# An Investigation of the Relationship between Serum Vitamin B12 Levels and Hepatic Fibrosis Scores Determined by Transient Elastography (FibroScan) in Nonalcoholic Fatty Liver Disease*

## *Alkole Bağlı Olmayan Karaciğer Yağlanmasında Transient Elastografi (FibroScan) Tekniğiyle Belirlenen Fibrozis Skoru ile Serum Vitamin B12 Arasındaki İlişkinin Araştırılması*

Filiz Fusun Bolukbas[1], Seyda Ignak[2], Ozlem Unay Demirel[3], Yavuz Furuncuoglu[1], Ferhan Mantar[4], Sercen Cansu Sertel[5], Filiz Yarımcan Saglam[6], Cengiz Bolukbas[1]

[1] Bahcesehir University Medical Faculty, Internal Medicine, Department of Gastroenterology, Istanbul Turkey
[2] Bahcesehir University Medical Faculty, Department of Medical Biology, Istanbul Turkey
[3] Bahcesehir University Medical Faculty, Department of Biochemistry, Istanbul Turkey
[4] Bahcesehir University Medical Faculty, Internal Medicine, Department of Endocrinology, Istanbul Turkey
[5] Bahcesehir University Medical Faculty
[6] Bahcesehir University Medical Faculty, Department of Medical Microbiology, Istanbul Turkey

**Abstract**

**Aim:** Non-alcoholic fatty liver disease (NAFLD) is the most frequent chronic liver disease. Because NAFLD is a complex disease, finding highly specific and sensitive biomarkers for diagnosis is very difficult. We investigated the possible relation between steatosis and fibrosis stages determined by FibroScan technique and serum vitamin B12 levels as a non-invasive biomarker in patients with NAFLD.

**Materials and Methods:** A total of 129 patients (45.68±12.9 years of age, 29 females) with NAFLD and 50 healthy subjects (43.44±15.3 years of age, 21 females) were included in this study. FibroScan was performed in all patients for the staging of fatty liver fibrosis. Liver enzymes were also analyzed in addition to serum vitamin B12 and C-reactive protein (CRP) levels.

**Results:** There was no difference with respect to age and gender between NAFLD and control groups. The serum alanine aminotransferase, aspartate aminotransferase, gamma-glutamyl transferase, and CRP levels were significantly higher in the patients with NAFLD than the controls ($p<0.05$). On the contrary, serum vitamin B12 vitamin levels were lower in the patients with NAFLD, compared to the controls (352.8±125.2pg/mL vs 435.2±134.4, $p<0.01$). There was a significant difference in mean serum B12 vitamin levels between the control group (435.2±134.4pg/mL) and the NAFLD subgroups with fibrozis staged F0 and F3 (366.17±129.7pg/mL, 285.22±101pg/mL, $p<0.01$).

**Discussion and Conclusion:** Serum vitamin B12 levels were found to be significantly low in the patients with NAFLD in comparison to the control group. This decline in serum vitamin B12 levels was even more prominent as hepatic inflammation and fibrosis stage increased (F0–F3), but not in advanced fibrosis stage (F4).

**Keywords:** nonalcoholic fatty liver disease; fibrosis; FibroScan; liver; steatosis; vitamin B12

**Öz**

**Amaç:** Alkole bağlı olmayan karaciğer yağlanması (NAFLD) en sık rastlanan kronik karaciğer hastalığıdır. NAFLD kompleks bir hastalık olduğu için tanıda kullanılacak spesifik ve duyarlı biyobelirteçler bulmak oldukça güçtür. Çalışmamızda NAFLD'li hastalarda kullanılabilecek invaziv olmayan bir biyobelirteç olarak transient elastografi (FibroScan) tekniğiyle belirlenen steatozis ve fibrozis skorları ile serum vitamin B12 düzeyleri arasındaki olası ilişki araştırılmıştır.

**Gereç ve Yöntemler:** Çalışmamıza toplam 129 NAFLD hastası (45,68±12,9 yaş, 29 kadın) ve 50 sağlıklı gönüllü (43,44±15,3 yaş, 21 kadın) dahil edilmiştir. Tüm hastaların fibrozis evresi FibroScan yöntemiyle belirlenmiştir. Serum vitamin B12 ve C reaktif protein (CRP) düzeylerinin yanı sıra çeşitli karaciğer enzimleri de incelenmiştir.

**Bulgular:** Yaş ve cinsiyet açısından NAFLD ile kontrol grupları uyumlu bulunmuştur. NAFLD hastalarında serum alanin aminotransferaz, aspartat aminotransferaz, gama-glutamil transferaz ve CRP düzeyleri kontrol grubuna göre anlamlı oranda ($p<0,05$) yüksek bulunurken, serum vitamin B12 düzeyleri ise düşük (352,8±125,2pg/mL ile 435,2±134,4; $p<0,01$) bulunmuştur. Ortalama serum B12 düzeylerinde kontrol grubu (435,2±134,4pg/mL) ve NAFLD fibrozis evre F0, F3 alt grupları (366,17±129,7pg/mL, 285,22±101pg/mL) karşılaştırıldığında anlamlı düzeyde ($p<0,01$) farklılık olduğu görülmüştür.

**Tartışma ve Sonuç:** Serum vitamin B12 düzeyinin NAFLD hasta grubunda kontrol grubuna göre anlamlı oranda düşük olduğu saptanmıştır. Serum vitamin B12 seviyesindeki bu düşüşün özellikle karaciğer enflamasyonu arttıkça ve fibrozis evresi yükseldikçe (F0–F3) daha belirgin olduğu, ancak ileri fibrozis düzeylerinde (F4) anlamlı bir fark görülmediği tespit edilmiştir.

**Anahtar Sözcükler:** alkole bağlı olmayan yağlı karaciğer hastalığı; fibrozis; FibroScan; karaciğer; steatozis; vitamin B12

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Sorumlu Yazar/*Corresponding Author*
Asst. Prof. Seyda Ignak
Medical Faculty of Bahcesehir University, Department of Medical Biology, Sahrayıcedid Mh. Batman Sk. No:66-68, Yenisahra-Göztepe, Istanbul Turkey
E-mail: seyda_ignak@hotmail.com

## INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is one of the most frequent chronic liver disease that occurs across all age groups around the world due to the growing prevalence of obesity and overweight. NAFLD may be asymptomatic in most patients, but it is usually related with obesity and signs of metabolic syndrome such as type 2 diabetes, dyslipidemia, visceral adiposity, and hypertension. NAFLD includes a spectrum of hepatic pathology from benign hepatocellular steatosis to inflammatory nonalcoholic steatohepatitis, liver fibrosis, and cirrhosis. It was also shown that hepatocellular carcinoma may develop in patients with NAFLD (1–3).

The etiology of NAFLD is complex and multifactorial. There are theories which explain development and progression of NAFLD. According to the "two-hit hypothesis," factors such as hepatic accumulation of lipid via sedentary lifestyle, the insulin resistance (IR), high fat diet, obesity sensitize the liver as the first hit and prepare a substructure for the second hit. The second hit activates inflammatory processes and fibrogenesis. Because this hypothesis cannot explain sufficiently the various molecular and metabolic changes in NAFLD, however, the "multiple-hit hypothesis" has been developed. This hypothesis considers multiple effects such as insulin resistance, hormones secreted from adipose tissue, nutritional factors, and gut microbiota, as well as genetic and epigenetic factors which work together on genetically predisposed subjects to induce NAFLD (4).

Because NAFLD is a complex disease and includes a spectrum of conditions, finding highly specific and sensitive biomarkers for diagnosis is very difficult. At the present time, there are a variety of scoring systems and panels for evaluating the progression of fatty liver to non-alcoholic steatohepatitis (NASH) and cirrhosis by using different non-invasive parameters such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), platelet count, age, body mass index, etc. But none of them is specific for NAFLD and each has different limitations to determine the exact progression of NAFLD (5).

Confirmation of NAFLD diagnosis is usually achieved by radiologic imaging techniques, and staging of the disease requires a liver biopsy. Liver biopsy is the gold standard for the diagnosis and prognosis of NAFLD. However liver biopsy is an invasive procedure and leads to serious complications such as pain and hypotension. Liver biopsy can also increase the length of hospitalization and costs. In recent years some different non-invasive methods such as serum biomarkers and imaging methods such as ultrasonography and transient elastography (FibroScan) are frequently used to assess fibrosis and thus reduce the number of NAFLD patients requiring liver biopsy (6,7).

FibroScan (transient elastometer) is a device to examine liver stiffness. Lately published studies have shown that transient elastography is a valuable tool to detect fibrosis and cirrhosis in chronic hepatitis but has limitations in overweight patients. Also in some studies it was reported that transient elastography was accurate enough to detect high stage of fibrosis and cirrhosis without baseline etiology evaluation. *Hassemi* et al. have indicated that considering transient elastography in advanced fibrosis have results close to liver histology and that, because it can demonstrate the progress of liver fibrosis, it would be suitable in the follow-up period (8).

In our study, we tried to reveal the possible relation between staging of steatosis and fibrosis determined by FibroScan technique and serum vitamin B12 levels as a biomarker in patients with NAFLD.

## MATERIALS AND METHODS

We conducted a retrospective study. Our experimental group consisted of 129 patients with NAFLD whereas the control group consisted of 50 healthy controls from check-up outpatient clinic. The study was conducted between January 2011 and May 2015 in patients admitted to the Hospital's Gastroenterology Clinic with several metabolic complaints compatible with liver disease. This study was carried out in accordance with the Helsinki Declaration and approved by institutional medical ethics committee. For research involving human hospital records, informed consent

**Table 1**. Biochemical test results and demographic characteristics of NAFLD and control groups.

| Parameters | NAFLD (n=129) | Controls (n=50) | *p* |
|---|---|---|---|
| **Age** | 45.68±12.9 | 43.44±15.3 | 0.381 |
| **Gender (F/M), n** | 29/100 | 21/29 | 0.517 |
| **Vitamin B12** | 352.8±125.2 | 435.2±134.4 | 0.001 |
| **CRP** | 7.08±13.3 | 2.45±2.6 | 0.001 |
| **ALT** | 44.12±33.9 | 23.56±12.6 | 0.001 |
| **AST** | 28.75±18.3 | 20.7±5.9 | 0.03 |
| **GGT** | 51.44±34.4 | 21.59±9.3 | 0.001 |
| **ALP** | 73.11±27.5 | 64.10±19.6 | 0.055 |
| **Total cholesterol** | 214.93±49.4 | 211.92±49 | 0.668 |
| **Triglycerides** | 158.70±89.9 | 124.20±72.0 | 0.199 |
| **LDL cholesterol** | 142.20±38.4 | 137.66±41.6 | 0.874 |
| **HDL cholesterol** | 41.12±9.6 | 49.42±11.2 | 0.143 |
| **Platelet count** | 240.80±78.6 | 241.16±50.8 | 0.977 |
| **Hemoglobin** | 14.56±2.0 | 13.14±1.5 | 0.208 |
| **MCV** | 87.71±6.1 | 85.00±6.1 | 0.518 |
| **Glucose** | 107.7±34.3 | 96.12±17.7 | 0.032 |
| **Folic acid** | 6.63±1.9 | 7.70±2.7 | 0.364 |
| **Uric acid** | 5.00±1.2 | 5.33±1.7 | 0.674 |
| **Body mass index** | 28.4±3.6 | 27.8±4.1 | 0.26 |

CRP: C-reactive protein; ALT: alanine aminotransferase; AST: aspartate aminotransferase; GGT: gamma-glutamyl transferase; ALP: alkaline phosphatase; MCV: Mean cell volume

from each subject was obtained. Presence of the following constituted the exclusion criteria: chronic liver disease due to viral hepatitis, autoimmune liver disease, and metabolic or inherited liver disease, ascites, pregnancy, history of atrophic gastritis, megaloblastic anemia, malabsorption, Crohn's disease, vitamin B12 replacement therapy, and taking medications lowering vitamin B12 concentrations.

The grading of fatty liver was performed by using four known criteria (hepatorenal echo contrast, liver brightness, deep attenuation, and vascular blurring) based on ultrasonography performed by two radiologists with more than 5 years of experience using the Aloka SSD-650CL ultrasound machine (Aloka Co., Ltd., Tokyo, Japan) (9,10). Classification of fatty liver with ultrasound was done in three grades: Mild (Grade 1): minimal diffuse increase in hepatic echogenicity, with normal diaphragm and intrahepatic vessel contour; Moderate (Grade 2): diffuse increase in hepatic echogenicity, slight deterioration in the visualization of the diaphragm and intrahepatic vessels; and Severe (Grade 3): marked increase in hepatic echogenicity, posterior segment of the right hepatic lobe is difficult to display, poor visualization of the intrahepatic vessels and diaphragm contour. Patients with Grade 1 to 3 hepatic steatosis on USG were included within the study.

FibroScan is also known as transient elastography that is used to evaluate the staging of steatosis and fibrosis. It is a reliable non-invasive technique and can be used as an alternative of liver biopsy (11). Liver stiffness evaluation (LSE) and liver steatosis detection and quantification were done with FibroScan device (Echosens touch 502 powered by VCTE, Paris, France) by a certificated hepatologist. LSE scores were measured as kilopascal (kPa). At least 10 successful measurements and a success rate over 60% with interquartile range/median ratios of <0.30 were obtained for a valid measurement. In our study, LSE cut-off scores for describing F0, F1, F2, F3, F4 were <6kPa, 6–7.2kPa, 7.2–9kPa, 9–11.8kPa, and >11.8kPa respectively. Controlled Attenuation Parameter (CAP) was used for detecting and quantifying steatosis and was described as dB/m.

Serum vitamin B12 levels were analyzed by using chemiluminescent microparticle immunoassay (CMIA) technology in Abbott Architect ci8200 system (Abbott Park, IL, USA).

In addition to serum vitamin B12 levels, blood samples of the subjects were evaluated in terms of liver enzymes including aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP); and C-reactive protein (CRP), total bilirubin, albumin, total cholesterol, triglycerides, high density lipoprotein (HDL), low density lipoprotein (LDL), platelet count, mean corpuscular volume (MCV), folic acid were analyzed by Sysmex XN-1000 hematology analyzer (Sysmex, Kobe, Japan).

To compare the parameters between test and control groups, independent t-test was used. The patients in the each group were compared with the control group and $p<0.05$ was accepted as statistically signifi-

**Table 2.** The biochemical test results according to ultrasonography grades in test and control groups

| Parameter | Ultrasonography | | | | | | |
|---|---|---|---|---|---|---|---|
| | Grade 1(n=83) | *p* | Grade 2(n=40) | *p* | Grade 3(n=6) | *p* | Control (n=50) |
| **Vitamin B12** | 351.0±125.2 | 0.01 | 360.15±126.6 | 0.01 | 322.0±96.0 | 0.05 | 435.28±134.4 |
| **CRP** | 7.5±14.2 | 0.01 | 5.7±12.0 | 0.06 | 10.7±11.8 | 0.01 | 2.44±2.6 |
| **ALT** | 43.8±35.2 | 0.01 | 60.9±38.1 | 0.01 | 110±47.7 | 0.01 | 23.56±12.6 |
| **AST** | 30.02±24.2 | 0.01 | 31.2±16.2 | 0.01 | 51.0±15.0 | 0.01 | 20.7±5.9 |
| **GGT** | 61.4±19.6 | 0.01 | 21.5±9.3 | 0.01 | 106.75±50.4 | 0.01 | 21.59±9.3 |
| **ALP** | 75.9±33.2 | 0.03 | 64.1±19.6 | 0.18 | 71.0±18.9 | 0.45 | 64.1±19.6 |

CRP: C-reactive protein; ALT: alanine aminotransferase; AST: aspartate aminotransferase; GGT: gamma-glutamyl transferase; ALP: alkaline phosphatase

cant. The data were analyzed by using SPSS for Windows 11.0 software.

## RESULTS

In our study, there was no statistical difference with respect to age and gender between the NAFLD and control groups. The serum ALT, AST and GGT levels were found significantly higher in the NAFLD patients than in the controls ($p<0.05$). On the contrary, serum vitamin B12 levels were lower in the patients with NAFLD compared with the controls (352.8±125.2 pg/mL vs. 435.2±134.4 $p<0.01$). Although both are in the normal reference interval range, vitamin B12 levels were significantly lower in our NAFLD group when compared to the control group ($p< 0.05$). BMI values were evaluated and found to be within normal limits. The values are similar in stages of fibrosis and the control group. Table 1 summarizes the results of the parameters analyzed in our study.

The mean values of vitamin B12 levels and other laboratory parameters that were found statistically significant between the NAFLD and control groups are presented in Table 2 according to staging by ultrasonography and in Table 3 according to staging by FibroScan.

According to the results there was a significant difference in the mean serum vitamin B12 levels between the controls (435.2±134.4pg/mL) and the NAFLD patients from the Grade 1 and 2 subgroups ($p<0.05$) determined by ultrasonography. But the difference was not statistically significant between the Grade 3 NAFLD patients and controls.

According to the results there was a significant difference in the mean serum vitamin B12 levels between the controls (435.2±134.4pg/mL) and NAFLD patients from F0 to F3 subgroups (366.17±129.7pg/mL, 285.22±101pg/mL, $p<0.01$) determined by FibroScan. But the difference was not statistically significant between the Grade F4 NAFLD patients and controls. The CRP levels of the controls and NAFLD patients from F0 to F4 were also compared, and it was found that there was statistically significant difference between the CRP levels of the NAFLD patients from F1, F3 and F4, but not F2.

The association between the B12 and CRP levels and steatosis grade from A0 to A3 in the NAFLD patients determined by FibroScan were also investigated. There was statistically significant difference between the vitamin B12 levels of the A0 and A3 subgroups of NAFLD patients and the control group (Table 4).

## DISCUSSION AND CONCLUSION

About a quarter of patients with fatty liver develop liver inflammation (nonalcoholic steatohepatitis-NASH) and over a quarter of NASH patients develop severe fibrosis, which is associated with a high mortality rate. Thus detection of inflammation in fatty liver is very important by means of managing NAFLD. Inflammation may be diagnosed by microscopic examination of liver biopsy specimens. However, liver biopsy is an invasive procedure that has a serious risk of complication (5). In the literature the mortality due to liver biopsy is reported to be as high as 2% (12).

**Table 3.** The biochemical test results according to FibroScan stages in test and control groups

| Parameter | FİBROSCAN (Fibrosis Stage) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | F0(n=72) | *p* | F1(n=17) | *p* | F2(n=12) | *p* | F3(n=9) | *p* | F4(n=19) | *p* | Control (n=50) |
| **Vitamin B12** | 366.17±129.7 | 0.01 | 309.59±101.4 | 0.01 | 337.73±108.8 | 0.04 | 285.22±101.0 | 0.01 | 368.24±124.8 | 0.19 | 435.28±134.4 |
| **CRP** | 5.1±7.2 | 0.02 | 17.02±27.8 | 0.01 | 2.4±1.2 | 0.94 | 12.4±13.7 | 0.01 | 4.86±5.0 | 0.05 | 2.44±2.6 |
| **ALT** | 46.2±34.7 | 0.01 | 50.00±31.8 | 0.01 | 79.1±50.4 | 0.01 | 39.71±25.7 | 0.01 | 65.64±51.0 | 0.01 | 23.56±12.6 |
| **AST** | 28.1±21.4 | 0.02 | 30.33±10.7 | 0.01 | 40.89±25.4 | 0.01 | 23.00±7.5 | 0.38 | 47.42±28.3 | 0.01 | 20.7±5.9 |
| **GGT** | 51.3±34.3 | 0.01 | 64.14±28.9 | 0.01 | 41.18±25.7 | 0.01 | 88.00±98.4 | 0.01 | 111.23±96.8 | 0.01 | 21.59±9.3 |
| **ALP** | 68.8±25.7 | 0.3 | 79.9±45.7 | 0.06 | 71.1±14.6 | 0.21 | 87.3±26.0 | 0.01 | 81.08±25.3 | 0.01 | 64.1±19.6 |

CRP: C-reactive protein; ALT: alanine aminotransferase; AST: aspartate aminotransferase; GGT: gamma-glutamyl transferase; ALP: alkaline phosphatase

While ultrasonography has many advantages such as safety, low cost, and repeatability (13), at the same time it has some important limitations including the relatively low sensitivity in detecting minor degrees of fatty liver changes, the low accuracy in obese patients, the ability to differentiate simple steatosis from NASH (14). FibroScan, on the other hand, measures liver stiffness which is well correlated with fibrosis stage in NASH, can be affected by elevated ALT levels, steatosis and may have technical problems especially by the presence of abdominal obesity (15,16).

According to the literature, one of the limitations of FibroScan is BMI and especially waist circumference measurement. There are two probes named as M and XL in FibroScan. Any misleading effect of obesity can lead to unreliable results that can be minimized significantly by probe choice (17,18). To overcome this limitation we used both M and XL probes when performing FibroScan procedure. When we used XL probe we detected LES scores, but not the Controlled Attenuation Parameter (CAP) score in obese patients. Also, there is no significant difference between our NAFLD and control groups in terms of age, sex and BMI. We observed hepatosteatosis in all patients in our NAFLD group determined by ultrasonography. On the other hand, the steatosis grade determined by FibroScan was within normal limits in 41 patients, and this group was classified as A0. In our A0 group, the level of ALT, GGT, CRP were higher and the vitamin B12 levels were significantly lower than those in the control group. Different types of probes are needed to be developed for the evaluation of these cases.

Vitamin B12 (cobalamin) is a water-soluble cobalt containing vitamin with an important role in biochemical processes. Ingested dietary vitamin B12 in food is released by the exposition of the low pH within the gastric lumen. After the release of vitamin B12, it is bound to haptocorrin (transcobalamin I) and carried to the duodenum. In the duodenum vitamin B12-transcobalamin complex undergo proteolytic cleavage, and vitamin B12 is bound to another carrier protein, intrinsic factor (IF) that is secreted by the gastric mucosa. This IF-cobalamin complex is absorbed by the terminal ileum. Then vitamin B12 dissociates from IF and enters the blood circulation where it binds transcobalamin II. Subsequently, vitamin B12 arrives in peripheral tissues and the liver via transcobalamin II. Vitamin B12 is mostly stored in the liver (19,20).

It is reported in several studies that liver diseases such as acute hepatitis, cirrhosis, hepatocellular carcinoma and metastatic liver disease are associated with major changes in vitamin B12 levels in the blood (19).

Ermens et al. have suggested that inflammation-induced cell degradation causes the release of stored cobalamin, which in the circulation predominantly binds to haptocorrin. They report that plasma vitamin B12 level can reach high values as the severity of liver cirrhosis progresses (21).

Joske et al. reported that cirrhosis might be accompanied by relative vitamin B12 deficiency, secondary to impaired liver storage. This might be due to the increased release during hepatic cytolysis and/or decreased clearance by the affected liver (22).

Holdsworth et al. observed in their study that cirrhotic patients had lower serum vitamin B12 level. They explain that the vitamin B12 stores of the cirrhotic liver are already severely depleted and that cellular necrosis could liberate less of the vitamin into the bloodstream or the actual rate of cellular damage and necrosis gets slower as the fibrosis progresses (23).

In our study the vitamin B12 level was significantly lower in the NAFLD group than in the control group, and especially the difference between the two groups became more significant as the fibrosis grade determined by FibroScan increased from F1 to F3. Although in the F4 group the mean vitamin B12 level is still lower than in the control group, the difference is not statistically significant. However, vitamin B12 level in the F4 group is relatively higher than in the F1–F3 groups. We think that as the severity of the fibrosis progresses from F1 to F3 the serum vitamin B12 level decreases due to the damage in the liver, and vitamin B12 stores in the liver are liberated slowly. In the F4 group, because of the severe hepatocyte necrosis, vitamin B12 level gets relatively higher than in the F3 group in serum. Since we have a small number of patients in the F4 group, further studies with more patients on vitamin B12 level in advanced fibrosis and cirrhosis are needed to be done to prove our hypothesis.

Cobalamin is a cofactor and coenzyme involved in many biochemical reactions such as DNA synthesis, and methionine synthesis from homocysteine (20). Homocysteine levels are measured in some studies as an independent risk factor for cardiovascular disease and atherosclerosis (24,25). Furthermore NAFLD and its strong relationship with metabolic syndrome has lead scientists to investigate the possible role of NAFLD in the development of cardiovascular disease (CVD). Recent studies also pointed out the association between NAFLD and high cardiovascular risk (2,27,28).

Pro-inflammatory cytokines including tumor necrosis factor-alpha (TNF-α) and IL-6 are the major stimuli responsible for increased hepatic production of C-reactive protein (CRP) in NAFLD (26,27,28). According to various studies, the severity of histological features of NAFLD is also strongly correlated to increased hs-CRP levels as well as to increased insulin resistance and higher prevalence of the metabolic syndrome (MetS). Inflammation, including CRP level, is strongly related to insulin resistance and/or MetS (29). Thus, in our study high CRP levels can indicate predisposition to MetS and its risks.

It has been shown that insulin may affect some enzymes involved in homocysteine (Hcy) turnover and induce hyperhomocysteinemia as a possible component of metabolic syndrome. Also, the low status of vitamin B12 may be a potential trigger contributing to the development of hyperhomocysteinemia. Gulsen et al. observed that plasma Hcy concentrations significantly correlated with both grade of activity and stage of fibrosis in NASH patients (30). Our study is limited by lack of Hcy level.

The literature includes many studies evaluating the relationship between liver diseases and serum vitamin B12 levels because of the role of the liver in vitamin B12 metabolism and storage. These studies especially

**Table 4.** The biochemical test results according to FibroScan grades in test and control groups

| Parameter | FIBROSCAN (Steatosis Grade) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | A0(n=41) | *p* | A1(n=19) | *p* | A2(n=21) | *p* | A3(n=48) | *p* | Control (n=50) |
| **Vitamin B12** | 345.58±119.8 | 0.01 | 396.89±127.7 | 0.28 | 354.7±157.7 | 0.47 | 338.26±112.3 | 0.01 | 435.28±134.4 |
| **CRP** | 7.88±14.1 | 0.01 | 6.4±9.0 | 0.01 | 3.97±4.9 | 0.12 | 7.82±15.9 | 0.02 | 2.44±2.6 |
| **ALT** | 39.5±31.3 | 0.01 | 33.35±15.3 | 0.01 | 75.65±39.6 | 0.01 | 60.28±44.4 | 0.01 | 23.56±12.6 |
| **AST** | 28.9±27.5 | 0.04 | 24.13±8.4 | 0.08 | 38.72±17.8 | 0.01 | 32.92±20.9 | 0.01 | 20.7±5.9 |
| **GGT** | 47.03±27.8 | 0.01 | 43.33±31.4 | 0.01 | 68.94±52.8 | 0.01 | 79.73±73.0 | 0.01 | 21.59±9.3 |
| **ALP** | 75.39±42.2 | 0.11 | 66.85±17.8 | 0.65 | 74.31±20.0 | 0.07 | 74.47±23.3 | 0.03 | 64.1±19.6 |

CRP: C-reactive protein; ALT: alanine aminotransferase; AST: aspartate aminotransferase; GGT: gamma-glutamyl transferase; ALP: alkaline phosphatase

focus on acute liver diseases, chronic liver diseases, and hepatocellular carcinoma (HCC) (20).

Koplay et al. compared 45 NAFLD patients with 30 healthy controls by means of ultrasonographic grading of fatty liver and assessing vitamin B12 levels. They found that the serum vitamin B12 levels were significantly lower in the NAFLD patients than in the control group, though levels still remained in the reference range. They proposed that low vitamin B12 levels might be associated with NAFLD, especially in grade 2 and grade 3 hepatosteatosis (31).

Nevertheless there are a few reports addressing the relationship of fibrosis in NAFLD and vitamin B12 levels. Polysoz et al. compared serum vitamin B12 levels in 30 liver biopsy-proven NAFLD patients with 24 healthy controls and investigated the association between B12 levels and disease severity. They did not find any significant difference between the two groups (32).

It has recently been reported that vitamin B12 level is an important indicator in the therapeutic process of HCV. It has also been emphasized that vitamin B12 deficiency causes impaired production of succinyl-CoA and methionine, increasing the risk of hepatic steatosis (33). The relation between vitamin B12 and steatosis observed in chronic hepatitis has not been clearly revealed yet. This subject is open to dispute.

If it had been possible to determine the plasma cytokeratin 18 (X-18) and its fragment levels in conjunction with FibroScan method in our study group, it would have strengthened our study. Because of the design of our study, we could not provide this progress. We hope further studies will focus not only on the diagnosis, but also on the treatment of NAFLD as well.

In conclusion, serum vitamin B12 levels were found to be significantly decreased in the patients with NAFLD than in the control group. The decline in the serum vitamin B12 levels was even more prominent as the liver inflammation and fibrosis stage increased, but not in advanced fibrosis stage. Further studies are needed to determine the role of serum vitamin B12 as a biological marker for NAFLD and an inflammatory marker in patients with severe liver fibrosis.

## REFERENCES


1. Abd El-Kader SM, El-Den Ashmawy EM. Non-alcoholic fatty liver disease: The diagnosis and management. World J Hepatol. 2015; 7(6):846–58.
2. Baffy G, Brunt EM, Caldwell SH. Hepatocellular carcinoma in non-alcoholic fatty liver disease: an emerging menace. J Hepatol. 2012;56(6):1384–91.
3. de Carvalho SC, Muniz MT, Siqueira MD, Siqueira ER, Gomes AV, Silva KA, et al. Plasmatic higher levels of homocysteine in non-alcoholic fatty liver disease (NAFLD). Nutr J. 2013;12:37.
4. Buzzetti E, Pinzani M, Tsochatzis EA. The multiple-hit pathogenesis of non-alcoholic fatty liver disease (NAFLD). Metabolism. 2016;65(8):1038–48.
5. Sanal MG. Biomarkers in nonalcoholic fatty liver disease-the emperor has no clothes? World J Gastroenterol. 2015;21(11):3223–31.
6. Farrell GC, Chitturi S, Lau GK, Sollano JD. Guidelines for the assessment and management of non-alcoholic fatty liver disease in the Asia-Pacific region: executive summary. J Gastroenterol Hepatol. 2007;22(6):775–7.
7. Loria P, Adinolfi LE, Bellentani S, Bugianesi E,Grieco A,Fargion S, et al. Practice guidelines for the diagnosis and management of nonalcoholic fatty liver disease. A decalogue from the Italian Association for the Study of the Liver (AISF) Expert Committee. Dig Liver Dis. 2010;42(4):272–82.
8. Hashemi SA, Alavian SM, Gholami-Fesharaki M. Assessment of transient elastography (FibroScan) for diagnosis of fibrosis in non-alcoholic fatty liver disease: a systematic review and meta-analysis. Caspian J Intern Med. 2016;7(4):242–252.
9. Osawa H, Mori Y. Sonographic diagnosis of fatty liver using a histogram technique that compares liver and renal cortical echo amplitudes. J Clin Ultrasound. 1996;24(1):25–9.
10. el-Hassan AY, Ibrahim EM, al-Mulhim FA, Nabhan AA, Chammas MY. Fatty infiltration of the liver: analysis of prevalence, radiological and clinical features and influence on patient management. Br J Radiol. 1992;65(777):774–8.
11. Wong VW, Vergniol J, Wong GL, Foucher J,Chan HL,Le Bail B, et al. Diagnosis of fibrosis and cirrhosis using liver stiffness measurement in nonalcoholic fatty liver disease. Hepatology. 2010;51(2):454–62.
12. Thampanitchawong P, Piratvisuth T. Liver biopsy: complications and risk factors. World J Gastroenterol. 1999;5(4):301–4.

13. Saadeh S, Younossi ZM, Remer EM, Gramlich T, Ong JP, Hurley M, et al. The utility of radiological imaging in nonalcoholic fatty liver disease. Gastroenterology. 2002;123(3):745–50.
14. Roldan-Valadez E, Favila R, Martínez-López M, Uribe M, Méndez-Sánchez N. Imaging techniques for assessing hepatic fat content in nonalcoholic fatty liver disease. Ann Hepatol. 2008;7(3):212–20.
15. Castéra L, Foucher J, Bernard PH, Carvalho F, Allaix D, Merrouche W, et al. Pitfalls of liver stiffness measurement: a 5-year prospective study of 13,369 examinations. Hepatology. 2010;51(3):828–35.
16. Wong GL. Update of liver fibrosis and steatosis with transient elastography (FibroScan). Gastroenterol Rep (Oxf). 2013;1(1):19–26.
17. de Lédinghen V, Wong VW, Vergniol J, Wong GL, Foucher J, Chu SH, et al. Diagnosis of liver fibrosis and cirrhosis using liver stiffness measurement: comparison between M and XL probe of FibroScan®. J Hepatol. 2012;56(4):833–9.
18. Wong VW, Vergniol J, Wong GL, Foucher J, Chan AW, Chermak F, et al. Liver stiffness measurement using XL probe in patients with nonalcoholic fatty liver disease. Am J Gastroenterol. 2012;107(12):1862–71.
19. Shipton MJ, Thachil J. Vitamin B12 deficiency - a 21st century perspective. Clin Med. 2015;15(2):145–50.
20. Andrès E, Serraj K, Zhu J, Vermorken AJ. The pathophysiology of elevated vitamin B12 in clinical practice. QJM. 2013;106(6):505–15.
21. Ermens AA, Vlasveld LT, Lindemans J. Significance of elevated cobalamin (vitamin B12) levels in blood. Clin Biochem. 2003;36(8):585–90.
22. Joske RA. The vitamin B12 content of human liver tissue obtaıned by aspiration biopsy. Gut. 1963;4:231–5.
23. Holdsworth CD, Atkinson M, Dossett JA, Hall R. Assessment of the diagnostic and prognostic value of serum vitamin B12 levels in liver disease. Gut. 1964;5:601–6.
24. Catena C, Colussi G, Nait F, Capobianco F, Sechi LA. Elevated homocysteine levels are associated with the metabolic syndrome and cardiovascular events in hypertensive patients. Am J Hypertens. 2015;28(7):943–50.
25. Leach NV, Dronca E, Vesa SC, Sampelean DP, Craciun EC, Lupsor M, et al. Serum homocysteine levels, oxidative stress and cardiovascular risk in non-alcoholic steatohepatitis. Eur J Intern Med. 2014;25(8):762–7.
26. Uchihara M, Izumi N. High-sensitivity C-reactive protein (hs-CRP): a promising biomarker for the screening of non-alcoholic steatohepatitis (NASH). Nihon Rinsho. 2006;64:1133–8.
27. Wieckowska A, Papouchado BG, Li Z, Lopez R, Zein NN, Feldstein AE. Increased hepatic and circulating interleukin-6 levels in human nonalcoholic steatohepatitis. Am J Gastroenterol. 2008;103(6):1372–9.
28. Yoneda M, Mawatari H, Fujita K, Iida H, Yonemitsu K, Kato S, et al. High-sensitivity C-reactive protein is an independent clinical feature of nonalcoholic steatohepatitis (NASH) and also of the severity of fibrosis in NASH. J Gastroenterol. 2007;42:573–82.
29. Marchesini G, Marzocchi R, Agostini F, Bugianesi E. Nonalcoholic fatty liver disease and the metabolic syndrome. Curr Opin Lipidol. 2005;16(4):421–7.
30. Gulsen M, Yesilova Z, Bagci S, Uygun A, Ozcan A, Ercin CN, et al. Elevated plasma homocysteine concentrations as a predictor of steatohepatitis in patients with nonalcoholic fatty liver disease. J Gastroenterol Hepatol. 2005;20(9):1448–55.
31. Koplay M, Gulcan E, Ozkan F. Association between serum vitamin B12 levels and the degree of steatosis in patients with nonalcoholic fatty liver disease. J Investig Med. 2011;59(7):1137–40.
32. Polyzos SA, Kountouras J, Patsiaoura K, Katsiki E, Zafeiriadou E, Zavos C, et al. Serum vitamin B12 and folate levels in patients with non-alcoholic fatty liver disease. Int J Food Sci Nutr. 2012;63(6):659–66.
33. Amer RM, Amin MA, Sabry D, Abd Elaziz A. Impact of serum level of vitamin B12 on response to combined interferon and ribavirin therapy for chronic HCV Infection. Med. J. Cairo Univ. 2016;84(1):529–35.