# SERUM HOMOCYSTEINE LEVELS IN PATIENTS WITH NON-ALCOHOLIC FATTY LIVER DISEASE

*Ali Çetinkaya[1], Bülent Kantarçeken[1], M Akif Büyükbeşe[2], Mustafa Çelik[3] , Fatma İ Tolun[4]*

Kahramanmaras Sutcu Imam University, Faculty of Medicine, Departments of Gastroenterology[1], Internal Medicine[2], Family Phycisian[3] and Biochemistry[4], Kahramanmaraş,Turkey

**Aim:** Non-alcoholic fatty liver disease (NAFLD) is a common disorder which causes serum liver enzyme elevation. Elevated homocysteine levels was demonstrated in fatty liver disease and chronic liver failure. However, enough data related with homocysteine levels in patients with NAFLD is not available. We aimed to find out whether there is an association between homocysteine levels and NAFLD.

**Methods:** Twenty patients (14 men, 6 women) with NAFLD and 20 healthy adults (13 men, 7 women) enrolled in the study. Fasting blood samples were obtained and serum homocysteine levels were measured by fluorescence polarization immunoassay (FPIA) technology. Oral glucose tolerance test was performed and serum insulin, c-peptide, and lipoprotein levels were also measured.

**Results:** The mean serum homocysteine levels (+/-SD) were 13.44±3.10 µmol/L and 11.62 ±1.34 µmol/L in NAFLD and the control group, respectively. Mean serum homocysteine level in the NAFLD group was significantly higher than in control group (p=0.015). Fasting blood glucose, insulin, total cholesterol and low density lipoprotein (LDL) cholesterol were all found higher than the control group.

**Conclusion:** The serum homocysteine levels were significantly higher in patients with NAFLD than in control group. This may point out that high homocysteine levels may be associated with NAFLD.

**Key words:** Homocysteine, non-alcoholic steatohepatitis, insulin resistance

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## INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) which was characterized by the association of fatty liver and lobular hepatitis and chronically elevated plasma levels of alanine transaminase (ALT) in patients with negligible alcohol intake was first identified by Ludwig et al (1). The etiology of NAFLD is not known completely, but it is found in a variety of clinical settings, particularly in those patients with obesity, diabetes or hyperlipidemia (2-4). However, predisposing factor is not defined in some cases. In addition, the pathogenesis of NAFLD remains unclear. A central role for cytotoxic free fatty acids released from the accumulation of intrahepatic triglycerides is one mechanism in the pathogenesis of NAFLD. Related to this hypothesis, obesity which gives rise to insulin resistance, hypertriglyceridemia and leptin resistance are thought to play an important role (5). Eventough potentially, NAFLD can progress to fibrosis, cirrhosis, and eventually terminal liver failure (6).

Homocysteine is a product of methionine metabolism. Hyperhomocysteinemia has been found in patients with type 2 and type 1 diabetes mellitus associated with premature atherosclerosis (7,8). Several observations suggest that there might be links between insulin resistance and hyperhomocysteinemia (9-11). Higher levels of homocysteine were also demonstrated in healthy non-obese subjects (9). However, homocysteine is an

***Correspondence:****Ali ÇETINKAYA*
*Kahramanmaras Sütçü İmam Üniversitesi, Tıp Fakültesi*
*Gastroenteroloji BD, 46050 Kahramanmaras–Turkey*
*Phone: 903442212337, Fax: 903442212371*
*E-mail: drcetinkaya@yahoo.com*

**Table 1. The results of main biochemical tests and demographic findings in groups.**

| Variable | Group NAFLD (n=20) | Controls (n=20) | p |
|---|---|---|---|
| Sex (M/F) | 6/14 | 7/13 | ns |
| Age, year (range) | 42.15 ± 10.22 (29-60) | 40.66 ± 7.95 (29-53) | ns |
| BMI, kg/m$^2$ (range) | 31.09 ± 4.33 (24.5-38) | 31.38 ± 5.18 (22.4-39) | ns |
| Waist circumference (cm) | 96.84 ± 10.56 | 96.33 ± 10.07 | ns |
| Homocysteine (μmol/L) | 13.44 ± 3.10 | 11.62 ± 1.34 | 0.015 |
| Vitamin $B_{12}$ (pg/mL) | 282.84 ± 92.22 | 226.33 ± 33.45 | ns |
| Folate (ng/mL) | 5.86 ± 1.43 | 4.94 ± 1.37 | ns |
| C-peptide (ng/mL) | 1.82 ± 0.42 | 1.58 ± 0.29 | ns |
| Insulin (ng/mL) | 12.36 ± 5.51 | 8.77 ± 2.37 | 0.009 |
| ALT (U/L) | 110.69 ± 19.58 | 50.66 ± 14.79 | 0.001 |
| AST (U/L) | 48.23 ± 13.10 | 24.77 ± 6.37 | 0.001 |
| Glucose (ng/dL) | 100.23 ± 8.96 | 87.66 ± 14.10 | 0.048 |
| Total cholesterol (mg/dL) | 200.76 ± 21.91 | 180.77 ± 14.52 | 0.041 |
| Triglyceride (mg/dL) | 154.61 ± 55.33 | 165.66 ± 87.87 | ns |
| LDL (mg/dL) | 126.53 ± 19.86 | 105.68 ± 39.10 | 0.049 |
| HDL (mg/dL) | 43.30 ± 9.72 | 50.77 ± 18.45 | ns |

*ns: nonsignificant*

atherogenic and thrombogenic risk factor (12) and may be involded hepatic fibrosis (13). Investigators reported in some studies that there was a link between homocysteine and alcoholic liver damage leading to fibrosis (14,15).

The aim of this study is to evaluate possible association of homocysteine with NAFLD.

## MATERIAL AND METHODS

A total of 20 patients with NAFLD and age, sex and BMI matched 20 healthy subjects as control group were enrolled in the study. NAFLD patients had been referred for a comprehensive assessment of liver function because of the presence of ultrasonographic evidence of hepatic steatosis and elevated transaminase concentrations (alanine transaminase [ALT]) in excess of twice the upper normal limits at least one occasion during the preceding 6 months. For the diagnoses of NAFLD and to rule out other possible liver diseases, all patients with NAFLD underwent a detailed clinical and laboratory evaluation, including liver function tests, hepatitis markers and autoantibodies. Fatty liver was classified as grade 1 (mild), grade 2 (moderate) and grade 3 (severe). Alcohol consumption was absent in all subjects. Since all patients in NAFLD group were non smokers, smokers were excluded from the control group. Renal functions of the subjects were within normal limits. The patients with a history of drug usage in the last 2 months were excluded from the study.

Informed written consent was obtained for each subject. All blood samples were collected in the morning after an overnight fast. Blood samples were drawn in a vacutainer blood-collecting tubes (Becton-Dickinson, Franklin Lakes, NJ) according to standard hospital guidelines for venipuncture and sample collection. Homocysteine specimens were placed on ice and all specimens were transported to the laboratory within 30 minutes of collection. Serum was obtained after centrifugation at 2000 x g for 10 minutes, frozen, and stored at –20 ºC until analysis. Serum total homocysteine concentrations were measured by using an IMX (Abbott diagn. USA) homocysteine assay. Assay is based on the fluorescence polarization immunoassay (FPIA) technology. Apart from that, serum insulin, c-peptide, serum lipoproteins and other biochemical tests were also evaluated in the NAFLD and control groups. All patients were asked to drink 75 gr glucose dissolved in 200 cc water and 2-hour glucose levels were detected. All results are expressed as mean ± SD. The mean homocysteine levels, vitamin $B_{12}$, insulin, c-peptide and other serum parameters in both groups were compared by Mann-Whitney U test as the distribution between the groups were not normal. The same test was also used for the comparison of homocysteine levels. The correlations were given by the Pearson correlation coefficient (r). All analyses were two tailed and were conducted using a computer-based statistics software (SPSS for Windows 9.0, 1998, SPSS, Chicago, IL). A p value of less than 0.05 was accepted as statistically significant.

## RESULTS

The results of main parameters studied in both groups are summarized in Table 1. In NAFLD Group, while there were 6 subjects

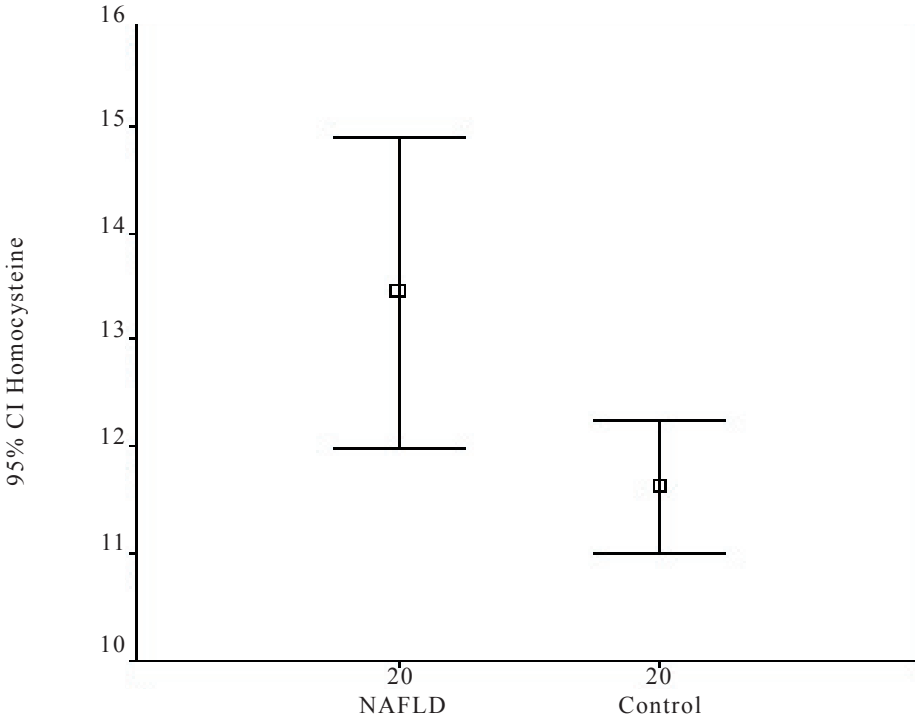


**Figure 1. The homocysteine levels in non-alcoholic fatty liver disease patients and controls (bar graphic with 95 % confidence interval for the mean)**

who had impaired glucose tolerance, no subjects had diabetes. Control group had neither impaired glucose tolerance nor diabetes. As another risk factor when obesity is taken into consideration for NAFLD, in the study group all subjects except for 5 subjecst had a BMI over 30. However, in the control group BMI less than 30 there were 4 subjects present. Serum homocysteine levels were significantly higher in patients with NAFLD (13.44±3.10 µmol/L) as compared with the control group (11.62 ± 1.34 µmol/L, p=0.015) (Figure 1). Serum vitamin $B_{12}$ and folate levels were within normal limits and no statistical difference was present between two groups.

The evaluation of liver via ultrasonography for the fatty liver in NAFLD group demonstrated 5 subjects in grade 1; 9 subjects in grade 2; and 6 subjects in grade 3 hepatosteatosis. The insulin levels of the patients with NAFLD was significantly higher when compared with controls (p=0.009), but c-peptide levels did not differ in both groups. In addition, distribution of insulin levels was shown in Figure 2. The serum ALT, AST, glucose, total cholesterol and low density lipoprotein (LDL) cholesterol levels were statistically higher in NAFLD patients than in controls (p<0.05). Of the biochemical parameters, ALT, AST and glucose had positive correlation with serum homocysteine levels, respectively [(r= 0.52, p= 0.01), (r= 0,58, p= 0.004), (r= 0.51, p= 0.014)].

## DISCUSSION

Nonalcoholic fatty liver disease has been reported in patients with type 2 diabetes and obese persons for a long time (16,17). Studies indicate that this syndrome has a relationship with obesity, diabetes and hyperlipidemia (3,18,19), however there has been a small group with normal weight having normal fasting glucose and normal glucose tolerance without dyslipidemia (4). Nonalcoholic steatohepatitis (NAFLD) has a more limited area than hepatosteatosis itself. Recently, it was proposed that toxic affects of free fatty acids, insulin resistance syndromes and associated metabolic abnormalities are the current mechanisms that may be responsible (20). Apart from this, recent investigations showed that homocysteine levels were increased in hyperinsulinemic subjects with obesity and in the states of insulin resistance (10,11). Giltay et al. demonstrated that plasma homocysteine levels were increased even in non-obese subjects with insulin resistance (9). All of these data indicate that high levels of homocysteine may have relationship with insulin resistance. In our study, significantly high levels of serum homocysteine were found

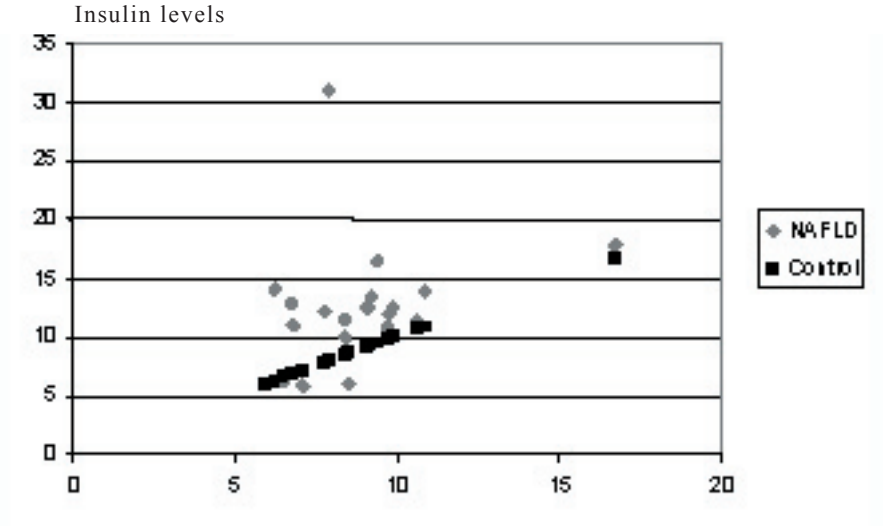


**Figure 2. Distribution of insulin levels in NAFLD and control groups.**

in NAFLD patients. Serum glucose, insulin, total cholesterol, and LDL cholesterol were all found significantly higher than the the same age and BMI matched control group. These results support insulin resistance and metabolic abnormalities in patients with NAFLD. In some studies relationship between homocysteine levels, insulin resistance and metabolic syndrome could not be demonstrated and even contrary findings to our results were obtained (21, 22). These observations may be indicative of the results of high homocysteine levels rather than the cause of insulin resistance. It seems there are still unexplained points on homocysteine metabolism and metabolic syndrome.

Whether serum homocysteine elevation has a metabolic effect in the development of steatosis and steatohepatitis is not clear at present. One of the important results of the study is that higher serum homocysteine levels were detected in patients with NAFLD compared with controls.. However, this study is not enough to explain a possible relationship between homocysteine and hepatic fat accumulation and hepatic injury. In chronic alcoholism, alcoholic cirrhosis and experimental liver damages hyperhomocysteinemia and fatty liver were also shown (15,23,24). It is also shown that severe hyperhomocysteinemia due to cystathionine β-synthase deficiency should lead to widespread hepatosteatosis and moreover to clinical manifestations like atherosclerosis, thrombosis, and osteoporosis (25,26). In one of their experimental studies Werstuck et al. found out that there has been a dysregulation in biosynthetic ways of cholesterol and triglyceride due to stress of homocysteine over endoplasmic reticulum and as a result of this, they concluded that progressive hepatosteatosis and probably atherosclerotic lesions existed (27). Recent studies demonstrated that serum homocysteine levels are increased in patients with liver cirrhosis (14,28,29). Higher levels of hyperhomocysteinemia is more prominent in alcoholic cirrhosis meanwhile increase in homocysteine levels are also present in non-alcoholic cirrhosis (29,30). Garcia-Tevijano et al. showed that homocyteine levels were increased in patients with liver cirrhosis and they advocated that this increase was the marker for a decrease in hepatic functions and had a role in the development of hepatic fibrosis (14).

Apart from this, a very broad distribution of values in homocysteine levels of the healthy subjects has been described with the same technique (97.5$^{th}$ percentile at 16.6 µmol/L) (31). In our study laboratory reference range for homocysteine was 5-12 µmol/L. Values that we have obtained were in consistent with mild hyperhomocysteinemia. We think that these results may be because of small number of our cases.

In conclusion; the data suggest that serum homocysteine level is increased in NAFLD patients. The increase in homocysteine levels may be associated with metabolic abnormalities of NAFLD. Additionaly, high homocysteine levels may be associated hepatic steatosis and steatohepatitis. However, further studies are needed to determine more clearly

the role of homocysteine on hepatic steatosis and steatohepatitis.

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