

# Serum thrombopoietin levels in patients with non-alcoholic fatty liver disease

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## ABSTRACT

**الأهداف:** مراقبة مستويات الثرومبوبويتين لدى المرضى المصابين بمرض الكبد الدهني غير الناتج عن تعاطي الكحول.

**الطريقة:** أُجريت هذه الدراسة في قسم الطب الباطني، كلية الطب، جامعة فاتيه، أنقرة، تركيا وذلك خلال الفترة من نوفمبر 2010م إلى مارس 2011م. شملت الدراسة 60 مريض ثبتت إصابتهم بمرض الكبد الدهني غير الناتج عن تعاطي الكحول (مجموعة الدراسة)، و28 من المتطوعين الأصحاء (مجموعة الشاهد). لقد قمنا بتقسيم مجموعة الدراسة إلى 3 مجموعات فرعية اعتماداً على التصوير بالصدى (الإيكو) وهي: مجموعة الكبد الدهني من الدرجة الدنيا، ومجموعة الكبد الدهني من الدرجة المتوسطة، ومجموعة الكبد الدهني من الدرجة القصوى. وقد قمنا بمقارنة مستويات الثرومبوبويتين بين مجموعات الدراسة ومجموعة الشاهد.

**النتائج:** أشارت نتائج الدراسة إلى ارتفاع مستويات الثرومبوبويتين في مجموعات الدراسة بشكل ملحوظ من الناحية الإحصائية مقارنة بمجموعة الشاهد. كما كانت مستويات الثرومبوبويتين في مجموعة الكبد الدهني من الدرجة 1 أعلى من مجموعة الشاهد.

**خاتمة:** أثبتت هذه الدراسة بأن ارتفاع مستويات الثرومبوبويتين في مجموعة الدراسة المصابة بالكبد الدهني غير الناتج عن تعاطي الكحول قد يكون بسبب استجابته لانخفاض الالتهاب وذلك بسبب كونه من العناصر المتفاعلة في المراحل الحرجة. ولهذا يجب على الأطباء الحذر والتنبيه لارتفاع مستويات الثرومبوبويتين لدى المرضى.

**Objectives:** To observe thrombopoietin (TPO) levels in patients with non-alcoholic fatty liver disease (NAFLD).

**Methods:** The study was performed between November 2010 and March 2011 at the Department of Internal Medicine, Faculty of Medicine, Fatih

University, Ankara, Turkey. A total of 60 consecutive patients with ultrasound proven NAFLD (study group), and 28 healthy volunteers (control study) were included in the study. The patient group was divided into 3 subgroups according to the ultrasonographic images as follows: minimal, intermediate, and marked hepatosteatosis. The TPO levels of the patient subgroups were compared with the healthy controls. All the data were collected prospectively, and recorded in FUHIS data collecting system, which is produced by our data-knowledge team. Quantitative measurements of thrombopoietin level were carried out by using the Human Thrombopoietin Quantikine ELISA Kit (R&D Systems, Minneapolis, Minnesota, USA).

**Results:** Thrombopoietin levels were significantly increased in the patient subgroups compared with the controls. The TPO levels were also higher in the patient subgroup of grade 1-non-alcoholic fatty liver disease (grade 1- NAFLD) compared with the control group.

**Conclusion:** The TPO increased in patients with NAFLD possibly as an acute phase reactant to decreased inflammation. In clinical practice, physicians should be alerted to increased TPO levels in patients.

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Thrombopoietin (TPO) is the major physiological regulator of the megakaryocytic line, and platelet production. Its molecular weight is 31-35 kilogram daltons. The TPO levels are inversely proportional to the platelet size, and count. Thrombopoietin is mainly produced in liver; but if there is thrombocytopenia, bone marrow and spleen may also excrete this molecule. Decreased TPO, and low platelet count are seen in patients with cirrhosis.<sup>1</sup> Some studies reported that TPO accelerates liver regeneration after partial hepatectomy via increasing the platelet count.<sup>2</sup> In addition, TPO behaves like an acute phase reactant in inflammatory conditions, and is the reason for thrombocytosis in some infections.<sup>3,4</sup> Non-alcoholic fatty liver disease (NAFLD) is a common disease of the liver. This condition greatly causes fibrosis (40%), hepatic insufficiency (10-24%), and terminates as cirrhosis (30%).<sup>5</sup> Steatosis usually has a benign course, but non-alcoholic steatohepatitis is the most common etiologic factor for cryptogenic cirrhosis.<sup>6,7</sup> Liver failure inevitably results in decreased serum concentrations of substances produced in by the liver. However, inflammatory events of the liver may cause an increase in the acute phase reactants, and some of them can affect the hemostatic mechanisms. Therefore, the objectives of the current study are to determine TPO levels, and other biochemical parameters in patients with NAFLD.

**Methods.** The study was conducted between November 2010 and March 2011 at the Department of Internal Medicine, Faculty of Medicine, Fatih University, Ankara, Turkey. Sixty patients ( $47.7 \pm 1.4$  years) with non-alcoholic fatty liver disease (NAFLD), and 28 healthy volunteers ( $48.1 \pm 2.6$  years) were included. The patient and control groups were collected from subjects admitted to the internal medicine polyclinic for routine check up examination. All the data were collected prospectively, and recorded in FUHIS data collecting system which is produced by data- knowledge team of our hospital. Informed consent was taken from each subject. The study was conducted in accordance with the Declaration of Helsinki and local ethics committee approved the study.

After overnight fasting, blood specimens were collected from the patient and control groups. Fasting blood glucose, aspartate aminotransferases (AST), alanine aminotransferases (ALT), alkaline phosphatase (ALP), gamma-glutamyl transpeptidase (GGT), total and direct bilirubin, albumin, iron, iron binding capacity, ferritin, TPO, and complete blood count were studied and evaluated. Quantitative measurements of TPO were carried out by using the Human Thrombopoietin Quantikine ELISA Kit (R&D Systems, Minneapolis, Minnesota, USA).

The diagnosis of fatty liver disease is based on the ultrasonographic findings of increased echogenicity in the liver, which divides the patient group into the following subgroups: minimal (grade 1), intermediate (grade 2), and marked (grade 3). Ultrasonographic evaluation of the controls confirmed that there was no NAFLD. Liver function tests were also normal in the control group.

Patients with known hepatic disease such as viral hepatitis, drug induced liver injury, alcohol abuse, hepatocellular carcinoma and other forms of chronic liver disease, positivity of human immune deficiency virus, diseases causing low platelet count, previous venous thrombosis, anticoagulant, and those on immunosuppressive therapy, and contraceptives were excluded from the study.

Statistical analysis was carried out using the Statistical Package for Social Sciences version 15.0 for Windows (SPSS Inc., Chicago, Illinois, USA). For all the statistical analyses,  $p < 0.05$  was considered statistically significant. The normality of the variables was assessed by using the Shapiro-Wilk W Test. The continuous variables between 2 groups were compared with Mann-Whitney U-test. The relationship among the variables was analyzed using Spearman's correlation test.

**Results.** A total of 60 patients with ultrasound proven NAFLD were included in this study, and their results were compared with those of 28 healthy controls. Characteristics of the patients and controls are shown in Table 1. The male ratio was higher in the NAFLD group compared with the controls ( $p = 0.017$ ), but age distribution was similar between the groups. Serum AST and ALT levels were different, however, total bilirubin, GGT, and ALP were not significantly different between the patient and control group (Table 1). However, there was no significant difference between the control and NAFLD groups for hemoglobin, white blood cells, and platelet counts. The grade 1-NAFLD group (minimal echogenicity of the liver according to the ultrasonography) had a significantly lower albumin level than the control group. Iron levels, and iron binding capacities were within the normal ranges in both the NAFLD and control groups. However, ferritin levels were significantly higher in the patient group ( $p = 0.01$ ). The TPO levels were significantly increased in the patients compared with the controls ( $p = 0.012$ ). The TPO levels were also higher in the grade 1-NAFLD group compared with the control group ( $p = 0.033$ ).

**Discussion.** Non-alcoholic fatty liver disease (NAFLD) covers a wide spectrum of liver pathologies ranging from fatty liver (steatosis), to non-alcoholic steatohepatitis (NASH), and cirrhosis. All the stages of

**Table 1** - The demographical and clinical features of patients with NAFLD and a control group.

Parameters	Study group (n=60) (mean±SD)	Control group (n=28) (mean±SD)	P-values
Age	47.7±1.42	48.1±2.61	>0.05
Gender (male/female)	41/19	10/18	0.017
ALT	41.1±25.4	24.20±23.3	0.02
AST	25.05±9.37	19.9±7.49	0.04
ALP	74.23±21.7	78.8±34.47	>0.05
GGT	40.06±34.39	27.03±24.4	>0.05
Total bilirubin	0.77±0.59	0.46±0.21	>0.05
Platelet	230.4±55	239±58.15	>0.05
Ferritin	133.18±107.82	45.08±40.81	0.01
TPO	97.97±39.57	76.11±35.38	0.012

NAFLD - non-alcoholic fatty liver disease, ALT - alanine aminotransferases, AST - aspartate aminotransferase, ALP - alkaline phosphatase, GGT - gamma-glutamyl transpeptidase, TPO - thrombopoietin.

the disease have, in common, the fatty infiltration in the hepatocytes. However, non-alcoholic steatohepatitis, which causes fat accumulation, is associated with varying degrees of inflammation (hepatitis), and fibrosis of the liver. The liver produces many substances regulating hemostatic functions such as anticoagulants, fibrinolytics, TPO, tissue plasminogen activator (t-PA), and urokinase plasminogen activators. Also, the clearance of many coagulation factors, and fibrinolytics, end products of fibrinogen-fibrin conversion reaction occur eventuates in the liver. Some hemostatic alterations occur in liver diseases.<sup>8</sup>

Thrombocytopenia is a known complication of liver failure. It may result from decreased TPO production, increased platelet sequestration in the liver with hypersplenism, cirrhosis, or some autoimmune mechanisms.<sup>9</sup> In the case of cirrhosis, platelet production is less than the normal because of the decreased volume of the liver. However there is limited data on the TPO levels in NAFLD. Also, TPO behaves like a positive acute phase reactant in inflammatory conditions.<sup>10</sup> In our study, we observed that TPO and ferritin levels were significantly higher in the NAFLD group when compared with the control group. Because these 2 molecules behave like positive acute phase reactants, the result is not surprising. Inflammatory conditions may cause increased acute phase reactants; so inflammatory component of non alcoholic fatty liver disease may cause increased TPO levels. Some studies have also proven in some studies that TPO accelerates liver regeneration after partial hepatectomy by increasing the platelet count. Based on this information, TPO can improve liver regeneration in cases of loss of functional liver volume.<sup>11</sup> Because of the fibrotic potential of NAFLD to progress to the stage of NASH, loss a functional volume of the liver is present in this disease. Therefore, TPO may be

increased to elicit liver regeneration, and to provide liver regeneration after the loss of active hepatocyte volume in NASH patients. Elevated TPO levels in response to fibrosis may contribute to thrombotic complications via increased platelet counts. Ferritin levels are commonly elevated in patients with NAFLD as a result of systemic inflammation, and increased iron stores, or both. Hyperferritinemia is a common finding in obesity-related chronic inflammatory conditions such as diabetes, and metabolic syndrome.<sup>12,13</sup> It has also been shown that elevated ferritin level is associated with hepatic iron deposition, a diagnostic criterion of NASH, and worsened histologic activity, and it is an independent predictor of severe hepatic fibrosis among patients with NAFLD.<sup>14</sup>

This pilot study has some limitations. Firstly, a higher number of patients group would provide more reliability. Control of hypothesis may be carried out via assessment of TPO levels before and after the treatment and lifestyle modifications to ensure relationship between TPO and inflammation. Consequently, we can not say that TPO is increased because of the liver regeneration. It may be elevated resulting as a result of ongoing inflammation. Therefore, further studies are required.

In conclusion, NASH is a disease that has an inflammatory pattern, and progresses to fibrosis. Although TPO levels in plasma decrease in cirrhosis, they increase in NAFLD, and other inflammatory diseases of the liver. As fatty liver disease is common in practice, clinicians must be alerted to the thrombotic complications. However, thrombocyte count must be elevated correlated with increase in TPO; but because of small study population, we could not show this correlation.

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## Illustrations, Figures, Photographs

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