Int J Clin Exp Med 2014;7(5):1430-1434
www.ijcem.com /ISSN:1940-5901/IJCEM0000311

Original Article

The utility of thrombopoietin in predicting liver fibrosis in chronic hepatitis B

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Received March 23, 2014; Accepted May 14, 2014; Epub May 15, 2014; Published May 30, 2014

Abstract: Many noninvasive serum markers have been studied to determine the liver fibrosis score (LFS). In this study, we aimed to investigate the association between thrombopoietin (TPO) levels and the stage of liver fibrosis in patients with chronic hepatitis B (CHB). Seventy-seven patients (64 active and 13 inactive) with CHB were included in this cross-sectional study. Patients were divided into three groups: In group 1, patients with mild or no fibrosis (F0, F1); in group 2, patients with significant fibrosis (F2-F4); and in group 3, inactive CHB carriers. Digital patient records were used to access pre-treatment laboratory findings including HBV DNA, HBeAg, ALT, AST, total bilirubin, PLT, albumin, INR. Liver biopsies were examined by experienced pathologists in our hospital who were blinded to the data of the patients. Serum TPO levels were measured using commercial ELISA kit. Serum TPO levels were significantly lower in patients with active CHB compared with the inactive carriers (528 vs 687.1 p=0.003). There was no statistically significant difference in TPO levels between the patients with and patients without significant fibrosis (568.9 vs 459.8 p=0.367). Correlation analysis with respect to ALT, AST, TPO, HBV-DNA level, platelet count, histological activity index (HAI) and liver fibrosis score was performed. TPO was only weakly positively correlated with AST, ALT and HBV-DNA levels (r=0.269 p=0.018; r=0.341 p=0.002; r=0.308 p=0.006; respectively) and no correlation in TPO with LFS and HAI was found (r=0.140 p=0.270, r=0.162 p=0.201; respectively). TPO was not associated with significant fibrosis (p=0.270). In conclusion, TPO levels were decreased in active CHB patients compared with inactive carriers but there was no correlation between TPO levels and the stage of fibrosis in active CHB.

Keywords: Thrombopoietin, chronic hepatitis B, liver fibrosis

Introduction

Chronic Hepatitis B virus (HBV) infection can lead to end-stage liver disease (ESLD) and hepatocellular carcinoma (HCC), and is an important public health problem. An estimated 350 million people are chronically infected with HBV in the world [1, 2]. Therefore, it is important to halt the progression to liver fibrosis and ultimately ESLD and HCC [3]. Although liver biopsy has some disadvantages, such as invasiveness, the possibility of sampling error, and potential complications (bleeding, infection, etc.). It is still the gold standard for detection of liver fibrosis to determine management strategies and prognosis of underlying liver disease [4]. Thus, there is an increasing need for reliable non-invasive histological predictors to assess the severity of liver fibrosis related to chronic hepatitis B (CHB). Serum aspartate aminotransferase to platelet ratio index (APRI), Forn's index, FIB-4, Fibrometer, Fibrotest, Hepascore and MPV [5-14] are designed for this purpose, however, none of these investigated non-invasive tests are feasible to replace liver biopsy.

Thrombopoietin (TPO) is a glycoprotein mainly produced by liver and has a primary regulatory role in megakaryo-thrombopoiesis [15]. Serum TPO levels are reported to be low or undetectable in patients with cirrhosis with various etiologies [16-18]. However, the association between serum TPO levels and the severity of liver fibrosis in patients with CHB has not been investigated. In this study, we aimed to investigate the association between serum TPO levels and the stage of liver fibrosis in patients with CHB.

Table 1. Characteristics of the patients with CHB

	Group 1 and 2 (Active) (n: 64)	Group 3 (Inactive HBV carriers) (n: 13)	*P* value
Age (Years)	45.0 (13.0)	44.7 (9.5)	0.734
Sex (M/F)	33/31	7/6	0.562
Duration of the disease, mean, years (SD)	6.6 (5.5)	6.6 (6.4)	0.743
Bilirubin, mg/dl (SD)	0.9 (0.6)	0.7 (0.3)	0.178
Albumin, gr/dl (SD)	4.2 (0.3)	4.4 (0.3)	0.162
INR	0.99 (0.08)	1.0 (0.27)	0.090
AST, IU/ml (SD)	46.7 (42.8)	26.4 (22.9)	<0.001
ALT, IU/ml (SD)	62.7 (65.7)	33.5 (33.0)	0.007
Platelet Count, /mm^3, (SD)	195.000 (59.000)	220.000 (77.000)	0.301
HBV-DNA, IU/ml, (SD)	2.329.017 (5.729.725)	424 (550)	<0.001
HBeAg negativity, n (%)	57 (89.1)	13 (100)	0.595
TPO, pg/ml (SD)	528.0 (±373.6)	687.1 (±478.6)	0.003

Materials and methods

The study was performed according to the guidelines of the Declaration of Helsinki, and it was approved by the local ethics review committee. In this cross-sectional study, 77 patients with CHB (13 of whom were inactive carriers) were included. Serum samples were collected at the time of liver biopsy, stored at -80°C and serum TPO levels were measured with a commercial ELISA kit (Ray biotech, Inc, Norcross GA). Digital patient records were used to access pre-treatment laboratory findings including HBV DNA, HBe-Ag, ALT, AST, total bilirubin, PLT, albumin, and INR. Liver biopsies were examined by experienced pathologists in our hospital who were blinded to the data of the patients. The METAVIR system was used to assess the severity of liver fibrosis. No fibrosis was defined as F0, portal fibrosis without septa as F1, portal fibrosis with rare septa as F2, numerous septa without cirrhosis as F3 and cirrhosis as F4 [16]. Patients were divided into three groups: Group 1 was composed of patients with mild or no fibrosis (F0, F1), Group 2 was composed of patients with significant fibrosis (F2-F4) and Group 3 was composed of inactive CHB carriers. Patients were not included if they had any of the following: other causes of chronic liver disease, prior antiviral therapy, fatty liver disease, alcohol intake, cirrhosis, HCC or other malignancies.

Statistical analysis

Data were analyzed with SPSS version 15.0 for Windows. The independent samples t-test was used for group comparisons. The Chi-square test was used to compare categorical variables. Pearson correlation was used to evaluate the association between TPO and histological findings or laboratory parameters. Statistical significance was defined as P<0.05.

Results

The patients' characteristics are summarized in **Table 1**. Comparisons of group 1 and 2 are summarized in **Table 2**.

The three groups were similar with respect to gender, mean age, bilirubin and albumin levels, INR and disease duration. In patients with significant fibrosis (group 2), AST and ALT levels were significantly higher than in patients with mild or no fibrosis (group 1) (p=0.013, 0.029; respectively). HBV DNA levels were significantly higher in active CHB patients compared with the inactive carriers (p<0.001). HBV DNA levels were significantly higher in group 2 (significant fibrosis) compared with group 1 (mild or no fibrosis), (p=0.003). Platelet counts were significantly lower in patients with significant fibrosis than in patients with mild or no fibrosis (p=0.028).

Serum TPO levels were significantly lower in patients with active CHB compared with the inactive carriers (p=0.003). There was no statistically significant difference in TPO levels between patients with and patients without significant fibrosis (p=0.367).

Correlation analysis with respect to ALT, AST, TPO, HBV-DNA level, platelet count, histological

Table 2. Characteristics of the patients with active CHB

	Group 1 (n: 24) F0-F1	Group 2 (n: 40) F2-F4	*P* value
Age (Years)	40.2 (9.7)	47.9 (14.0)	0.142
Sex (M/F)	11/13	22/18	0.986
Duration of the disease, mean, years (SD)	5.8 (5.0)	7.1 (5.8)	0.280
Bilirubin, mg/dl (SD)	0.8 (0.5)	1.0 (0.6)	0.054
Albumin, gr/dl (SD)	4.2 (0.3)	4.2 (0.4)	0.895
AST, IU/ml (SD)	33.2 (21.5)	54.9 (50.0)	0.013
ALT, IU/ml (SD)	43.5 (38.5)	74.3 (75.7)	0.029
INR	0.97 (0.07)	1.00 (0.08)	0.185
Platelet Count, /mm^3, (SD)	209.000 (61.000)	186.000 (57.000)	0.028
HBV-DNA, IU/ml, (SD)	1.324.451 (3.804.374)	2.931.757 (6.595.720)	0.003
HBeAg negativity, n (%)	23 (95.8)	34 (85.0)	0.185
TPO, pg/ml (SD)	459.8 (235.7)	568.9 (433.7)	0.367

activity index (HAI) and liver fibrosis score (LFS) was performed. TPO was only weakly positively correlated with AST, ALT and HBV-DNA levels and no correlation in TPO with LFS and HAI was found (**Table 3**). TPO was not associated with significant fibrosis (p=0.270).

Discussion

In this study, we found that TPO was significantly lower in active CHB patients than in inactive carriers. However, TPO levels in this study were not associated with the stage of fibrosis in patients with active CHB.

Chronic HBV infection is an important health problem worldwide which is one of the main causes of chronic liver diseases, and determination of the degree of fibrosis is necessary for initiation and guidance of therapy [19]. Liver biopsy has been the gold standard for staging liver fibrosis, but has some disadvantages and potential complications [4]. Therefore, many investigators have been searching for noninvasive tests to get realistic information about the liver fibrosis stage in patients with chronic liver diseases. Unfortunately, these tests are usually expensive and are not feasible in clinical practice.

TPO is a glycoprotein which has a primary regulatory role in the maturation of megakaryocytes and thrombocytes [15]. However, its role is not completely understood. Although TPO is mainly produced in the liver, it is also expressed elsewhere such as in kidney, bone marrow and spleen [20]. Serum TPO levels are directly regulated when TPO binds to platelets [18, 23]. Thrombocytopenia is a common complication of advanced liver diseases and is usually considered as secondary to portal hypertension or as a result of reduced production of thrombopoietin in the liver [21, 22]. Panasiuk et al [21] and Kawasaki et al [22] showed a positive correlation between TPO levels and platelet count. In contrast, Rios et al reported no correlation between TPO levels and platelet counts [18]. In our study, we likewise did not find a correlation between TPO levels and platelet counts. Our results suggest that other factors such as sequestration of platelets in spleen may have a role in thrombocytopenia of cirrhotic patients. Three different studies revealed that in patients with cirrhosis, serum TPO levels were low or undetectable [16-18]. This suggests that the decrease in TPO levels in cirrhotic patients is a result of the decrease in the number of hepatocytes, as the disease progresses from mild fibrosis to cirrhosis. However, Shimodaira et al reported unchanged levels of TPO in cirrhosis of various etiologies compared to those in normal controls [24]. To the best of our knowledge, our study is the first to investigate whether TPO levels are associated with fibrosis stage in patients with CHB, and whether TPO levels might be used as a non-invasive predictor of liver fibrosis.

Although TPO levels were significantly lower in active CHB patients compared with inactive carriers, no significant correlation between serum TPO levels and liver fibrosis stages in active CHB patients was found. A possible explanation for this might be increased TPO production by other tissues such as kidney,

Table 3. Correlation analysis among the groups

	TPO	HBV DNA	ALT	AST	Platelet count	HAI	STAGE
TPO	r: 1	r: 0.308* p:0.006	r: 0.341* p:0.002	r: 0.269* p:0.018	r: -0.74 p:0.520	r: 0.162 p:0.201	r: 0.140 p:0.270
HBV DNA	r: 0.308* p:0.006	r: 1	r: 0.264* p:0.020	r: 0.235* p:0.030	r: -.158 p:0.169	r: 0.225 p:0.074	r: 0.204 p:0.105
ALT	r: 0.341* p:0.002	r: 0.264 p:0.020	r: 1	r: 0.919** p:<0.001	r: -.076* p:0.015	r: 0.237* p:0.060	r: 0209 p:0.098
AST	r: 0.269* p:0.018	r: 0.235* p:0.030	r: 0.919** p:<0.001	r: 1	r: -0268* p:0.018	r: 0.306* p:0.014	r: 0.283* p:0.023
Platelet count	r: -0.74 p:0.520	r: -.158 p:0.169	r: -.076* p:0.015	r: -0268* p:0.018	r: 1	r: -0.61 p:0.632	r: -0.068 p:0.592
HAI	r: 0.162 p:0.201	r: 0.225 p:0.074	r: 0.237* p:0.060	r: 0.306* p:0.014	r: -0.61 p:0.632	r: 1	r: 0.649** p:<0.001
STAGE	r: 0.140 p:0.270	r: 0.204 p:0.105	r: 0209 p:0.098	r: 0.283* p:0.023	r: -0.068 p:0.592	r: 0.649** p:<0.001	r: 1

*revealed weak correlation, whereas **strong correlation according to the Pearson correlation analysis.

bone marrow and spleen as hepatocytes decline in number.

In conclusion, in our study, TPO levels were decreased in active CHB patients compared with inactive carriers but no correlation was observed between TPO levels and fibrosis stages in active CHB. Further studies on larger number of subjects are needed to elucidate the relation between TPO levels and liver fibrosis.

Acknowledgements

The study was performed according to the guidelines of the Declaration of Helsinki, and it was approved by the local ethics review committee.

Disclosure of conflict of interest

None.

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