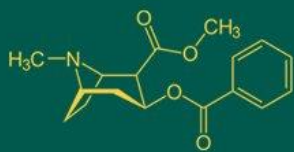


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Evaluation of serum homocysteine levels among patients with non-alcoholic fatty liver disease in Basrah

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Abstract

Non-Alcoholic fatty liver disease (NAFLD) is closely associated with obesity and insulin resistance (IR). Homocysteine (Hcy) is a sulphur-containing amino acid derived from the metabolism of methionine, and linked to oxidative stress, endoplasmic reticulum stress, progression of NAFLD, and liver fibrosis.

Objective: To assess serum Hcy levels in patients with NAFLD and to investigate its association with biochemical markers such as liver enzymes, lipid profile, fasting plasma glucose (FPG), insulin, insulin resistance (measured by HOMA-IR), and pancreatic B-cell function (measured by HOMA-B).

Methods: This is a case-control study that included 90 patients and 90 apparently healthy individuals as controls. Body mass index (BMI), blood pressure (BP), waist circumference (WC), Hcy levels, fasting plasma glucose (FPG), insulin, HOMA-IR, HOMA-B, liver enzymes, and lipid profile were all measured.

Results: Patients with NAFLD had significantly higher BMI, WC, SBP, DBP, Hcy levels, ALT, AST, FPG, insulin, HOMA-IR, HOMA-B, TC, TG, and LDL-C ($P < 0.001$) than controls. Whilst HDL-C was lower compared to control ($p < 0.001$). Hcy levels are significantly higher in patients with NAFLD and T2D compared to those without T2D ($P < 0.01$).

Conclusion: A strong relationship was encountered between Hcy level and NAFLD. Elevated Hcy concentrations make patients with NAFLD at considerable risk of a variety of adverse consequences, including cardiovascular, thromboembolic, and neurological sequelae.

Keywords: NAFLD, homocysteine, HOMA-IR

Introduction

NAFLD is the most common liver disorder worldwide [1]. It is described as the accumulation of fat in more than 5% of liver cells without drinking large amounts of alcohol or any other known cause [2]. Fat accumulation in liver cells may occur for several reasons, including increased amounts of free fatty acids (FFA) reaching the liver, decreased oxidation of fatty acids (FA), and increased production or decreased excretion of very low-density lipoprotein (VLDL-C) [3]. Metabolic, genetic, and therapeutic status may affect NAFLD, for example, lipodystrophies, mitochondrial disease, polycystic ovary syndrome, and Wilson disease, as well as some treatments such as methotrexate and amiodarone [4, 5].

The prevalence of NAFLD varies significantly across populations. A study conducted between 2016 and 2019 indicates that the prevalence of NAFLD was approximately 38.2%. The prevalence is higher in certain regions, reaching 44.4% in Latin America, 33.8% in the Middle East, and North Africa. Prevalence rates are higher in men than in women, but after the menopause, the rate increases in women [6-8].

Hcy is a non-essential sulfur-containing amino acid derivative from the metabolic processing of dietary methionine (Met) [9]. Hcy can then be remethylated back to Met or broken down through transsulfuration to cysteine and other metabolites. This conversion process requires several enzymes and three essential vitamins: folic acid, vitamin B12, and vitamin B6 [10].

Hyperhomocysteinemia (HHcy) is a metabolic disorder in which Hcy levels rise above 15 $\mu\text{mol/L}$ [11]. HHcy varies and is influenced by geographical location, dietary habits, genetic factors (particularly the MTHFR polymorphism), insufficient intake or malabsorption of vitamins (B12, B6, and folic acid), lifestyle, and environmental factors such as smoking, an

unhealthy diet, and physical inactivity^[12]. HHcy is associated with alterations in inflammatory markers and levels of oxidative stress, which are believed to increase the risk of cardiovascular disease (CVD), thromboembolic disease, oxidative assault, and the progression of NAFLD. HHcy has a negative effect on mitochondria through its impact on the oxygen transport chain. Consequently, it may cause DNA damage, leading to a reduction in ATP production^[13-15].

The aim of the study was to evaluate Hcy levels among patients with NAFLD.

Patients and methods

This is a case-control study conducted between January, 2025 throughout August, 2025 and included 90 patients with NAFLD (diagnosed with the aid of fibroscan), 44 males and 46 females, 25 - 67 years of age. Among patients, 40 had T2D, and 50 without T2D. The control group included 90 apparently healthy individuals, 42 males and 48 females, 22-

71years of age. The study was conducted at the GIT and Hepatobiliary Hospital, Basrah, Iraq.

Waist circumference (WC), systolic blood pressure (SBP), diastolic blood pressure (DBP), and body mass index (BMI) were measured for all participants. Venous blood samples were obtained from NAFLD patients and the control group after an 8-10-hour fast for laboratory tests, which included Hcy, FPG, insulin, lipid profile, and liver enzymes. In addition, HOMA-IR and HOMA-B were also determined.

Statistical analyses were carried out using SPSS software, $P < 0.05$ was considered statistically significant.

Results

Table1 presents the characteristics and physiological measurements. There was no significant difference in terms of age ($P > 0.05$). On the other hand, SBP, DBP, BMI, and WC were significantly higher among patients with NAFLD than controls ($P < 0.001$).

Table 1: Physiological measurements among the study groups

Characteristic	NAFLD group (n=90)	Control group (n=90)	P-value
Age (years)	48.43±8.47	47.79±9.35	> 0.05
SBP (mmHg)	124.8±12.26	117±2.98	< 0.001
DBP (mmHg)	80.99±7.077	76.67±2.37	< 0.001
BMI (kg/m ²)	31.84±5.723	22.96±1.38	< 0.001
WC (cm)	106.4±13.10	81.15±5.95	< 0.001

Data are expressed as mean ±SD.

Biochemical parameters presented in Table 2 showed a significant increase in Hcy levels, ALT, AST, TC, TG, and LDL-C ($P < 0.001$). The results indicated a significant

decrease in HDL-C levels in NAFLD patients compared to the control group ($P < 0.001$).

Table 2: Biochemical parameters

Parameter	NAFLD group (n=90)	Control group (n=90)	P-value
Hcy (μmol/L)	20.25±5.48	8.45±2.07	< 0.001
ALT (U/L)	48.67±19.86	22.81±7.90	< 0.001
AST (U/L)	28.17±11.93	20.85±6.47	< 0.001
TC (mg/dL)	177.6±29.23	129.9±23.47	< 0.001
TG (mg/dL)	233.3±82.54	93.80±26.65	< 0.001
LDL-C (mg/dL)	114.2±16.00	55.84±19.15	< 0.001
HDL-C(mg/dL)	33.38±5.26	49.45±5.26	< 0.001

Data are expressed as mean ±SD.

The data presented in Table 3 revealed a significant increase in Hcy levels among patients with NAFLD and T2D compared with those without T2D ($P < 0.01$).

Table 3: HCY concentration among patients with NAFLD with regard to the existence of T2D

Parameter	NAFLD with T2D (n=40)	NAFLD without T2D (n=50)	P-Value
Hcy (μmol/L)	22.14±5.60	18.73±4.94	< 0.01

Data are expressed as mean ±SD.

**: $P < 0.01$ (Patients with NAFLD without T2D Vs. Patients with NAFLD with T2D).

As presented in Table 4, there was a significant increase in FPG, insulin, HOMA-IR, and HOMA-B among patients with NAFLD in comparison to controls, $p < 0.001$.

Table 4: FPG, insulin, and Homeostatic model assessment

Parameter	NAFLD group (n=90)	Control group (n=90)	P-value
FPG (mg/dL)	138.0±51.18	84.61±3.09	< 0.001
Insulin (μIU/L)	29.13±12.46	7.83±1.121	< 0.001
HOMA-IR	11.01±7.973	1.64±0.270	< 0.001
HOMA-B	175.6±84.55	131.7±18.30	< 0.001

Data are expressed as mean ±SD.

Discussion

The present study clearly illustrates the presence of general and central obesity as well as elevated B.P among patients with NAFLD, Table 1. On the other hand, there is no significant difference in terms of age. These findings are consistent with the prevailing view that NAFLD is closely associated with metabolic syndrome ^[16]. The accumulation of visceral fat, which increases BMI and WC, releases large amounts of FFAs that exceed the liver's metabolic capacity. Consequently, fat accumulation leads to the progression of NAFLD. Similarly, high blood pressure increases the risk of CVD ^[17].

Significant elevation of Hcy is demonstrated in the present study, Table 2. As the liver is the primary organ involved in Met metabolism and contains the enzymes required for transmethylation, HHcy may affect this process ^[18]. The liver cell damage markers ALT and AST were significantly elevated in NAFLD patients compared to the control group. This is consistent with hepatocellular damage and inflammation that is seen in NAFLD, for which aminotransferases, specifically ALT, are sensitive biochemical markers of hepatic steatosis and inflammation. These findings agree with other studies indicating that ALT and AST are markedly higher in NAFLD patients than in those without NAFLD ^[19, 20]. Concerning lipid profiles, there was a significant increase in TG, TC, and LDL-C, and a decrease in HDL-C. This supports the metabolic dysfunction in patients with NAFLD, which is an indication of the existence of inflammation and oxidative stress. Dyslipidemia is a common feature in patients with NAFLD and a predictor of disease progression and CVD ^[21] (21).

Significantly higher Hcy levels in NAFLD patients with T2D compared to NAFLD patients without T2D, Table 3. This may indicate that the combination of T2D and NAFLD may exacerbate Hcy abnormalities, which in turn may increase oxidative stress and metabolic disturbances in these patients ^[22]. This is consistent with the concept that IR may elevate Hcy levels in patients with NAFLD. This supports the notion that elevated Hcy levels contribute to metabolic damage in these patients ^[23].

FPG and insulin levels of the patients with NAFLD were significantly higher than control group, Table 4. This evidence indicates that patients with NAFLD exhibit significant alterations in the metabolism of glucose and the sensitivity to glucose. Increased FPG and hyperinsulinemia confirm an IR condition, which is known to be metabolically linked to hepatic fat accumulation and glucose homeostasis ^[24]. As well as HOMA-IR and HOMA-B were elevated, indicating a significant increase in IR and a compensatory increase in beta-cell function (compensatory response to IR in the early stages of metabolic dysfunction). This confirms that increased IR is a major factor in the progression of NAFLD ^[25-26].

In conclusion, patients with NAFLD demonstrate significantly elevated Hcy levels; therefore, those patients are at substantial risk of CVD, thrombotic events, and neurological complications. This implies that patients with NAFLD should receive active therapeutic interventions together with regular monitoring of cardio-metabolic profile for the earlier detection and subsequent management of corresponding complications.

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