Original Article

Are bone morphogenetic protein-7 (BMP-7) serum levels correlated with development of hepatic fibrosis?

Nazlim Aktug Demir[1], Servet Kolgelier[2], Ahmet Cagkan Inkaya[3], Sua Sumer[1], Lutfi Saltuk Demir[4], Fatma Seher Pehlivan[5], Mahmure Arslan[6], Abdullah Arpaci[7]

[1] *Department of Infectious Diseases and Clinical Microbiology, Selcuk University, Faculty of Medicine, Konya, Turkey*
[2] *Department of Infectious Diseases and Clinical Microbiology, Adiyaman University, Faculty of Medicine, Adiyaman, Turkey*
[3] *Department of Infectious Diseases and Clinical Microbiology, Hacettepe University, Faculty of Medicine, Ankara, Turkey*
[4] *Department of Public Health, Necmettin Erbakan University,Meram Faculty of Medicine, Konya, Turkey*
[5] *Department of Pathology, Adiyaman State Hospital, Adiyaman, Turkey*
[6] *Department of Biochemistry, Adiyaman State Hospital, Adiyaman, Turkey*
[7] *Department of Biochemistry, Adiyaman University, Faculty of Medicine, Adiyaman, Turkey*

Abstract

Introduction: Bone morphogenetic protein-7 (BMP-7) is a key protein in organogenesis and liver development. The protein has been studied in the context of liver fibrosis and regeneration. The aim of the present study was to explore any possible association between fibrosis levels (as revealed by liver biopsy) and serum BMP-7 levels.

Methodology: A total of 189 patients with chronic hepatitis B and 51 healthy controls were enrolled in the study.

Results: The study group contained 120 (63.5%) males and 69 (36.5%) females, and the control group contained 25 males (49.0%) and 26 females (51%). In general, serum BMP-7 values of patients were higher than those of controls ($p = 0.001$). Serum BMP-7 values of patients with liver fibrosis of stages 1, 2, 3, or 4 were higher than control values (all p values = 0.01), but the serum BMP-7 levels of patients with stage 5 fibrosis were similar to that of controls. Associations between fibrosis stage and the serum levels of BMP-7, ALT, HBVDNA, platelets, and albumin were all statistically significant ($p = 0.001$). The AUROC for the BMP-7 level in advanced stage fibrosis was found to be 0.23. The data were analyzed using the binary logistic regression analysis (backward stepwise method) and BMP-7, HBVDNA, and platelet levels were found to be risk factors associated with fibrosis (p values 0.031, 0.040, and 0.001, respectively).

Conclusions: BMP-7 may play anti-inflammatory and anti-fibrogenic roles in the pathogenesis of chronic hepatitis B infection.

Key words: BMP-7; chronic hepatitis B; fibrosis

J Infect Dev Ctries 2014; 8(5):605-610. doi:10.3855/jidc.4033

(Received 24 July 2013 – Accepted 01 December 2013)

Introduction

Hepatitis B virus (HBV) infection remains of great concern; the disease is very prevalent and chronic liver disease may result. Chronic hepatitis B (CHB) infection may cause hepatic fibrosis and progress to cirrhosis and hepatocellular carcinoma (HCCA) [1]; however, it is possible to stop the progress of fibrosis with specific treatment. It is thus very important to understand the pathogenetic mechanisms of fibrosis [2].

Bone morphogenetic protein-7 (BMP-7), also termed osteogenic protein-1 (OP-1), a member of the transforming growth factor β (TGF-β) superfamily, plays a key role in liver organogenesis and development [3,4]. BMP-7 has anti-apoptotic, anti-inflammatory, and proliferation-stimulating effects [5,6]. The normal concentration of BMP-7 in blood is 100–300 pg/mL. BMP-7 is believed to act as a regulator of endogens controlling hepatocyte proliferation and liver homeostasis in adult hepatocytes [7,8].

Recent experimental studies have shown that serum BMP-7 levels increase in patients with liver damage because of increased expression of BMP-7 in hepatocytes [3-5]. Another study found that BMP-7 levels increased in animal models of liver cirrhosis [6]. However, only a few studies have measured BMP-7 levels in diseased states.

The purpose of the present study was to evaluate whether serum BMP-7 levels were good predictors of

liver damage and to investigate a possible relationship between development of hepatic fibrosis and serum BMP-7 levels; the extent of fibrosis was assessed by analysis of liver biopsy samples.

Methodology

A total of 189 CHB patients and 51 healthy controls admitted to the infectious disease clinics of the Adiyaman State Hospital, the Adiyaman 82nd Year State Hospital, and Adana State Hospital between 1 January 2010 and 31 December 2010 were studied.

The biopsy criteria of the American Association for the Study of Liver Diseases (AASLD) was used. The Ethics Committee's approval was obtained for the study and 5 mL blood samples were taken from each patient and control after written informed consent was obtained. Serum was separated via centrifugation at 5000 cycles/g for three minutes. Serum samples were stored at –80°C. Patients' demographic data, which included serum ALT, AST, AFP, PLT, HBV DNA, and albumin levels, as well as biopsy results, were recorded. The control group contained healthy volunteers whose ALT and AST values were normal, whose HBsAg status was negative, and who lacked antibodies against HCV.

Liver biopsies from patients diagnosed with CHB were placed in a fixation solution of formalin and sent to the pathology laboratory. All samples were aspired using needles and were between one and three centimeters in length. Sections were stained with hematoxylin-eosin, reticulin, and Masson trichrome, and examined under a light microscope by two pathologists who applied the Ishak modified histologic activity index. Histologic activity indices and fibrosis scores were recorded.

Serum BMP-7 levels were measured using RayBio ELH-BMP-/001 kits. One hundred microliter amounts of standard solutions or serum samples were added to tubes and incubated at room temperature for 2.5 hours. Next, 100 µL of biotinylated anticore was added to each tube and incubated at room temperature for 1 hour. One hundred microliters of a streptavidin-containing solution was added to each tube and incubated at room temperature for a further 45 minutes. One hundred microliters of TMB were added, incubation was continued for 30 minutes, 50 µL of stop solution were added, and absorbance values were read at 450 nm.

Data were analysed using SPSS version 16.0. Descriptive statistics calculated were means ± standard deviations, medians (with minimum and maximum values), and percentage distributions. The Chi-square test, the independent samples T-test, and the Mann-Whitney U test, were used to conduct statistical analysis. The Mann-Whitney U test (after application of the Bonferroni correction when the Kruskal-Wallis test had been performed) was used to explore the possible association of liver disease biopsy stage with serum BMP-7 level. The Spearman approach was used to search for correlations among the levels of different variables. $P < 0.05$ was considered to reflect statistical significance.

Results

The study group contained 120 (63.5%)males and 69 (36.5%) females, and the control group contained 25 males (49.0%) and 26 females (51%). The mean patient age was 34.6±10.8 years and the mean age of the control group was 33.8±9.3 years.

In general, serum BMP-7 values of patients were higher than those of controls ($p = 0.001$). Serum BMP-7 values of patients with liver fibrosis of stages 1, 2, 3, or 4 were higher than those of controls (all p values = 0.01), but the serum BMP-7 levels of patients with stage 5 fibrosis were similar to those of controls (Figure 1). The serum BMP-7 levels in patients with grade 1 fibrosis were lower than in those with grade 2 fibrosis ($p = 0.01$), and the BMP-7 levels in the latter patients were higher than in those with grade 5 fibrosis ($p = 0.01$). No other statistically significant association was detected between fibrosis grade and serum BMP-7 levels (Figure 2). The associations between fibrosis stage and serum levels of BMP-7, ALT, HBVDNA, platelets, and albumin were all statistically significant ($p = 0.001$) (Table 1).

For advanced stage fibrosis, the area under the ROC curve (AUROC) was used to calculate the BMP-7 level. The AUROC for the BMP-7 level in advanced stage fibrosis was found to be 0.23 (Figure 3).

In order to determine the risk factors in patients with advanced stage fibrosis (stage 5), the data were analyzed using the binary logistic regression analysis (backward stepwise method). Although there were 12 patients with advanced stage fibrosis, the regression analysis was carried out on 9 patients whose data were complete. At the end of the analysis, BMP-7, HBVDNA, and platelet levels were found to be risk factors associated with fibrosis (p values 0.031, 0.040, and 0.001, respectively) (Table 2).

Table 1. BMP-7, ALT, HBV DNA, AFP, PLT, and albumin levels plotted against fibrosis stage as assessed by examination of liver biopsies

	Stage 1 n=40	Stage 2 n=77	Stage 3 n=37	Stage 4 n=23	Stage 5 n=12	P value
BMP-7	23.1 (0.01-3019.6)	79.1 (14.7-554.9)	48.4 (0.01-2664.4)	53.6 (1.0-5606.7)	31.3 (0.01-60.3)	**0.001**
ALT	54 (32-144)	56 (32-124)	82 (32-164)	69 (43-145)	87 (54-144)	**0.001**
HBVDNA	$6.7x10^4$ ($1.2x10^4$-$1.1x10^8$)	$9.8x10^4$ ($1.2x10^4$-$1.1x10^8$)	$1.1x10^9$ ($1.2x10^4$-$9.8x10^{10}$)	$6x10^7$ ($4.5x10^5$-$1.2x10^{10}$)	$1.1x10^9$ ($2.3x10^6$-$3.2x10^9$)	**0.001**
AFP	5 (3-8)	5 (3-8)	6 (3-8)	6 (3-13)	6 (3-34)	0.122
Platelet	$2.8x10^5$ ($2.4x10^4$-$4.3x10^5$)	$2.8x10^5$ ($1.9x10^5$-$4.3x10^5$)	$2.9x10^5$ ($1.9x10^5$-$3.6x10^5$)	$2.7x10^5$ ($1.1x10^5$-$2.5\ x10^5$)	$1.8x10^5$ ($1.1x10^5$-$2.8x10^5$)	**0.001**
Albumin	4.0 (3.9-4.5)	4.0 (3.9-4.6)	4.0 (3.8-4.6)	3.9 (3.1-4.0)	3.5 (3.0-4.1)	**0.001**

Table 2. Results of backward binary logistic regression analysis

	B	S.E	wald	P	Exp (B)	95% CI Lower	95% CI Upper
BMP-7	-0.064	0.030	4.66	0.031	0.938	0.885	0.994
HBVDNA	0.001	0.001	4.21	0.040	1.0	1.0	1.0
Platelet	0.001	0.001	11.56	0.001	1.0	1.0	1.0

Figure 1. BMP-7 values of patient and control groups

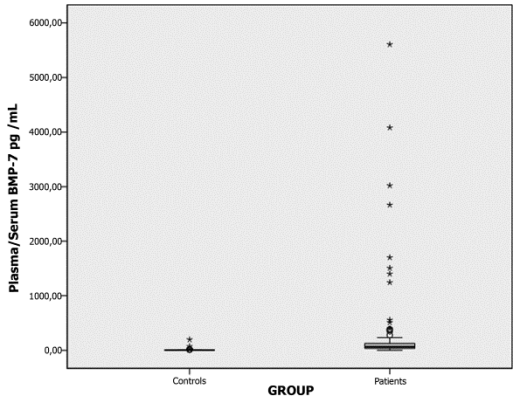


Figure 2. Change in BMP-7 levels according to fibrosis stages

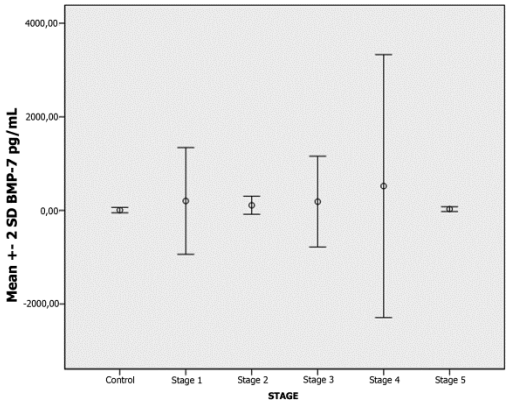
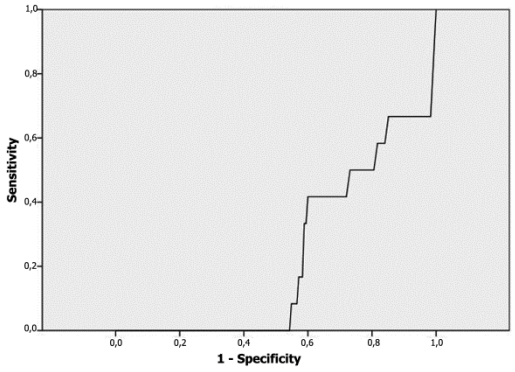


Figure 3. ROC analysis



Discussion

The extent of fibrosis in CHB patients is the most important parameter both in terms of selection of treatment and evaluation of the response to treatment. CHB is a common cause of chronic hepatitis. If CHB is specifically treated, fibrosis may regress and the viral load may decrease. In this manner, disease progression may be slowed and serious consequences such as cirrhosis and HCCA prevented [1].

The gold standard diagnostic test for fibrosis is a liver biopsy. Biopsy complications, problems with histopathological evaluation of samples, inadequate sample size (in some instances), and an inability to take a biopsy if medically contraindicated, mean that the utility of biopsy remains controversial [8-10].

Experimental studies conducted in recent years have shown that a high serum BMP-7 level is an excellent indicator of the presence of fibrosis [11,12]. The principal purpose of the present study was to measure BMP-7 levels in CHB patients, and our PUBMED search indicates that the sample size of our study is the largest of all published reports on the topic.

Clinical and experimental studies conducted over the last 20 years have examined fibrotic liver tissue and the structure and contents of the normal extracellular matrix (ECM) [13,14]. The liver ECM is composed of proteins (collagen, elastin), glucoconjugates (structural proteins, proteoglycans), and glycosaminoglycans (hyaluronan). Fibrogenesis in hepatocytes commences with necrosis and apoptosis triggered by inflammation attributable to various reasons (including infection with hepatitis viruses, alcohol ingestion, and metabolic problems). Activation of hepatic stellate cells, with subsequent inflammation, elevates TGF-β levels; this material mediates fibrogenesis. In turn, ECM deposition rises. After several steps of activation, myofibroblasts are formed from stellate cells in the process termed the epithelial-mesenchymal transition (EMT). This process is believed to precede development of cirrhosis. Myofibroblast formation is followed by fibrosis, cirrhosis, and development of primary HCCA. Myofibroblast activity causes the levels collagen, elastin, glycoproteins, proteoglycans, and hyaluronans to all rise during development of cirrhosis [5].

BMP-7 antagonizes some of the activities of TGF-β [12,15]. BMP-7 stops ECM synthesis and the hepatocyte proliferation induced by TGF-β [16,17]. Also, BMP-7 can reverse the EMT [5,16]. This balance between the levels of TGF-β and BMP-7 is critical in terms of development of chronic liver disease and initiation of fibrosis [12]. Overexpression of BMP-7 or addition of recombinant BMP-7 has been shown to exert an antifibrotic effect [6,17-19]. In animal studies, defective organ development and deaths have been reported in BMP-7-deficient mice [20]. Sugimoto *et al*. [6] observed marked increases in hepatic regeneration and proliferation in partially hepatectomized mice after administration of BMP-7. *In vitro*, Kinoshita *et al*. [17] showed that hepatic stellate cellsoverexpressed BMP-7. In another study, Sakamato *et al*. [11] found that BMP-7 reduced the intracellular HCV subgenome level, in a dose-dependent manner, in cultures of HCVJFH1 cells. The cited authors also found that BMP-7 acted synergistically with interferon to inhibit HCV replication *in vitro*.

BMP-7 is an antifibrogenetic molecule, and the serum BMP-7 level is higher in patients with chronic liver disease than in healthy controls [7,21]. Tacke *et al*. [7] studied 111 cirrhotic patients and 96 healthy controls and found that the serum BMP-7 level was higher in the cirrhotic patients. In the cited study, immunohistochemistry was used to reveal that BMP-7 levels were higher in cirrhotic human hepatocytes than in healthy cells. We compared the BMP-7 levels of 189 CHB patients and 51 healthy controls and detected higher levels in CHB patients ($p = 0.001$). When BMP-7 levels were compared by grade of fibrosis, we found that the levels in patients with fibrosis of grades 1, 2, 3, and 4 were higher than in controls (all p values = 0.01), but this was not true of grade 5 patients with severe hepatic failure. We concluded that fibrosis advanced because BMP-7 expression decreased because of severe hepatocyte loss; BMP-7 was unable to exert therapeutic anti-apoptotic, anti-inflammatory, and antifibrotic effects.

Only a few human studies have compared increased serum BMP-7 levels with the severity of fibrosis. Tacke *et al*. [7] evaluated 111 patients with chronic liver disease and found that BMP-7 levels were increased in cirrhotic patients. The cited authors also graded such patients using the Child classification and found that the BMP-7 levels of Child C patients were higher than those of Child A patients. Also, the cited authors found that increased serum BMP-7 levels correlated with rises in hepatocyte BMP-7 concentrations in the livers of cirrhotic patients. We found only a single study that explored a possible relationship between development of CHB and BMP-7 levels; this was the work of Cao *et al*. [21]. In a study involving 81 CHB patients, the cited authors compared fibrosis levels (as revealed by liver biopsy) and serum

BMP-7 levels, and found that BMP-7 levels were high in patients with severe fibrosis independent of the extent of inflammation. Similarly, the cited authors found that serum BMP-7 levels were high in patients with severe inflammation, independent of the extent of fibrosis. We compared serum BMP-7 levels in 189 CHB patients with biopsy data from those patients. The serum BMP-7 levels in patients with grade 1 fibrosis were lower than in those with grade 2 fibrosis ($p = 0.01$), and the BMP-7 levels in the latter patients were higher than in those with grade 5 fibrosis ($p = 0.01$). No other statistically significant association was detected between fibrosis grade and serum BMP-7 level. We suggest that our inability to detect higher BMP-7 levels was attributable to the death of healthy hepatocytes that could express BMP-7 because such cells were replaced by cirrhotic nodules. We found that fibrosis stage was associated with the serum levels of BMP-7, ALT, HBVDNA, platelets, and albumin; these associations were statistically significant.

Through the binary logistic regression analysis (backward stepwise method), the risk factors associated with fibrosis were found to be the BMP-7, HBVDNA, and platelet levels in patients with advanced stage fibrosis (stage 5) (p values 0.031, 0.040, and 0.001, respectively). Additionally, AUROC was found to be 0.23 in this patient group as a result of the ROC analysis. This was linked to the scarcity of patients with stage 5 fibrosis, which is the major limitation of our study. There is a need for studies to be conducted with a large number of patients with advanced stage fibrosis to obtain more accurate data on the issue.

In conclusion, although increases in both serum BMP-7 level and hepatocyte expression of the protein in patients with liver disease have been previously reported, any role played by BMP-7 in the development of fibrosis in CHB patients remains unclear. More studies are needed; only a few reports on humans have been published.

References

1. Anastasiou J, Alisa A, Virtue S, Portmann B, Murray-Lyon I, Williams R (2010) Noninvasive markers of fibrosis and inflammation in clinical practice: prospective comparison with liver biopsy. Eur J Gastroenterol Hepatol 22: 474-480.
2. Zeisberg M, Yang C, Martino M, Duncan MB, Rieder F, Tanjore H, Kalluri R (2007) Fibroblasts derive from hepatocytes in liver fibrosis via epithelial to mesenchymal transition. J Biol Chem 282: 23337-23347.
3. Weiskirchen R, Meurer SK (2007) Bone morphogenetic protein-7 in focus: a member of the transforming growth factor-beta superfamily is implicated in the maintenance of liver health. Hepatology 45: 1324-1325.
4. Ozkaynak E, Rueger DC, Drier EA, Corbett C, Ridge RJ, Sampath TK, Oppermann H (1990) OP-1 cDNA encodes an osteogenic protein in the TGF-beta family. EMBO J 9: 2085-2093.
5. Gressner OA, Weiskirchen R, Gressner AM (2007) Evolving concepts of liver fibrogenesis provide new diagnostic and therapeutic options. Comp Hepatol 6: 7.
6. Sugimoto H, Yang C, LeBleu VS, Soubasakos MA, Giraldo M, Zeisberg M, Kalluri R (2007) BMP-7 functions as a novel hormone to facilitate liver regeneration. FASEB J 21: 256-264.
7. Tacke F, Gäbele E, Bataille F, Schwabe RF, Hellerbrand C, Klebl F, Straub RH, Luedde T, Manns MP, Trautwein C, Brenner DA, Schölmerich J, Schnabl B (2007) Bone morphogenetic protein 7 is elevated in patients with chronic liver disease and exerts fibrogenic effects on human hepatic stellate cells. Dig Dis Sci 52: 3404-3415.
8. Uyar C, Akcam FZ, Ciris M, Kaya O, Kockar C, Isler M (2010). Comparison of FibroTest-ActiTest with histopathology in demonstrating fibrosis and necroinflammatory activity in chronic hepatitis B and C. Indian J Pathol Microbiol 53: 470-475.
9. Poynard T, Ratziu V, Bedossa P (2000) Appropriateness of liver biopsy. Can J Gastroenterol 14: 543-548.
10. Regev A, Berho M, Jeffers LJ, Milikowski C, Molina EG, Pyrsopoulos NT, Feng ZZ, Reddy KR, Schiff ER (2002) Am J Gastroenterol 97: 2614-2618.
11. Sakamoto N, Yoshimura M, Kimura T, Toyama K, Sekine-Osajima Y, Watanabe M, Muramatsu M (2007) Bone morphogenetic protein-7 and interferon-alpha synergistically suppress hepatitis C virus replicon. Biochem Biophys Res Commun 357: 467-473.
12. Gressner OA, Gressner AM (2008) Connective tissue growth factor: a fibrogenic master switch in fibrotic liver diseases. Liver Int 28: 1065-1079.
13. Schuppan D, Ruehl M, Somasundaram R, Hahn EG (2001) Matrix as a modulator of hepatic fibrogenesis. Semin Liver Dis 21: 351-372.
14. Gressner AM, Weiskirchen R (2006) Modern pathogenetic concepts of liver fibrosis suggest stellate cells and TGF-beta as major players and therapeutic targets. J Cell Mol Med 10: 76-99.
15. Chen D, Zhao M, Mundy GR (2004) Bone morphogenetic proteins. Growth Factors 22: 233-241.
16. Zeisberg M, Hanai J, Sugimoto H, Mammoto T, Charytan D, Strutz F, Kalluri R (2003) BMP-7 counteracts TGF-beta1-induced epithelial-to-mesenchymal transition and reverses chronic renal injury. Nat Med 9: 964-968.
17. Kinoshita K, Iimuro Y, Otogawa K, Saika S, Inagaki Y, Nakajima Y, Kawada N, Fujimoto J, Friedman SL, Ikeda K

(2007) Adenovirus-mediated expression of BMP-7 suppresses the development of liver fibrosis in rats. Gut 56: 706-714.
18. Gressner OA, Rizk MS, Kovalenko E, Weiskirchen R, Gressner AM (2008) Changing the pathogenetic roadmap of liver fibrosis? Where did it start; where will it go? J Gastroenterol Hepatol 23: 1024-1035.
19. Zeisberg M, Yang C, Martino M, Duncan MB, Rieder F, Tanjore H, Kalluri R (2007) Fibroblasts derive from hepatocytes in liver fibrosis via epithelial to mesenchymal transition. J Biol Chem 282: 23337-23347.
20. Luo G, Hofmann C, Bronckers AL, Sohocki M, Bradley A, Karsenty G (1995) BMP-7 is an inducer of nephrogenesis, and is also required for eye development and skeletal patterning. Genes Dev 9: 2808-2820.
21. Cao H, Shu X, Chen LB, Zhang K, Xu QH, Li G (2010). The relationship of expression of BMP-7 in the liver and hepatic inflammation and fibrosis in patients with chronic HBV infection. Zhonghua Shi Yan He Lin Chuang Bing Du Xue Za Zhi 24: 101-103.

Corresponding author
Sua Sumer, MD
Faculty of Medicine, Department of Infectious Diseases and Clinical Microbiology
Selcuk University, Selcuklu/Konya, Turkey
Phone: +90 505 8746251
Fax: +90 332 2412184
Email: suasumer@gmail.com

Conflict of interests: No conflict of interests is declared.