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Research Article

Investigating the Association Between Serum Testosterone Level and Metabolic Profile in Patients with Non-alcoholic Fatty Liver Disease: A Cross-sectional Study

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Abstract

Background: Considering the impact of serum testosterone levels on metabolic diseases, this study aims to investigate testosterone levels in patients with non-alcoholic fatty liver disease (NAFLD).

Methods: A prospective cohort study was performed between two groups: Group A, 58 patients with NAFLD, and Group B, 59 patients without NAFLD. Fatty liver was diagnosed based on Abdominal ultrasound using the Hamaguchi score. Blood specimens were obtained from all patients between 8 and 10 AM and analyzed for testosterone, aspartate aminotransferase, alanine transaminase, serum lipid profile, ferritin, and fasting blood sugar levels.

Results: The mean weight and body mass index were significantly higher in the study group (Group A) (*P*-value = 0.0001). The mean aspartate aminotransferase and alanine transaminase were 37.7 and 56.6 in Group A, respectively, and were significantly higher than the control group (Group B) (*P*-value = 0.0001). Fasting blood sugar, lipid profile, and serum ferritin differed between the two groups. The mean serum testosterone level was 3.38 ± 0.72 in Group A and 4.79 ± 0.88 ng/dL in Group B (*P*-value = 0.0001). The testosterone level negatively correlated with age and hepatic steatosis grade (*P*-value = 0.0001). However, it has a weak and positive correlation with BMI (*P*-value = 0.454).

Conclusion: This study revealed that the patients with NAFLD had a significantly lower level of testosterone compared to the other individuals. This study highlights the role of NAFLD as a potential cause of hypogonadism in men.

Keywords: Fatty Liver; Non-alcoholic fatty liver disease; NAFLD; Testosterone; Hypogonadism.

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Introduction

Excess triglyceride accumulates in the liver parenchyma in the absence of excessive alcohol consumption, leading to non-alcoholic fatty liver disease (NAFLD) (1). With an estimated prevalence of about 10 to 35 %, NAFLD is the most common cause of chronic liver disease. Furthermore, the NAFLD prevalence has been increasing in recent years, which can be attributed to the uncontrolled spread of obesity and diabetes (2,3). The pathogenesis of NAFLD is not fully understood, but traditionally, two hypotheses have been considered. The first hypothesis suggests that the accumulation of triglycerides and free fatty acids in the liver, due to an imbalance between their entry into the liver, synthesis, and export, beta-oxidation, leads to NAFLD formation. This imbalance exposes the liver to damage, such as inflammation, mitochondrial dysfunction, and oxidative stress. The second hypothesis proposes that free fatty acids alone can cause damage to hepatocytes. In reaction with glycerol, they form triglycerides, ultimately activating inflammatory pathways (4). Although the underlying mechanism leading to NAFLD development is still unknown, factors such as obesity, metabolic syndrome, insulin resistance, and dyslipidemia may play a role in NAFLD formation. However, it seems that NAFLD is the hepatic manifestation of the metabolic syndrome, associated with increased visceral fat tissue, insulin resistance, and dyslipidemia (2,5). About 35 % of males with hypogonadotropic hypogonadism have NAFLD. Males with NAFLD have lower serum testosterone, serum sex hormone-binding globulin, sperm concentration, and motility (6). By increasing insulin resistance, low serum testosterone causes an increase in liver lipogenesis, which contributes to NAFLD progression (7). Visceral fat accumulation can induce liver inflammation by circulating cytokines such as tumor necrosing factor and interleukin 6, leading to less hormone secretion of the testis and pituitary glands (8,9).

Numerous studies have demonstrated that NAFLD increases the risk of cardiovascular diseases, diabetes, and related mortality. In men, a decrease in testosterone levels has been associated with increased visceral fat and insulin resistance (10). Also, testosterone replacement therapy has shown promise in reducing visceral fat (11). Considering the effect of NAFLD on serum testosterone levels and the association between testosterone insufficiency and the severity of NAFLD, this study aims to investigate testosterone levels in patients with NAFLD (12). The blood specimen was obtained between 8 to 10 am after at least 8 hours of fasting because several studies have shown that serum testosterone level reduces after 9 am and 7 to 10 am is the optimal time for achieving blood specimen (13).

Methods

The study group was selected among patients with NAFLD who presented to the gastroenterology departments of the Rasoul-e-Akram Hospital (Tehran, Iran). Also, the control group was chosen randomly among patients who presented to the urology department of the Firoozgar Hospital (Tehran, Iran) from Jun 2020 to March 2021. Fifty-eight male NAFLD patients who met the inclusion criteria were considered the study group (Group A). A control group of 59 patients were selected randomly among patients without NAFLD who presented due to kidney stones, varicocele, and inguinal hernia (Group B). All 117 patients were informed about the study criteria.

Inclusion Criteria: 20- to 60-year-old male patients with NAFLD diagnosis based on medical history, physical examination, and liver ultrasound were included.

Exclusion Criteria: Patients with viral or autoimmune hepatitis, patients using more than 10 g ethanol per day, patients with iron and copper overload (Wilson and hemochromatosis disease), patients with alpha-1 antitrypsin deficiency, and patients with congenital hyper or hypogonadism disorders were excluded. Also, patients under treatment with medications affecting testosterone levels, such as finasteride, anabolic steroids, and drugs affecting GNRH levels, were excluded.

NAFLD and biochemical assessments

A skilled radiologist performed the abdominal ultrasound. Images were assessed again by a gastro-intestine-hepatologist. The Hamaguchi score assessing hepatic steatosis by hepatorenal echo contrast, bright liver, and vessel blurring was used to diagnose NAFLD (14, 18).

Blood specimens were drawn between 8 and 10 AM after at least 8 hours of fasting. Obtained samples were sent to the Firoozgar Hospital's laboratory, and the

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analyses were performed using the Free Testosterone AccuBind ELISA Kit-96 wells (Monobind Inc ®, USA). Also, aspartate aminotransferase (AST), alanine transaminase (ALT), triglyceride (TG), cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), ferritin, and fasting blood sugar (FBS) levels were measured.

Statistics

Data were analyzed by Statistical Package for the Social Science (SPSS) software, version 26. The quantitative variables were reported as mean and standard deviation (SD), and qualitative variables were reported as frequency and percentage. Qualitative data were compared using Chi-Square and Fisher exact tests. Also, for quantitative variables, Pearson Correlation and Spearman tests were used. Independent sample T-test and Mann-Whitney U test were used to compare quantitative and qualitative variables. A P-value of < 0.05 was considered as statistically significant.

Ethical Consideration and consent to participate

Iran University of Medical Sciences ethics committee approved this prospective cohort study in June 2021. The ethical code is: (IR.IUMS.REC.1400.280). The informed consent was obtained from all patients for participation. Also, Patients were assured that non-participation in the study would not affect their treatment process.

Results

Among 117 patients, 58 were in the study group (Group A), and 59 were in the control group (Group B). The mean age was 43.4 ± 9.09 years (27 to 60 years). The mean body mass index (BMI) was 26.23 (SD: ± 2.94) kg/m^2. Among 58 patients with NAFLD, 40 (69%) had grade 3, 13 (22.4 %) grade 2, and 5 (8.6 5) grade 3 fatty liver. The mean testosterone level of all individuals was 4.09 ± 1.07 ng/dL. Also, the mean AST and ALT levels were 33.8 ± 10.2 and 44.8 ± 21.0 units/litre respectively. The mean FBS, TG, cholesterol, LDL, HDL, and serum ferritin levels were 165 ± 14.01, 285 ± 41.85, 301 ± 35.22, 199 ±23.25, 55 ± 4.83, and 358 ± 68.36 respectively.

The age and height did not differ between the two groups significantly (P-value = 0.773 and 0.631), but the mean weight and BMI were significantly higher in Group A (P-value = 0.0001).

The mean AST and ALT were 37.7 and 56.6 in Group A and were considerably higher than Group B (P-value = 0.0001). As shown in Table 1, FBS, lipid profile, and serum ferritin differed significantly between the two groups. The mean serum testosterone level was 3.38 ± 0.72 in Group A and 4.79 ± 0.88 ng/dL in Group B (P-value = 0.0001) (Figure 1).

Based on the Pearson Correlation test, the testosterone level was negatively associated with age and hepatic steatosis grade (P-value = 0.0001). However, it has a weak and positive correlation with BMI (P-value = 0.454). As shown in Table 2, the mean serum testosterone level negatively correlated with FBS and TG (r = - 0.357 and – 0.297).

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Table 1 Patient's characteristics and laboratory tests

Parameter	Mean	SD (±)	Group A (± SD)	Group B (± SD)	P-value
Age (years)	42.43	9.09	42.18 (9.01)	42.67 (9.24)	0.773
Height (cm)	174.53	6.21	174.25 (6.28)	174.81 (6.18)	0.631
Weight (Kg)	80.14	11.70	84.31 (11.21)	76.05 (10.77)	0.0001
BMI (Kg/m^2)	26.23	2.94	27.68 (2.55)	25.80 (2.60)	0.0001
AST	33.84	10.22	38.70 (12.44)	29.06 (3.19)	0.0001
ALT	44.83	21.08	56.60 (24.80)	33.27 (3.10)	0.0001
FBS	107.80	14.01	112.39 (15.60)	103.28 (10.57)	0.0001
TG	167.35	41.85	180.41 (51.51)	154.52 (23.61)	0.001
Cholesterol	178.55	35.22	195.29 (37.90)	162.10 (22.67)	0.0001
LDL	115.24	23.25	124.14 (26.39)	106.49 (15.47)	0.0001
HDL	42.01	4.83	40 (4.61)	44 (4.20)	0.0001
Ferritin	75.58	68.36	110.29 (82.07)	41.47 (18.42)	0.0001
Testosterone	4.09	1.07	3.38 (0.72)	4.79 (0.88)	0.0001

Footnote: Standard deviation (SD), aspartate aminotransferase (AST), alanine transaminase (ALT), fasting blood sugar (FBS), triglyceride (TG), cholesterol, high-density lipoprotein (HDL), and low-density lipoprotein (LDL).

Table 2 Pearson correlation test for testosterone, patient's characteristics, and laboratory tests

Parameter	P-value	r	Correlation
Age	0.0001	- 0.693	Negative, strong
Height	0.489	0.093	Positive, extremely weak
Weight	0.359	0.123	Positive, weak
BMI	0.454	0.100	Positive, weak
Grade of NAFLD	0.0001	- 0.555	Negative, moderate
AST	0.168	- 0.183	Negative, weak
ALT	0.152	- 0.190	Negative, weak
FBS	0.006	- 0.357	Negative, moderate
TG	0.024	- 0.297	Negative, moderate
Cholesterol	0.794	0.035	Positive, extremely weak
LDL	0.553	- 0.080	negative, extremely weak
HDL	0.938	0.010	Positive, extremely weak
Ferritin	0.563	0.078	Positive, extremely weak

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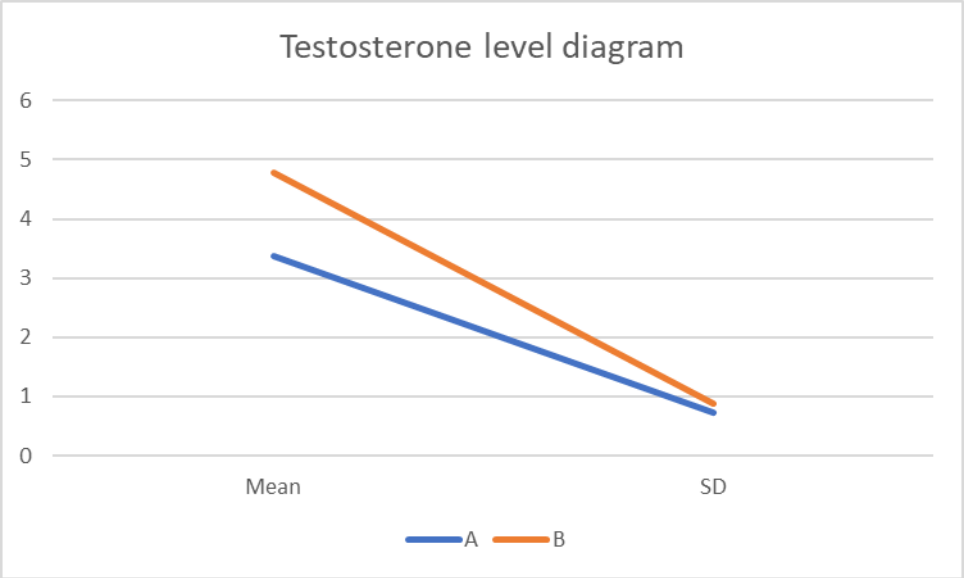

Figure 1: Testosterone level comparison between two groups: Group A: Patients with non-alcoholic fatty liver disease (NAFLD), Group B: Patients without NAFLD

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Discussion

The present study compared the weight, height, testosterone level, and liver function of patients with and without non-alcoholic fatty liver disease (NAFLD). We found that NAFLD patients had higher weight, body mass index (BMI), aspartate aminotransferase (AST), alanine aminotransferase (ALT), triglycerides (TG), total cholesterol, and low-density lipoprotein (LDL), but lower testosterone level than non-NAFLD patients. We also observed a negative correlation between testosterone level and age, liver steatosis grade, fasting blood sugar (FBS), and TG in NAFLD patients.

Previous studies have suggested that testosterone deficiency may contribute to liver steatosis and that testosterone replacement may reduce visceral fat and improve NAFLD. Moreover, the association between NAFLD and testosterone level may differ by sex and menopausal status (15,16). For instance, Polyzos et al. examined the grade of liver steatosis in two groups with high testosterone and low testosterone levels. The mentioned study stated that patients with lower testosterone levels develop non-invasive NAFLD more frequently compared with patients with higher testosterone levels (17).

Nikolaenko et al. revealed that testosterone deficiency can increase liver steatosis. Also, their study on male rats showed that testosterone replacement can decrease visceral fat, leading to NAFLD improvement (16).

This relationship between NAFLD and testosterone does not belong to men, as Park et al. reported a higher prevalence of NAFLD in postmenopausal women compared with premenopausal individuals (33 % and 19.2 %). They suggest that in contrast with postmenopausal women, testosterone level is

positively associated with NAFLD in premenopausal females (15).

Kim et al. examined 251 patients. They revealed that patients with NAFLD had more inappropriate metabolic profile such as FBS, TG, and cholesterol. Also, they revealed that serum testosterone level has an association with NAFLD independently (11).

This study showed that patients with non-alcoholic fatty liver disease (NAFLD) had lower testosterone levels, higher body mass index (BMI), and poorer metabolic profile than patients without NAFLD. They also had impaired liver function, as indicated by elevated aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels. Future research should investigate how NAFLD affects sex hormones and male fertility, and how testosterone levels are related to NAFLD in women.

Conclusion

This study revealed that the patients with NAFLD had a significantly lower level of testosterone compared to the other individuals. This study highlights the role of NAFLD as a potential cause of hypogonadism in men.

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Data acquisition, Data analysis: HN

Drafting of the manuscript: HN

Critical revision of the manuscript: AM

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