal and the Col 2-domain at the carboxyterminal end of the propeptide (Plebani and Burlina, 1991). PIIINP is cleared from the circulation by scavenger receptors in liver endothelial cells. PIIINP is an indicator of collagen degradation excreted by fibroblasts and represents an accurate marker of liver fibrosis (Rosenberg *et al*, 2004). Data regarding the association between HBsAg levels and liver fibrosis are limited. Our objective was to evaluate the correlation between HBsAg and PIIINP levels in patients with CHB infection.

MATERIALS AND METHODS

We conducted a cross sectional prospective study among CHB patients seen for treatment at King Chulalongkorn Memorial Hospital from 2010 to 2012. The research protocol was approved by the Institutional Review Board (IRB number 515/53) of the Faculty of Medicine, Chulalongkorn University. The objective of the study was explained to patients who gave written informed consent prior to participation.

Subjects

Eighty-four patients with CHB infection without previous antiviral treatment were recruited into the study. Clinical, demographic, and laboratory data were collected. Patients with evidence of hepatitis C virus or human immunodefiency virus co-infection, alcoholic liver disease, chronic liver disease due to other causes or acute hepatitis B infection were excluded from the study.

Specimen collection

Blood samples were obtained from patients and the clotted blood was separated from the serum within 6 hours. All specimens were kept at -70°C until tested. A percutaneous needle liver biopsy was obtained from each patient. Hepatic fibrosis was staged using a 5-point Metavir scale (F0: no fibrosis; F1: minimal fibrosis; F2: fibrosis with a few septa; F3: numerous bridging fibroses without cirrhosis; F4: cirrhosis or advanced severe fibrosis). Inflammation was also describe (Knodel *et al*, 1981): 0 = no inflammation, 1-4 = minimal inflammation, 5-8 = mild inflammation, 9-12 = moderate inflammation, 13-18 = marked inflammation.

Clinical assessment

The patients were classified into 3 groups based on the stages of CHB infection: Group 1, HBeAg positive (immunotolerance and immunoclearance); Group 2, HBeAg negative with a low hepatitis B virus (HBV) DNA concentration (<2,000 IU/ml) (low replicative phase) and Group 3, HBeAg negative with a high HBV DNA concentration (>2,000 IU/ml) (reactivation phase).

Liver stiffness measurement

Transient elastography was performed using a FibroScan 502 (Echosens, Paris, France). The median value of 10 validated scores was considered the elastic modulus of the liver and was expressed in kilopascals (kPa).

Laboratory method

We determined the HBsAg titer by ELISA (Elecsys, Roche Diagnostics, Indianapolis, IN) and the HBV DNA concentration by quantitative real time PCR

Table 1
Comparison of demographic and laboratory data between among the study groups.

	HBeAg positive (*N*=28)	HBeAg negative, HBV VL<2,000 IU/ml (*N*=28)	HBeAg negative, HBV VL>2,000 IU/ml (*N*=28)	*p*-value
Median age (years)	29.5	49	41	0.07
Male gender	19/28 (67.8%)	15/28 (53.5%)	24/28 (85.7%)	0.08
Mean ALT(U/l)	97.1 ± 69.7	25.2 ± 11.8	85.4 ± 79.5	<0.01
HBV DNA (logIU/ml)	7.1 ± 1.3	2.2 ± 0.7	5.5 ± 1.3	<0.01
HBsAg titer (IU/ml)	20,496.1 ± 21,847.8	3,765.4 ± 7,389.6	5,189.3 ± 9,833.9	<0.01
PIIINP (ng/ml)	163.5 ± 6.5	137.4 ± 43.9	160.5 ± 7.3	<0.01
Liver stiffness (kPa)	7.1 ± 2.3	4.9 ± 1.4	7.2 ± 3.3	<0.01
Median METAVIR fibrosis score (range)	1(0-3) (*n*=25)	-	2(0-3) (*n*=25)	0.55
Mean histological activity index (HAI)	4.81 ± 2.2	-	4.3 ± 3.2	0.5

Plus–minus values are means ±SD for all comparisons.

(The Abbott m2000sp RealTime System, Des Plaines, IL). The PIIINP level (USCN, Houston, TX) was determined by ELISA.

Statistical analysis

Continuous variables were compared between groups using unpaired *t*-test and one-way ANOVA test. Categorical variables were compared between groups using a chi-square or Fisher's exact test. Pearson's correlation coeffiient was used to describe the correlation between two continuous, normally distributed variables. Spearman's correlation was used where variables were not normally distributed. All statistical analyses were performed using SPSS, version 16 (IBM Corp, Armonk, NY).

RESULTS

Fifty-eight males and 26 females with a median age of 40 years were included in the study. There were 28 patients in each group. The mean PIIINP levels in Groups 1, 2 and 3 were 163, 137 and 160 ng/ml, respectively. The mean PIIINP level in Group 2 was significantly lower than in Groups 1 ($p<0.01$) and 3 ($p=0.014$). The mean HBsAg level in Group 1 was 20,496 IU/ml, which was significantly higher than in Group 2 (3,765 IU/ml, $p<0.01$) and Group 3 (5,189.3 IU/ml, $p<0.01$) (Table 1). Overall, HBsAg titer was significantly correlated with PIIINP level ($r=0.548$; $p<0.01$) (Fig 1). The correlation between the mean HBsAg titer and mean PIIINP level in Group 2 ($r=0.808$) was significantly greater ($p<0.01$) than the correlations in Groups 1 ($r=0.615$) and 3 ($r=0.528$) (Fig 1).

The mean liver stiffness levels by transient elastography in Groups 1, 2 and 3 were 7.1, 4.9 and 7.2 kPa, respectively (Table 1). The stiffness level in Group 2 was significantly lower than in Groups 1 and 3 ($p<0.01$). The overall mean PIIINP level was significantly correlated with the overall mean liver stiffness ($r=0.32$; $p<0.01$) (Fig 2). The correlation between the mean

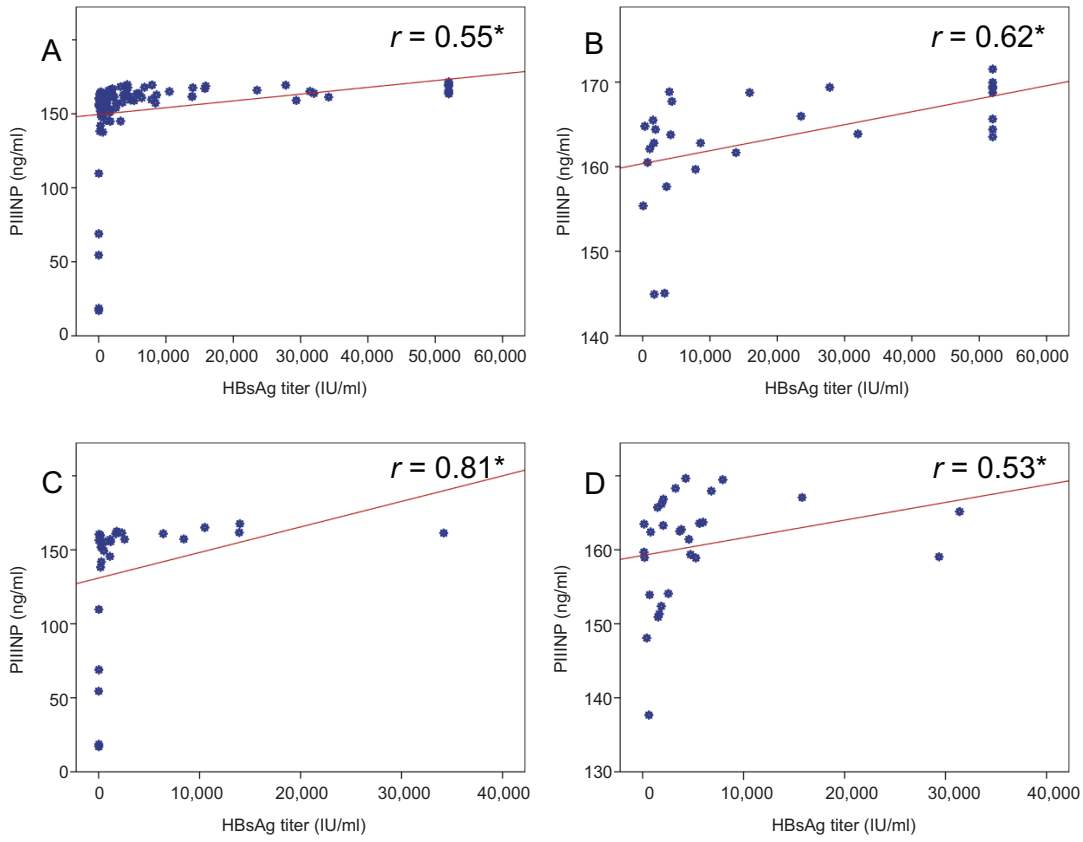

Fig 1–Correlation between HBsAg titer and procollagen type III N-terminal peptide in each stage of disease. A. all groups; B. Group 1, HBeAg positive; C. Group 2, low replicative (LR); D. Group 3, reactivation phase. *Statistically significant.

PIIINP levels and liver stiffness levels were not significantly different by Group.

Liver biopsy was done in 50 patients, 25/28 in Group 1 and 25/28 in Group 3. The median METAVIR Fibrosis scores were 1 (range 0-3) for Groups 1 and 2 (range 0-3) for Group 3. The mean Knodell histological activity index (HAI) for Group 1 was 4.81±2.2 and for Group 3 was 4.3±3.2. No significant correlations were seen between the mean PIIINP level and the Knodell HAI (Fig 3).

DISCUSSION

The clinical course of CHB is classified into four stages: (immunotolerance, immunoclearance, low replicative and reactivation stage) using the HBeAg status, ALT level and HBV DNA level (European Association For The Study of The Liver, 2009). Each stage of hepatitis B infection displays distinct viral kinetics and host immune responses, resulting in different levels of liver necroinflammation and fibrosis. HBsAg levels are different for

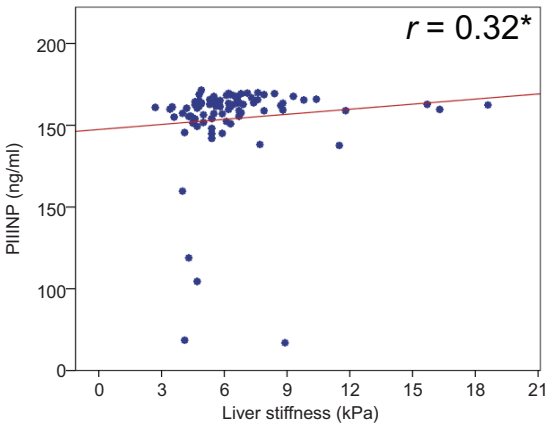

Fig 2–Correlation between liver stiffness (kPa) and procollagen type III N-terminal peptide among all subjects. *Statistically significant.

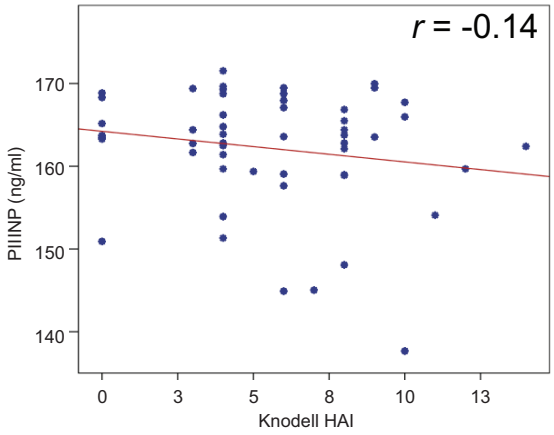

Fig 3–Correlation between Knodell liver histological activity index and procollagen type III N-terminal peptide for Group 1 and Group 3 (*N*=50).

the different stages of CHB (Nguyen *et al*, 2010). In our study, the HBsAg level correlated well with the PIIINP level. This correlation was especially good in the low replicative stage of CHB. This finding may explain the results of a previous study that found high HBsAg levels (>1,000 IU/ml) were associated with liver cirrhosis in CHB infection long term cohorts, especially during the LR stage (Tseng *et al*, 2013).

The serum fibrosis markers hyaluronic acid (HA), tissue inhibitor of matrix metalloproteinase 1 (MMP1) and PIIINP have been used as a non-invasive evaluation of liver fibrosis; the results of which are sensitive, specific, and reproducible (Rosenberg *et al*, 2004). PIIINP can quantify fibrosis and it is a biological component involved in fibrogenesis; therefore, it facilitates discriminating between patients with significant inflammation and fibrosis and those without. A scoring system involving ALT level, PIIINP level and HA level can serve as an accurate non-invasive predictor of inflammatory activity among patients with CHB (Cho *et al*, 2012).

In patients with advanced liver necroinflammation without significant fibrosis, PIIINP levels are higher than in patients without liver necroinflammation, such as with non-alcohol fatty liver disease. Thus, PIIINP helps to discriminate between patients with simple steatosis and those with non-alcoholic steatotic hepatitis or advanced fibrosis (Tanwar *et al*, 2013). PIIINP levels increase in response to persistent fibrosis and fibrogenesis.

In conclusion, our data show that HBsAg levels correlate well with PIIINP levels, particularly during the low replicative phase of chronic HBV infection. HBsAg is associated with liver fibrogenesis and should be considered as a disease activity parameter in patients with chronic hepatitis B infection.

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