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Vitamin D Levels in Non-alcoholic Fatty Liver Disease in Type II Diabetic and Non-Diabetic Patients

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ABSTRACT

Background: Non-alcoholic fatty liver disease (NAFLD) individuals are more prone to acquire type 2 diabetes (T2DM). However, the knowledge regarding this phenomenon is limited to individuals without diabetes mellitus (DM). Vitamin D deficiency may lead to a more pronounced shift in an individual's glucose metabolism. Moreover, hepatocytes and peripheral tissues may potentially benefit from vitamin D in terms of reducing insulin resistance induced by free fatty acids.

Objective: In this study, serum vitamin D levels were examined in patients with NAFLD with T2DM, and its relationship with NAFLD without DM was evaluated.

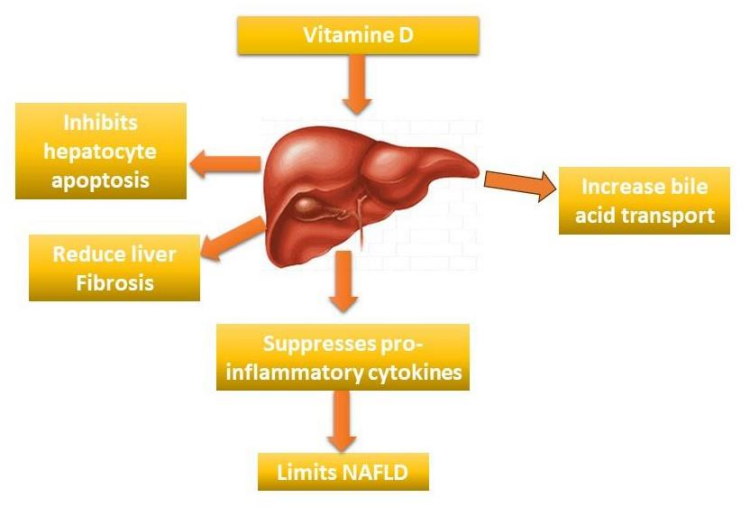
Subjects and Methods: A total of 120 subjects were included in this study, divided into three groups: Group I included 30 healthy subjects matched for sex and age, Group II included 50 patients with NAFLD who had diabetes, and Group III

included 40 subjects without diabetes. Three groups were compared regarding clinical and biochemical characteristics concerning serum 25-hydroxyvitamin D levels using enzyme-linked immunosorbent assays.

Results: According to this study, people with NAFLD diagnosed by ultrasound examination Group II had significantly reduced serum levels of 25(OH) vitamin D compared to patients without NAFLD diagnosed by ultrasound examination Group II. There was a statistically significant difference between groups and 25(OH) vitamin D levels than group III. In addition, a highly significant difference in serum 25(OH) vitamin D levels was observed between groups II and III and the control group. Moreover, 25(OH) vitamin D is an independent predictor of T2DM in patients with NAFLD.

Conclusion: Serum 25(OH) vitamin D levels were lower in patients with NAFLD and DM, indicating that low 25(OH) vitamin D status contributes to the onset and progression of diabetes mellitus in NAFLD.

Keywords: 25-hydroxy vitamin D[25(OH)D)], Non-alcoholic fatty liver disease (NAFLD), diabetes mellitus.



INTRODUCTION

The liver is a crucial organ for maintaining the balance of blood glucose metabolism and oversees metabolizing glucose [1]. Hepatic gluconeogenesis and fat accumulation depend on insulin sensitivity because insulin suppresses hepatic gluconeogenesis and regulates adipogenesis. Diabetes mellitus (DM) and hyperglycemia are caused by liver insulin resistance [2, 3].

Patients with various NAFLD phenotypes may appear with various clinical symptoms because NAFLD has varied etiology [4]. Insulin resistance often occurs in conjunction with NAFLD. The etiology of NAFLD is mostly characterized by insulin resistance [5]. Type 2 diabetes mellitus (T2DM) patients are more likely to develop NAFLD [6]. Additionally, those who have DM with NAFLD die at a rate equivalent to those who have NAFLD three times without T2DM [7, 8]. Including DM in most non-invasive composite prediction scores for advanced fibrosis and non-alcoholic steatohepatitis (NASH) illustrates the significance of DM in NAFLD [9]. According to the American Society's recommendations, the NAFLD Fibrosis Score (NFS) is a composite predictive score that can be used to predict advanced fibrosis in NAFLD [10, 11]. Unfortunately, some T2DM patients acquire NAFLD,

and others develop NASH; these individuals may develop secondary liver cirrhosis or possibly liver cancer [12, 13].

Vitamin D has a crucial impact on insulin secretion via direct and indirect mechanisms. Insulin release is activated by the expression of Vit D receptors (VDRs) by pancreatic β cells. The influx of Ca^{+2} into the β cell occurs directly through the cell's membrane and from intracellular organelles indirectly [14]. According to [14], calcium^{+2} activates the calcium-dependent endopeptidase responsible for insulin generation from proinsulin 19 [15]. The liver and skeletal muscles contain Vit D receptors outside the pancreas. The VDR influences gene and receptor activity to mediate the effect of Vit D on insulin sensitivity. By increasing Ca^{+2} influx into adipose tissue, secondary hyperparathyroidism causes insulin resistance and lipogenesis. As a result, it also reduces insulin release by affecting β cells [16]. This study aims to examine the relationship between serum Vit D levels in patients with NAFLD and diabetes and those without diabetes.

SUBJECTS AND METHODS:

Study design: The study involved 120 subjects split into three groups: the control group (30 patients), the NAFLD with diabetes group (50 patients), and the NAFLD without diabetes group (40 patients). From July 2018 to October 2020, patients aged 35 to 65 attended the endocrinology and internal medicine outpatient clinics at Al-Zahara University Hospital, Cairo, Egypt.

Clinical data: Consent was obtained from the patients after explaining the aim of the study and its benefits to them. Patients with hepatitis C virus (HCV), B virus (HBV), alcoholic patients, and those with autoimmune hepatitis were excluded from this study. According to the American Diabetes Association (ADA) criteria, diabetes mellitus (DM) was identified [17]. Body mass index (BMI) was calculated, and measurements were taken for HbA1C, total cholesterol (Chol), triglycerides (TGs), LDL, HDL, serum bilirubin, albumin, ALP, AST, and ALT levels. Additionally, insulin resistance was assessed using the homeostatic model assessment of insulin resistance (HOMA-IR) formula, which includes insulin (U/mL), fasting glucose (mg/dL), and was calculated as described in reference [18].The following criteria were used to diagnose NAFLD: 1) No history of alcohol misuse, viral hepatitis, cholestasis, metabolic liver disease, or other liver illnesses; increased transaminase values (AST and/or ALT); and positive findings from a confirmatory B-ultrasound since liver biopsy is difficult to do clinically. Using ultrasonography, the fatty liver diagnosis may be made based on changes in the liver echo, differences between the liver and kidney echo, and vascular blurring.

According to a previously published methodology, NAFLD fibrosis score (NFS) was computed as follows: NFS = 0.037 × age (years) +1.675 + + 1.13 × impaired fasting glycemia or DM (yes =1; no = 0) + 0.094 × BMI (kg/m^2) + 0.99 × AST/ALT ratio - 0.66 × albumin (g/dL) - 0.013 × platelet (10^9/L) [19].

Using the NFS for fibrosis classification, the cutoff points were less than −1.455, −1.455 to 0.676, a high probability of advanced fibrosis, and more than 0.676 for low, indeterminate [19, 20].

A score of 2 or above is indicative of advanced fibrosis. The BARD score was also computed using a 4-point scale created from the weighted total of three factors (AST/ALT ratio > 0.8 = 2 points, DM = 1 point, BMI > 28 = 1 point) [21]. Similarly, it has been proposed that an AST/ALT ratio of greater than 0.8 may help indicate advanced fibrosis [22].

Statistical analysis: For data collection, review, coding, and input, the Statistical Package for the Social Sciences (SPSS) version 28 was used. Means, standard deviations, and ranges were used to report quantitative data to compare the study groups, and a one-way analysis of variance was used [23]. Variables were ranked favorably or negatively concerning one another using the Spearman correlation coefficient test. The allowed error margin was changed to 5%, and the confidence interval

was set at 95%. As a result, p-values more than 0.05 were regarded as unimportant, those < 0.05 as significant, and those < 0.01 as highly significant.

RESULTS

In this study, 120 patients met the inclusion criteria and were recruited from 130 patients screened for eligibility. There were 30 participants in the control group, 50 in group II, and 40 in group III (Fig. 1). The informed consent of 10 patients was withdrawn before the study was completed. Between the groups, there were no significant differences in the mean age. In the control group, 22 (73.3%) were men, and 8 (26.7%) were women; in group II, 31 (62.0%) were men, and 19 (38.0%) were women; and in group III, 19 (47.5%) were women, and 21 (52.5%) were men. All patients had well-preserved BMI, insulin, HOMA-IR, fasting blood sugar (FBS), and HGA1c. Group II demonstrated a significant rise in BMI, insulin, HOMA-IR, HGA1c, and FBS (48.50%, 214.29%, 133.33%, 60.87%, 113.82%, and 137.93%, respectively), compared with group I (Fig. 1). Similarly, group III had a significant increase in BMI by 40.24%, insulin by 131.25%, HOMA-IR by 71.43%, HGA1c 21.74%, and FBS by 34.68% compared with group I.

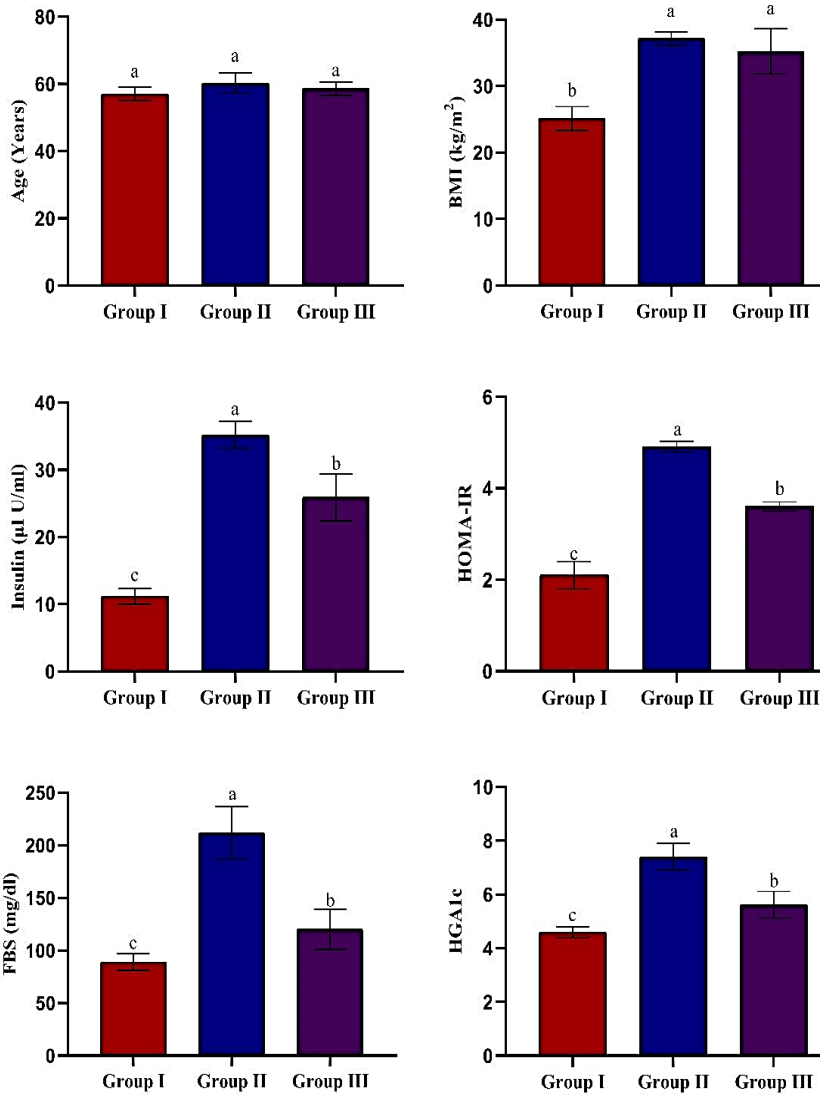


Figure 1. Comparison among all groups in terms of age, BMI, insulin, HOMA-IR, FBS, and HGA1c. According to the Fisher test, the various letters (i.e., a, b, and c) are significantly different at 0.05%. The standard deviations of the means are shown as vertical bars.

As shown in Figure 2, 2HPP, TG, cholesterol, and LDL increased by 113.82%, 42.75%, 65.04%, and 12.38%, respectively, in group II compared with those in group III. In contrast, platelet count and HDL decreased in group II. Furthermore, group III increased 2HPP, TG, cholesterol, and LDL compared with group II (Fig. 2).

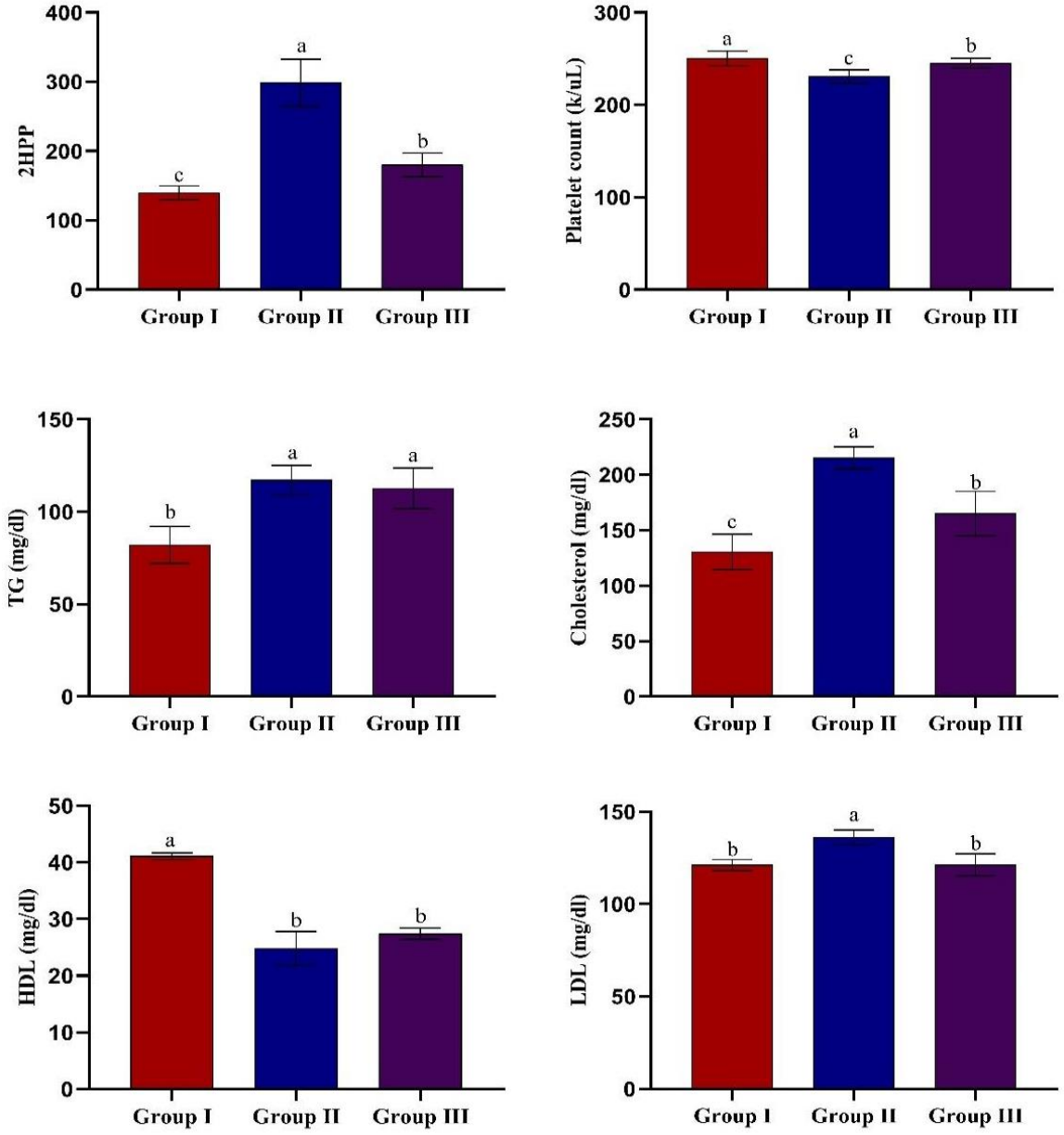


Figure 2. All groups compared 2HPP, platelet count, TG, cholesterol, HDL, and LDL. According to the Fisher test, the various letters (i.e., a, b, and c) are significantly different at 0.05%. The standard deviations of the means are shown as vertical bars.

Group II demonstrated a significant rise in the AST, AST/ALT ratio, albumin, and urea by 11.39%, 60.93%, 6.89%, and 4.33%, respectively, compared with the control group. Interestingly, group II also demonstrated a significant increase in ALT by 126.78% and creatinine by 45.45% compared with group I. Figure (4) illustrates that group II significantly decreased levels of 25(OH) vitamin D_3 than group II. Furthermore, a significant reduction in serum Vit D levels was discovered in groups II and III compared to group I.

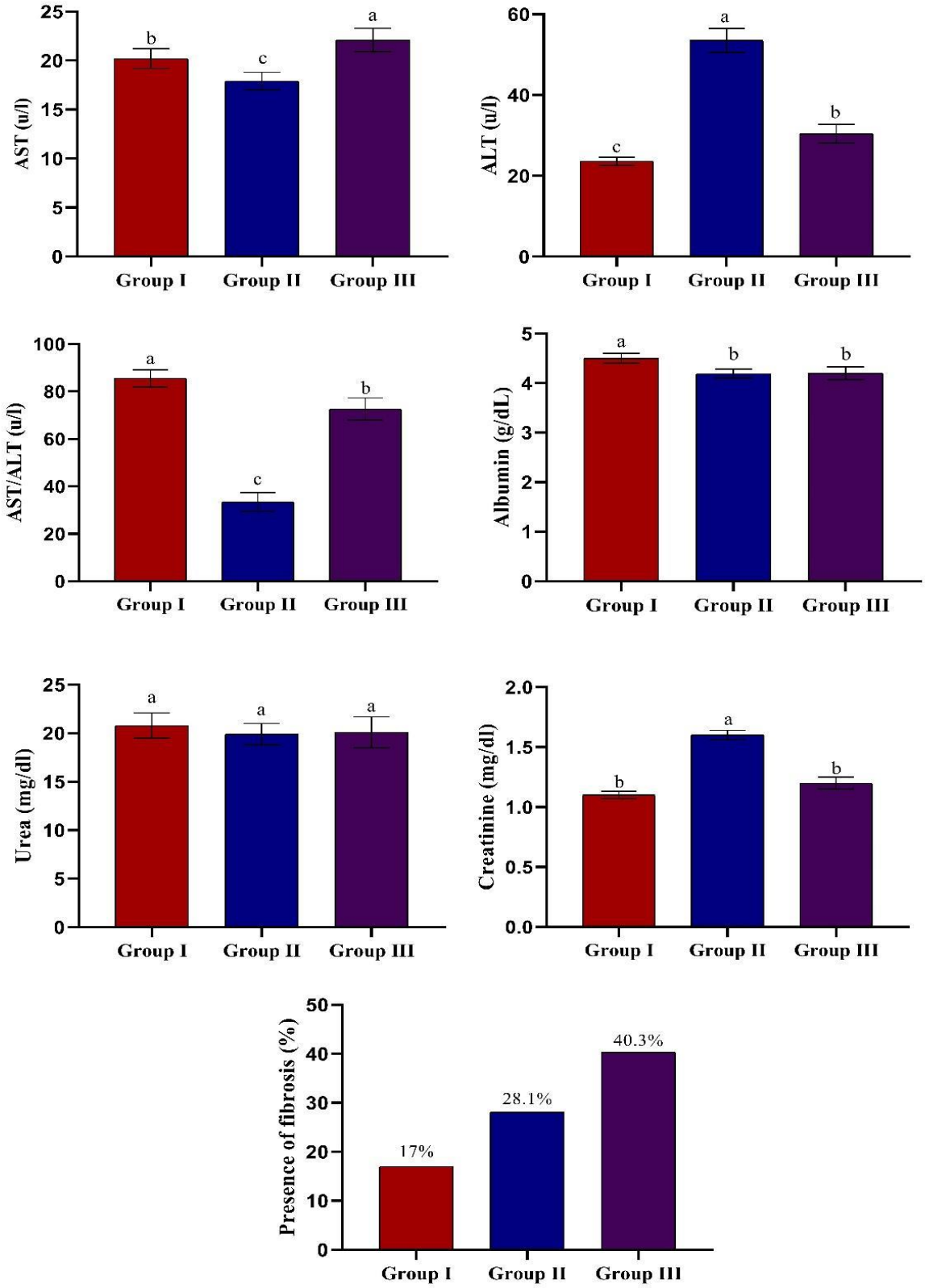


Figure 3. Comparison among all groups in terms of AST, ALT, AST/ALT ratio, albumin, urea, creatinine, and the presence of fibrosis. According to the Fisher test, the various letters (i.e., a, b, and c) are significantly different at 0.05%. The standard deviations of the means are shown as vertical bars.

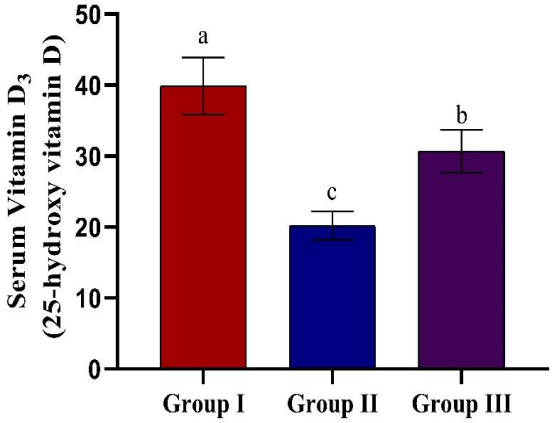


Figure 4. Comparison among all groups in terms of serum vitamin D_3 levels. According to the Fisher test, the various letters (i.e., a, b, and c) differ significantly at 0.05%. The standard deviations of the means are shown as vertical bars.

Correlation Analysis: In group II, the result of the correlation analysis between 25(OH) Vit D_3 and all parameters showed that 25(OH) vitamin D_3 and TG, LDL had a significant negative correlation. However, no significant correlation existed between any of the parameters and groups (Table 1).

Table 1. Pearson correlation analysis between 25(OH) Vit D_3 and all parameters among groups I, II, and III. r = Pearson correlation

Variable	Group I		Group II		Group III	
	r	p	r	p	r	p
Insulin	0.23	0.662	0.19	0.062	0.20	0.752
HOMA-IR	0.020	0.452	0.149	0.32	0.043	0.874
FBS	−0.41	0.541	0.012	0.19	−0.30	0.368
2HPP	0.12	0.352	0.08	0.57	0.11	0.541
TG	0.170	0.432	-0.38	0.034	0.20	0.332
Cholesterol	−0.19	0.412	0.21	0.545	−0.20	0.412
HDL	0.18	0.897	0.05	0.685	0.20	0.333
LDL	−010	0.774	-0.49	0.015	−0.15	0.15
AST	0.24	0.254	0.05	0.412	0.29	0.541
ALT	0.20	0.496	0.24	0.541	0.22	0.360
ALP	0.11	0.742	0.09	0.870	0.10	0.892
UREA	0.08	0.995	0.24	0.745	0.12	0.971
Creatinine	0.20	0.870	0.21	0.661	0.21	0.255

According to Table (2), individuals without diabetes had lower correlation coefficients for the different components used to construct the NFS in relation to fibrosis than patients with diabetes did. Therefore, this research evaluated the AST/ALT ratio and BARD score, two additional fibrosis measures created for NAFLD, for usage in patients without and with DM.

Table 2. Patients with NAFLD and those without diabetes mellitus were correlated with fibrosis using the Pearson method.

		Age	BMI	Platelet	Albumin	AST/ALT
NAFLD + DM	r	0.20	−0.71	−0.32	−0.17	0.35
	p	0.269	<0.001	0.007	<0.001	0.025
NAFLD	r	0.20	0.05	−0.19	−0.14	0.31
	p	0.459	0.003	0.027	<0.001	0.003

It was determined which indices showed a statistically significant correlation with NAFLD and diabetes had the highest area under the curve (AUC) using receiver operating characteristic (ROC) curves. To determine which indices provided the maximum sensitivity and specificity, 25(OH) vitamin D3 was used. Table (3) and Figure (5) show that the AUC for various levels of 25(OH) vitamin D_3 for diagnosis was 0.901. The best cutoff value for 25(OH) vitamin D_3 was ≤ 22. The sensitivity and specificity of 25(OH) vitamin D_3 levels were 100% and 77.8%, respectively.

Table 3. Sensitivity and specificity to diagnose NAFLD with DM using 25(OH) vitamin D_3 and ROC curve.

Cutoff point	Area under the ROC curve (AUC)	Sensitivity	Specificity	Accuracy	P-value
≤22	0.901	100.00	77.8	89.6%	0.001

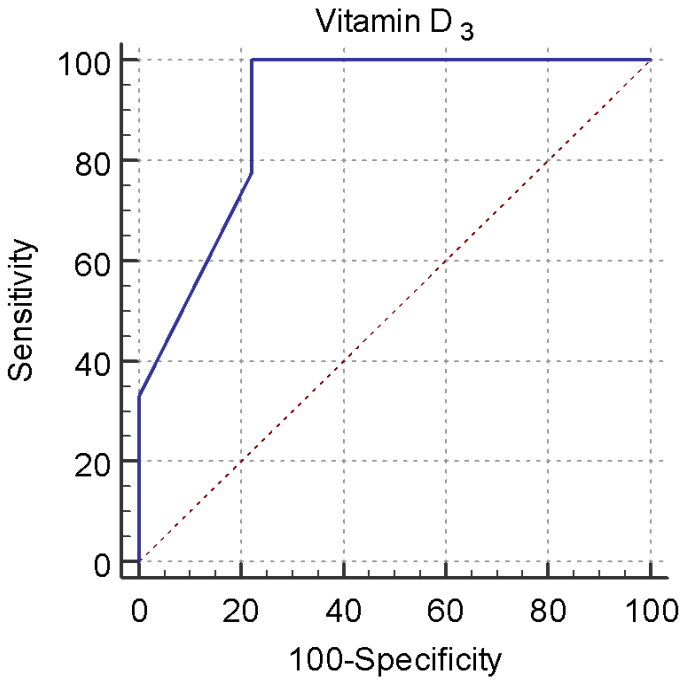


Figure 5. ROC curve for detecting NAFLD with DM using 25(OH) vitamin D_3

ROC curves were drawn to determine the maximum sensitivity and specificity for indices that showed statistically significant correlation with NAFLD without DM using 25(OH) vitamin D_3 to determine which one provided the maximum AUC. Table (4) and Figure (6) show that the AUC for various levels of 25(OH) vitamin D_3 for the diagnosis of NAFLD with DM was 0.877. The best cutoff value for 25(OH) vitamin D_3 levels was ≤ 34. The sensitivity and specificity of 25(OH) vitamin D_3 were 100% and 70.23%, respectively.

Table 4. Sensitivity and specificity to diagnose NAFLD without DM using 25(OH) vitamin D_3 and ROC curve.

Cutoff point	Area under the ROC curve (AUC)	Sensitivity	Specificity	Accuracy	P-value
≤34	0.877	100.00	70.23	84.2%	0.003

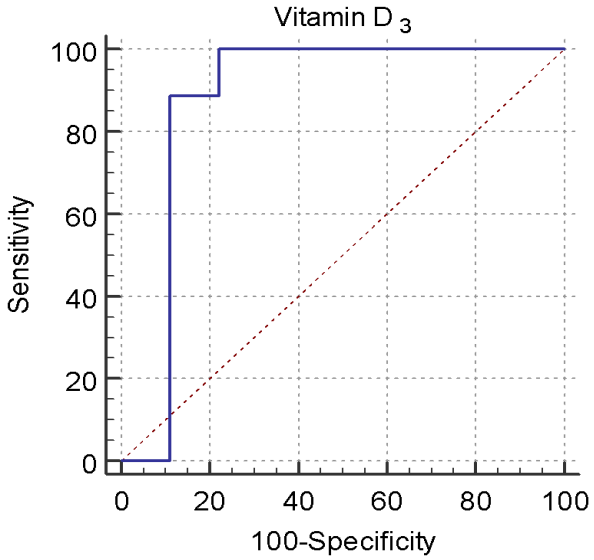


Figure 6. ROC curve for detecting NAFLD without DM using 25(OH) vitamin D_3.

DISCUSSION

Comparing patients with DM and no NAFLD to the general population, those with NAFLD had a mortality risk that was twice as high. Similar to individuals with NAFLD without DM, those with NAFLD with DM had a 22-fold rise in the risk of liver-related death and a threefold increase in total mortality risk [24].

According to the study's findings, NAFLD in individuals with and without DM is correlated with blood Vit D levels. Individuals with NAFLD with DM had considerably lower Vit D levels than patients with NAFLD alone. This is in line with Xiu, et al. [25], which discovered that individuals with T2DM exacerbated by NAFLD had decreased Vit D levels than those with T2DM alone.

Numerous pathologic systems benefit from Vit D. Lack of Vit D may cause oxidative stress, increase systemic inflammation, and raise the risk of developing NAFLD. Liver disorders and vitamin D interact with one another. Autophagy is boosted by vitamin D, and hepatic steatosis is avoided [10]. Lack of vitamin D may raise the

expression of resistin, hepatitis, oxidative stress genes, and insulin resistance, all of which can raise the risk of developing NAFLD [26].

The NFS, a globally accepted measure, may determine the level of liver fibrosis in DM patients. To detect liver fibrosis in patients with NAFLD and DM, we employed the biomarker mentioned above since liver biopsy cannot be extensively used in clinical practice. We discovered that patients with DM had lower blood Vit D levels than those without DM. This research also implies a connection between Vit D levels and the severity of liver disease. According to [27], patients with moderate and severe NAFLD had levels of 25-(OH) Vit D that were lower than those of the healthy control group. Serum 25-(OH) Vit D levels in individuals with liver fibrosis decreased considerably [28]. When gender, FBG, BMI, age, and other variables were considered, 25-(OH) D was shown to have a negative correlation with both NAFLD and progressive liver fibrosis [14, 29].

This is in line with [30], which discovered a connection between Vit D insufficiency and the severity of fibrosis, viral comorbidities, and liver function. A lack of vitamin D has also been associated with cancer development, especially liver cancer. Consequently, it is crucial to maintain normal blood vitamin D levels [31, 32].

In this investigation, groups II and III had higher levels of insulin, HOMA-IR, and blood sugar (FBS and 2HPP) compared to the control group ($p < 0.001$). TGs, total cholesterol, and HDL levels in groups II and III were considerably higher than in the control group. Additionally, there was a statistically significant difference in creatinine levels across the three groups. However, there were no significant variations in AST, LDL, ALP, ALT, or urea levels between any of the groups. The research of [33], which comprised 211 consecutive participants and found that 154 had NAFLD and 57 did not, contradicts this. The FBS ($p = 0.005$), ALP ($p = 0.028$), insulin ($p = 0.016$), HOMA-IR ($p = 0.003$), total cholesterol ($p < 0.001$), and TG ($p < 0.001$) values were all higher in the NAFLD group.

In this research, groups II and III had decreased serum 25(OH) vitamin D levels than the control group, which was statistically significant. This is consistent with the work of [34], which showed that blood 25(OH) vitamin D levels in individuals with NAFLD were lower than those in healthy control participants.

This investigation discovered no association between Vit D and blood glucose, HOMA-IR, or HDL levels in the control group. In contrast, statistically significant indirect associations between 25(OH) vitamin D and LDL and TG were seen in group I. This is supported by [35, 36], who examined 100 healthy control volunteers and 162 people with NAFLD. Their research showed that HDL, FBS, AST, and ALT had no bearing on the connection between NAFLD and low 25(OH) Vit D levels. Low Vit D levels were shown to be independently linked to metabolic syndrome and insulin resistance in the research [37], even in Caucasians without diabetes mellitus. They also served as a predictor of a higher 10-year risk of developing hyperglycemia and insulin resistance.

CONCLUSION

Our findings showed a significant correlation between Vit D insufficiency and NAFLD, both with and without DM, as determined by ultrasound examination. However, there was only evidence of an indirect relationship between Vit D, LDL, and TG in group II. Further research is required to determine the effect of Vit D supplementation in preventing or delaying the advancement of NAFLD, as well as the involvement of Vit D deficiency in the pathophysiology of the disease.

List of Abbreviations: ALP: Alkaline phosphatase, ALT: Alanine transaminase, AST: Aspartate transaminase, TG: Triglyceride, NAFLD: Non-alcoholic fatty liver disease, HDL: High-density lipoprotein, Vit D: Vitamin D, T2DM:

Type 2 diabetes mellitus, ALT: alanine aminotransferase, DM: diabetes mellitus.

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