Turk J Gastroenterol 2007; 18 (2): 95-99

The role of thrombopoietin and spleen volume in thrombocytopenia of patients with noncirrhotic and cirrhotic portal hypertension

Sirotik ve nonsirotik portal hipertansiyonlu hastalarda trombopoetin ve dalak hacminin rolü

Filiz AKYÜZ[1], Ensar YEKELER[2], Sabahattin KAYMAKOĞLU[1], Sibel HORASANLI[3], Duygu İBRİŞİM[1], Kadir DEMİR[1], Nevzat AKSOY[1], Şule POTUROĞLU[1], Selim BADUR[3], Atilla ÖKTEN[1]

Departments of [1]Gastroenterohepatology, [2]Radiology and [3]Microbiology, İstanbul University, İstanbul Faculty of Medicine, İstanbul

Background/aims: *To determine the role of thrombopoietin and spleen volume in thrombocytopenia diagnosed in cirrhotic and noncirrhotic portal hypertensive patients.* ***Methods:*** *Seventy-four portal hypertensive patients (group 1: 28 noncirrhotic; group 2: 46 cirrhotic) were enrolled into this study. Spleen volume was measured by magnetic resonance imaging. Thrombopoietin and hyaluronic acid were detected by ELISA in sera.* ***Results:*** *Splenic volume was significantly higher in group 1 (1375±658.74 ml) than group 2 (981.78±512.39 ml). In group 1, thrombopoietin and hyaluronic acid levels were 76.6±30.39 pg/ml and 78.17±66.67 ng/ml, respectively. These values were significantly higher in group 2, at 99.89±38.5 pg/ml and 271.97±197.34 ng/ml, respectively (p<0.05). Platelet counts and thrombopoietin levels had a negative correlation with spleen volume in both groups (p<0.05). Serum thrombopoietin levels were not correlated with platelet counts in cirrhotic and noncirrhotic groups; however, thrombopoietin levels were negatively correlated with splenic volume in the whole group (p= 0.044, r= - 0.23). Although spleen volume was significantly larger in noncirrhotic patients, platelet counts were similar in both groups.* ***Conclusions:*** *This study confirms that splenic sequestration is the main factor in the thrombocytopenia in portal hypertensive patients. The balance of thrombopoietin production and degradation may be more important for platelet counts than decreasing synthesis.*

Key words: Thrombopoietin, thrombocytopenia, portal hypertension

Amaç: *Sirotik ve nonsirotik portal hipertansiyon tanısı konulan hastalarda trombopoetin ve dalak hacminin trombositopenideki etkisini araştırmaktır.* ***Yöntem:*** *Çalışmaya portal hipertansiyonu olan 74 hasta (grup 1: 28 nonsirotik; grup 2: 46 sirotik) alındı. Dalak hacmi magnetik rezonans görüntüleme ile hesaplandı. Trombopoetin ve hyaluronik asid serumda ELISA ile tayin edildi.* ***Bulgular:*** *Dalak hacmi grup 1'de (1375±658.74 mL) grup 2'ye (981.78±512.39 mL) göre anlamlı olarak büyüktü. Grup 1'de trombopoetin düzeyi 76.6±30.39 pg/ml, hyaluronik asid düzeyi 78.17±66.67 ng/ml olarak bulundu. Trombopoetin (99.89±38.5 pg/ml) ve hyaluronik asid (271.97±197.34 ng/ml) düzeyleri grup 2'de grup 1'den daha yüksekti (p<0.05). Her iki grupta da trombosit ve trombopoetin düzeyleri ile dalak hacmi arasında negatif korelasyon saptandı (p <0.05). Sirotik ve nonsirotik gruplarda serum trombopoetin düzeyleri ile trombosit düzeyleri arasında korelasyon yoktu. Fakat tüm grupta trombopoetin düzeyi ile dalak hacmi arasında negatif korelasyon saptandı (p=0.044, r= - 0.23). Dalak hacmi nonsirotik hastalarda anlamlı olarak daha büyük olmasına rağmen, trombosit düzeyleri her iki grupta da benzerdi.* ***Sonuç:*** *Bu çalışma portal hipertansif hastalarda trombositopeninin esas nedeninin dalaktaki sekestrasyon olduğunu göstermiştir. Bu sonuçlar trombosit düzeyleri üzerinde trombopoetin sentezindeki azalmadan daha çok trombopoetin üretimi ve yıkımı arasındaki dengenin önemli olabileceğini düşündürmüştür.*

Anahtar kelimeler: Trombopoetin, trombositopeni, portal hipertansiyon

INTRODUCTION

Thrombocytopenia is one of the most frequent hematological disorders in cirrhotic and noncirrhotic portal hypertension (1, 2). The mechanism of thrombocytopenia classically is thought to be caused by splenic sequestration and destruction of platelets (3). Portal hypertension resulting in hypersplenism is considered to play a role in splenic platelet sequestration. In clinical experience,

Address for correspondence: Filiz AKYÜZ
Department of Gastroenterohepatology, İstanbul University, İstanbul Faculty of Medicine, İstanbul, Turkey
Phone: +90 212 414 20 00/31140 • Fax: +90 212 631 97 43
E-mail: filizakyuz@hotmail.com

Manuscript received: 07.12.2006 **Accepted:** 03.05.2007

some cirrhotic patients have normal platelet counts or some of them are thrombocytopenic without splenomegaly (4). Thus, there may be other mechanisms, such as humoral and genetic factors, in the pathogenesis of thrombocytopenia. Thrombopoietin (TPO) is a thrombopoietic growth factor that stimulates megakaryocytopoiesis and platelet maturation. In thrombocytopenic states, TPO levels have been demonstrated to increase as a compensatory mechanism (5). There are some studies about serum TPO levels in patients diagnosed with cirrhosis and chronic hepatitis (3-9). It has recently been suggested that liver fibrosis may play a role in determining relatively low TPO serum levels among patients with chronic hepatitis (2), and it has been shown that TPO levels were significantly lower in cirrhotic patients as compared to chronic hepatitis patients (10). However, there has been no study that investigated the relationships between serum TPO levels, splenomegaly and thrombocytopenia in cirrhotic and noncirrhotic portal hypertensive patients.

We aimed to determine the role of TPO and spleen volume in the development of thrombocytopenia in patients with cirrhotic and noncirrhotic portal hypertension.

MATERIALS AND METHODS

Seventy-four consecutive portal hypertensive patients were enrolled into this prospective study. Twenty-eight of them were noncirrhotic (group 1) and 46 were cirrhotic (group 2). Informed consent was obtained from each patient and the study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the local committee. The diagnosis of liver cirrhosis was confirmed by liver biopsy or peritoneoscopy in all patients. The severity of liver cirrhosis was assessed according to the Child-Pugh classification (11). The evaluation of all noncirrhotic portal hypertensive patients included assessment of liver parenchyma by liver biopsy or peritoneoscopy; imaging of the portal system including hepatic, portal and splenic veins by Doppler ultrasound or portal venography; and demonstration of the absence of the stigmata of chronic liver failure by clinical evaluation and appropriate liver function tests. Arterial portography or computed tomography techniques were used for portal venography. Idiopathic portal hypertension was diagnosed by the criteria adopted by the Japan Idiopathic Portal Hypertension Research Committee including histological and/or peritoneoscopic findings of the liver in all cases (12). Portal vein thrombosis was considered in patients who had a normal liver in biopsy or peritoneoscopy and a thrombosis within the portal vein lumen on portal venography. Hepatitis B surface antigen, antibody to hepatitis B surface antigen, antibody to hepatitis C virus and anti delta total were analyzed by immuno-enzymatic assays (Organon Teknika, Holland) and autoantibodies were analyzed by enzyme linked immunosorbent assay (ELISA) to determine the etiology in the cirrhotic group. Magnetic resonance imaging (MRI) was performed to calculate splenic size and volume in axial and coronal planes by using T2-weighted HASTE (half-fourier single-shot turbo spin echo imaging) sequences with respiratory navigator. Slice thickness was chosen as 10 mm and whole spleen was covered in both axial and coronal images. Total splenic volume was measured by hand tracing the spleen outline on the axial images and summing all areas obtained from each axial image.

Blood was drawn from all patients and samples were centrifuged at 2500 rpm for 5 minutes and separated serum samples were stored at –85°C. TPO (Quantikine, RD Systems, Wiebaden-Nordenstandt, Germany) was detected by ELISA in patients' sera. Hyaluronic acid (Corgenix inc. Kit, United Kingdom), which is a marker for hepatic fibrosis, was also detected by ELISA in patients' sera in order to confirm that there was no marked fibrosis in noncirrhotic patients.

Statistical analysis was done with non-parametric Mann-Whitney test, correlation tests, regression and receiver operating characteristic (ROC) analysis using SPSS for Windows (version 10.0; Chicago, IL, USA) where appropriate. The results are expressed as mean ±SEM. A value of $p<0.05$ was considered statistically significant.

RESULTS

Patient characteristics and splenic sizes are shown in Table 1. Mean age and sex distribution were comparable between groups 1 and 2. Mean platelet counts were similar in cirrhotic and noncirrhotic groups. Etiologic distribution of patients was as follows: group 1: idiopathic (19) and portal vein thrombosis (9); group 2: hepatitis B virus (24), cryptogenic (11), hepatitis C virus (6), alcohol (2), Budd-Chiari syndrome (2) and hepatitis D virus (1). Of the cirrhotic patients, 17 were in Child's A, 18 in Child's B, and 11 in Child's C.

Table 1. Clinical and laboratory features of the studied groups

n	Noncirrhotic PHT* (group I-28)	Cirrhotic PHT* (group II-46)	p
Age (mean, year)	40.7±14.5	48.2±12.7	>0.05
Gender (F/M)	18/10	20/26	>0.05
ALP (IU/L)	224±103	285±149	>0.05
GGT (IU/L)	145±31	96±117	>0.05
AST (IU/L)	33±12	70±42	**<0.05**
ALT (IU/L)	31±15	59±41	**<0.05**
Total bilirubin (mg/dl)	1.4±1.2	2.1±1.4	**<0.05**
Albumin (g/dl)	4±0.4	3.5±0.5	**<0.05**
Gamma globulin (g/dl)	1.5±0.4	2.1±0.6	**<0.05**
Leukocyte /mm^3	3454±1626	4620±2194	**<0.05**
Hemoglobin (g/dl)	11.3±1.5	11.7±1.5	>0.05
Thrombocyte /mm^3	85178.6±53811	89108.7±61583.9	>0.05
Thrombopoietin (pg/ml)	76.6±30.39	99.89±38.5	**<0.05**
Hyaluronic acid (ng/ml)	78.17±66.67	271.97±197.34	**<0.05**
Spleen volume (ml)	1375±658.74	981.78±512.39	**<0.05**

*PHT: Portal hypertension, ALP: Alkaline phosphatase, GGT: Gamma-glutamyl transferase, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase

Splenic volume was significantly larger in group 1 (1375±658.74 ml) than group 2 (981.78±512.39 ml). Serum TPO and hyaluronic acid levels were significantly higher in group 2 compared to group 1 (p<0.05). In the cirrhotic group, serum hyaluronic acid levels were 276.3±57.6 ng/ml in Child A, 239.6±24 ng/ml in Child B and 318.4±72.4 ng/ml in Child C patients. In logistic regression analysis, a positive linear correlation was found between serum hyaluronic acid levels and Child stages (p<0.05). In ROC analysis, cut-off level for hyaluronic acid was 120 pg/ml (area under curve 0.810; 95% confidence interval-CI 0.703-0.916).

Thrombopoietin levels were not correlated with Child-Pugh stage. Platelet counts had a negative correlation with spleen volume in both groups (p= 0.006, r= -0.314) (Figure 1), and were not correlated with TPO levels. However, TPO levels were negatively correlated with splenic volume in the whole group (p= 0.044, r= - 0.23) (Figure 2). TPO levels were not different in patients diagnosed with portal vein thrombosis and idiopathic noncirrhotic portal hypertension. Platelet counts were lower in idiopathic portal hypertension compared to portal vein thrombosis, but the difference did not reach statistical significance (p=0.051).

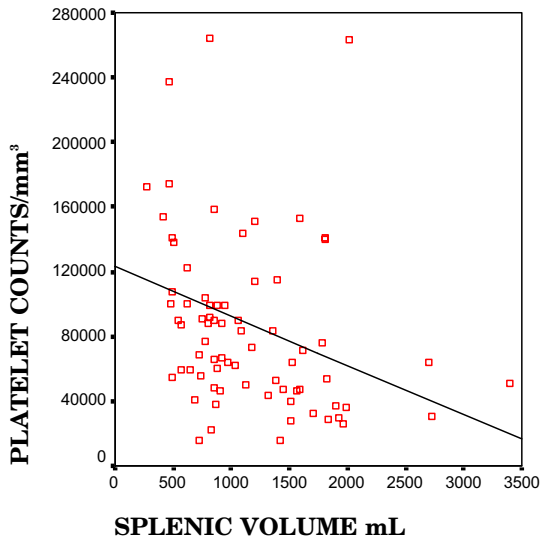

Figure 1. Negative correlation between platelet counts and splenic volume

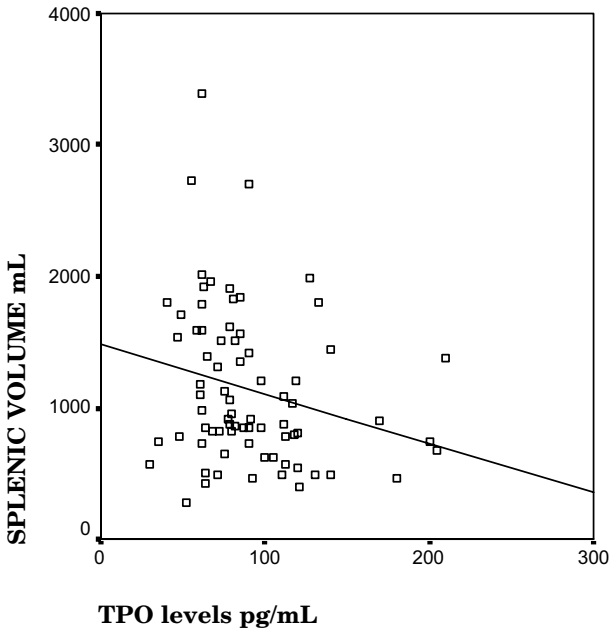

Figure 2. Negative correlation between TPO and splenic volume

DISCUSSION

Thrombocytopenic disorders have been classified into disorders of production, distribution (splenic pool) or destruction (immune or consumption) (2). Thrombocytopenia seen in portal hypertension is thought to be caused by sequestration and destruction of platelets within a large spleen (3). This classic hypothesis has been questioned on the basis of the failure to normalize low platelet counts by splenectomy and portosystemic shunt in some

patients (13, 15). However, platelet counts may be normal in some patients with splenomegaly or low in normal splenic volume. With these findings, it is considered that splenic sequestration is not the only mechanism in thrombocytopenia in these patients. Our data confirm that spleen size is inversely correlated with the platelet count. Recently, TPO levels were investigated by several centers, but their results were contradictory (3, 5-7, 15). These studies investigated only cirrhotic or chronic viral hepatitis patients. Some studies showed low plasma levels of TPO in adult cirrhotic patients with thrombocytopenia and reduced hepatic TPO mRNA levels in cirrhotic children (16-18). Our study contrasts Adinolfi et al.'s study (2) that showed correlation between fibrosis and TPO levels. However, we analyzed thrombopoietin levels in noncirrhotic and cirrhotic patients. Although we did not analyze serum TPO levels in healthy subjects, TPO levels in both groups (76.6±30.29 pg/ml in noncirrhotic and 99.89±38.5 pg/ml in cirrhotic patients) were lower than previously reported serum TPO levels (120 pg/ml) in healthy subjects (9). Although splenic volumes were not as large in cirrhotic patients as in noncirrhotics, platelet counts were nearly similar. This may be explained in two ways:

a) Impaired Balance of TPO Production and Degradation

TPO produced in hepatocytes and bound to platelets undergoes proteolysis and further degradation (19). Rios et al. (9) showed that TPO level was related to the splenic size. After partial splenic embolization, TPO levels and platelet counts significantly increased. We also found negative correlation between TPO levels and splenic size, compatible with Rios et al.'s study. In our study, splenic volume was significantly higher in noncirrhotic patients but serum platelet levels were similar in cirrhotic and noncirrhotic patients. TPO levels were also significantly lower in noncirrhotic patients. These results suggested that degradation of TPO is higher in noncirrhotic patients because of higher splenic volume as compared with cirrhotic patients. These results clearly showed that degradation of TPO is more important than TPO production in portal hypertension.

b) Related With Activated Stellate Cells

Our data also showed that hyaluronic acid levels were significantly higher in cirrhotic patients and positively correlated with Child-Pugh stage. These results confirmed previous studies reporting that hyaluronic acid was a valuable diagnostic and prognostic marker for cirrhotic patients (20, 21). Hyaluronate is synthesized by activated stellate cells in the liver, and activated stellate cells also synthesize other proteins such as growth factors and cytokines (22, 23). TPO mRNA was shown in hepatocytes (24), and our study revealed that TPO levels were elevated along with high hyaluronic acid in cirrhotic patients. Freni et al. (5) reported that serum TPO levels were higher in patients with chronic liver disease than normal controls. Schiodt et al. (25) showed normal and increased serum levels of TPO in acute liver failure. Whether TPO may be synthesized by activated stellate cells in the liver must be determined by further investigations.

In conclusion, serum TPO levels were not correlated with platelet levels in cirrhotic or noncirrhotic patients, but they were negatively correlated with splenic size. This study confirms that sequestration is the main factor in the thrombocytopenia in portal hypertensive patients.

REFERENCES

1. Shah SHA, Hayes PC, Allan P, et al. Measurement of spleen size and its relation to hypersplenism and portal hemodynamics in portal hypertension due to hepatic cirrhosis. Am J Gastroenterol 1996; 91: 2580-3.
2. Adinolfi LE, Giordano MG, Andreana A, et al. Hepatic fibrosis plays a central role in the pathogenesis of thrombocytopenia in patients with chronic viral hepatitis. Br J Haematol 2001; 113: 590-5.
3. Sanjo A, Satoi J, Ohnishi A, et al. Role of elevated platelet-associated immunoglobulin G and hypersplenism in thrombocytopenia of chronic liver diseases. J Gastroenterol Hepatol 2003; 18: 638-44.
4. Sezai S, Kamisaka K, Ikegami F, et al. Regulation of hepatic thrombopoietin production by portal hemodynamics in liver cirrhosis. Am J Gastroenterol 1998; 93: 80-2.
5. Freni MA, Spadaro A, Ajello A, et al. Serum thrombopoietin in chronic liver disease: relation to severity of the disease and spleen size. Hepatogastroenterology 2002; 49: 1382-5.
6. Koruk M, Onuk MD, Akcay F, Savas MC. Serum thrombopoietin levels in patients with chronic hepatitis and liver cirrhosis, and its relationship with circulating thrombocyte counts. Hepatogastroenterology 2002; 49: 1645-8.
7. Kawasaki T, Takeshita A, Souda K, et al. Serum thrombopoietin levels in patients with chronic hepatitis and liver cirrhosis. Am J Gastroenterol 1999; 94: 1918-22.
8. Schiodt FV, Balko J, Schilsky M, et al. Acute Liver Failure Study Group. Thrombopoietin in acute liver failure. Hepatology 2003; 37: 558-61.

9. Rios R, Sangro B, Herroro I, et al. The role of thrombopoietin in the thrombocytopenia of patients with liver cirrhosis. Am J Gastroenterol 2005; 100: 1311-6.
10. Giannini E, Botta F, Borro P, et al. Relationship between thrombopoietin serum levels and liver function in patients with chronic liver disease related to hepatitis C virus infection. Am J Gastroenterol 2003; 98: 2516-20.
11. Pugh RHN, Murray-Lyon IM, Dawson JL, et al. Transection of the oesophagus for bleeding oesophageal varices. Br J Surg 1973; 60: 646-9.
12. Okuda K. Idiopathic portal hypertension. In: Thomas HC, Jones EA, eds. Recent advances in hepatology. 2nd ed. Edinburg: Churchill Livingstone, 1986; 93-108.
13. Ferrara J, Ellison EC, Martin EW Jr, Cooperman M. Correction of hypersplenism following distal splenorenal shunt. Surgery 1979; 86: 4068-71.
14. Alvarez OA, Lopera GA, Patel V, et al. Improvement of thrombocytopenia due to hypertension after transjugular intrahepatic portosystemic shunt placement in cirrhotic patients. Am J Gastroenterol 1996; 91: 134-7.
15. Peck-Radosavljevic M, Zacherl J, Meng YG, et al. Is inadequate thrombopoietin production a major cause of thrombocytopenia in cirrhosis of the liver? J Hepatol 1997; 27: 127-31.
16. Wolber EM, Ganshow R, Burdelski M, Jelkman W. Hepatic thrombopoietin mRNA levels in acute and chronic liver failure of childhood. Hepatology 1999; 29: 1739-42.
17. Martin TG, Somberg KA, Meng YG, et al. Thrombopoietin levels in patients with cirrhosis before and after orthotopic liver transplantation. Ann Intern Med 1997; 127: 285-8.
18. Ishikawa T, Ichida T, Matsuda Y, et al. Reduced expression of thrombopoietin is involved in thrombocytopenia in human and rat liver cirrhosis. J Gastroenterol Hepatol 1998; 13: 907-13.
19. Kato T, Matsumoto A, Ogami K, et al. Native thrombopoietin: structure and function. Stem Cells 1998; 16: 322-8.
20. Plevris JN, Haydon GH, Simpson KJ, et al. Serum hyaluronan--a non-invasive test for diagnosing liver cirrhosis. Eur J Gastroenterol Hepatol 2000; 12: 1121-7.
21. Guechot J, Serfaty L, Bonnand AM, et al. Prognostic value of serum hyaluronan in patients with compensated HCV cirrhosis. J Hepatol 2000; 32: 447-52.
22. Murata K, Ochiai Y, Akashio K. Polydispersity of acidic glycosaminoglycan components in human liver and the changes at different stages in liver cirrhosis. Gastroenterology 1985; 89: 1248-57.
23. Yamashita Y, Shiota A, Fujise N, et al. Effects of the deleted form of hepatocyte growth factor on serum hyaluronate levels in rats with liver cirrhosis. J Vet Med Sci 1998; 60: 359-60.
24. Shimada Y, Kato T, Ogami K, et al. Production of thrombopoietin (TPO) by rat hepatocytes and hepatoma cell lines. Exp Hematol 1995; 23: 1388-96.
25. Schiodt FV, Balko J, Schilsky M, et al. Thrombopoietin in acute liver failure. Hepatology 2003; 37: 558-61.