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Plasma Osteopontin Level and Nonalcoholic Fatty Liver Disease in Patients with Type 2 Diabetes Mellitus

Mahmoud Rizk*, Mohamed Ahmed El Assal, Mohammed Awad Sakr, Ahmed Refaat Mohammed, Mohamed Abd Ellatif Afifi

Department of Internal Medicine, Faculty of Medicine, Benha University, Benha, Egypt

***Corresponding author:** Mahmoud Rizk, **Mobile:** (+20) 01117454801, **E-mail:** mahmoudrizk70@yahoo.com

ABSTRACT

Background: Type 2 diabetes mellitus (T2DM) and nonalcoholic fatty liver disease (NAFLD) are frequently linked. NAFLD has grown to be a major health problem. A glycoprotein called osteopontin (OPN) has been linked to the aetiology of NAFLD.

Objective: This study aimed to determine if there is a connection between the level of plasma OPN and the occurrence of NAFLD in people with T2DM.

Patients and Methods: This case-control study was conducted in the outpatient clinic of Internal Medicine Department at Benha University Hospital through the period between November 2022 and April 2023. It included 138 participants divided into three groups: Group 1 included 46 patients with NAFLD and T2DM, group 2 included 46 patients with NAFLD without T2DM, and group 3 that contained 46 healthy individuals as control group.

Results: There were significantly higher plasma OPN concentrations in both groups 1 and 2 than in group 3. OPN levels were higher in group 1 compared to group 2. A positive correlation was found between OPN and body mass index (BMI) in both NAFLD groups. In group 2, plasma OPN correlated positively with uric acid and high-density lipoproteins (HDL) levels, while a negative correlation was observed with systolic blood pressure (SBP).

Conclusion: Patient's with NAFLD and T2DM had higher plasma OPN levels more than those without diabetes who have NAFLD. Additionally, our study showed a favorable association between plasma OPN level and BMI, uric acid (UA), and HDL, suggesting that plasma OPN may have a role in the adjustment of the metabolic state.

Keywords: Osteopontin, NAFLD, Type 2 diabetes mellitus.

INTRODUCTION

One of the most common causes of chronic liver disease (CLD) in the globe is NAFLD. In the absence of alcohol misuse, drug adverse effects, or viral hepatitis, NAFLD is characterised by an increased intrahepatic triglyceride (TG) concentration, with or without inflammation and fibrosis [(1)].

Steatosis alone, which is often benign, can advance to steatohepatitis, which has inflammation and fibrosis, followed by cirrhosis, liver failure, and in rare circumstances, hepatocellular cancer. Obesity, DM, and metabolic syndrome are frequently linked to NAFLD. It is regarded as a component of the metabolic syndrome as well [(2)].

T2DM is characterised by hyperglycemia brought on by a variety of pathophysiological elements, most notably insulin resistance and insufficient insulin production. Diabetes patients are more likely to experience accelerated atherosclerosis, which can lead to coronary artery disease, peripheral artery disease, and cerebrovascular diseases, all of which have a significant negative impact on morbidity and mortality[(3)].

Diabetes is expected to be substantially more common in NAFL and nonalcoholic steatohepatitis (NASH) patients than in the general population (8.5%), with prevalence rates of 22.51% and 43.63%, respectively [(4)].

Inflammation, immunity, angiogenesis, fibrogenesis, and carcinogenesis in different tissues are all strongly hypothesised to play a role in the complex bidirectional association between the progression of NAFLD and T2DM, and their interaction may lead to 22 an increase in both hepatic and diabetic mortalities in cases with concurrent NAFLD and T2D [(5)].

The crucial role of OPN in cell signaling, including the control of cell proliferation, migration, inflammation, fibrosis, and tumour growth, has been well studied. OPN may have a role in the development of NAFLD and NASH, according to earlier investigations. In morbidly obese people, elevated OPN expression in the liver substantially linked with steatosis and insulin resistance [(6)]. Our study aimed at determination if there is a connection between the level of plasma OPN and the occurrence of NAFLD in people with T2DM.

PATIENTS AND METHODS

This study was conducted in the outpatient clinic of Internal Medicine Department at Benha University Hospital through the period between November 2022 and April 2023. It included 138 participants divided into three groups. Group 1 included 46 cases with NAFLD and T2DM, group 2 included 46 cases with NAFLD without T2DM, and group 3 contained 46 healthy subjects as control group.

Inclusion criteria: Patients aged ≥ 18 years, diagnosed patients with NAFLD by abdominal ultrasonography based on World Gastroenterology Organisation Global Guidelines [(7)].

Exclusion criteria: Patients with secondary causes of liver steatosis [(7)]. Patients who have gestational

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diabetes, type 1 diabetes, or other particular forms of diabetes, patients who take any steatogenic medications like (amiodarone, valproic acid, corticosteroids and tetracyclines), people with any history of alcohol use, persistent liver illness brought on by other factors, viral hepatitis C or B, hemochromatosis, drug-induced hepatitis, known cases of Wilson disease or autoimmune hepatitis, patients with malignant tumours, severe cardiovascular illness, severe cerebrovascular disease, thyroid dysfunction, polycystic kidney disease, chronic kidney disease, or chronic polycystic kidney disease were not included in this research.

All participants were subjected to:

1. Detailed history taking focusing on history of diabetes mellitus, medications and past history of any medical condition or previous hospital admissions.

2. Complete clinical examination including BMI (kg/m^2) was determined by measuring the subject's height and weight. BMI was computed by dividing the weight in kilograms by the square of the height in meter (kg/m^2). A professionally trained nurse took the individual's blood pressure while they were sitting using a mercury sphygmomanometer.

3. Laboratory investigations:

After 10–12 h of overnight fasting, before breakfast, skilled nurses took venous blood samples into vacuum tubes from the antecubital vein. Blood samples underwent centrifugation at 3000 rpm, divided into portions, and kept at -80^{o}C. A Cobas 6000 biomedical analysis device performed all biochemical assays. Fasting glucose (FG), total cholesterol (TC), low-density lipoprotein (LDL), and triglycerides (TG) were tested using the glucose oxidase and enzymatic techniques, respectively and high-density lipoprotein cholesterol (HDL-C) was measured utilizing the clearance method.

The following investigations were performed to all participants:

Hemoglobin concentration (Hb%), red blood cells (RBCs) count, white blood cells (WBCs) count, and platelet count are all included in the complete blood picture (CBC). Alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin and bilirubin are the liver function tests. Lipid profile: serum TG, LDL, HDL, and total cholesterol. Ion exchange high-performance chromatography was used to quantify glycosylated hemoglobin (HbA1c). The homeostasis model assessment of insulin resistance (HOMA-IR) formula for plasma insulin was as follows: Fasting insulin (FIns, mU/L) divided by fasting blood glucose (FBG, mmol/L) equals HOMA-IR [(8)].

Tests for kidney function: Blood urea levels and serum creatinine. Uridine in the blood. Level of fasting blood sugar. Employing an enzyme immunoassay (EISA) to detect viral markers (HBsAg, anti-HCV Ab). ELISA measurements of plasma OPN utilising OPN ELISA kits that made available by Bioneovan Co., Ltd. at 18 Keyuan Road, DaXing Industry Zone, Beijing, China.

Ethical approval

Benha Medical Ethics Committee of Benha Faculty of Medicine gave its approval to this study. All participants gave written consents after receiving all information. The Helsinki Declaration was followed throughout the study's conduction.

Sample size: Epiinfo program was used to calculate the least sample size at 0.05 level of significance and power 0.8, and it was 138 subjects divided into 46 in each group.

Statistical analysis

The SPSS version 26 statistical analysis programme was used. Using the unpaired Student's t-test and ANOVA (F) test, quantitative variables were given as means and standard deviation (SD) and compared between the groups. The Chi-square test or Fisher's exact test was used to analyse qualitative variables, which were provided as frequency and percentage (%). With non-parametric quantitative data, Spearman's correlation was utilised to examine the relationship between two variables. The examination of receiver operating characteristics (ROC) was also carried out. Statistical significance was defined as a two tailed P value ≤ 0.05.

RESULTS

No significant difference existed between the three groups as regards age, sex and smoking habit. The mean BMI was significantly higher in groups 1 and 2 than in group 3 ($P < 0.001$), with no significant difference in between (Table 1).

Table (1): Comparison of the demographics between the three groups

Variables	Group 1 (NAFLD with T2DM) (n=46)	Group 2 (NAFLD without T2DM) (n=46)	Group 3 (Control group) (n=46)	Test of significance	Multiple comparisons
Age (years) [Mean ± SD]	47.24 ± 8.09	48.70 ± 8.80	47.41 ± 8.28	F = 0.413 P = 0.663	P_1=0.684 P_2 = 0.995 P_3= 0.745
Gender [n (%)]				c^2 = 0.764 P= 0.683	P_1=0.608 P_2 = 0.884 P_3= 0.510
Male	25 (54.3%)	28 (60.9%)	24 (52.2%)		
Female	21 (45.7%)	18 (39.1%)	22 (47.8%)		
BMI (kg/m^2) [Mean ± SD]	29.09 ± 5.08	28.74 ± 3.23	22.97 ± 0.71	F = 44.354 P <0.001 *	P_1=0.880 P_2<0.001 * P_3<0.001 *
Smoking [n (%)]				c^2 = 1.270 P= 0.530	P_1=0.460 P_2 = 0.934 P_3= 0.318
No	37 (80.4%)	40 (87%)	36 (78.3%)		
Yes	9 (19.6%)	6 (13%)	10 (21.7%)		

F: One-way ANOVA test, P: General intergroup significance, P1: Comparing between Group 1 (NAFLD with T2DM) and Group 2 (NAFLD without T2DM), P2: Comparing between Group 1 (NAFLD with T2DM) and Group 3 (Control group), P3: Comparing between Group 2 (NAFLD without T2DM) and Group 3 (Control group), *: Statistically significant (p≤ 0.05).

A non-significant difference existed among the three groups regarding the mean DBP. However, the mean SBP was significantly higher in groups 1 and 2 than in group 3 (P = 0.001), with no significant difference in between **(Figure 1).**

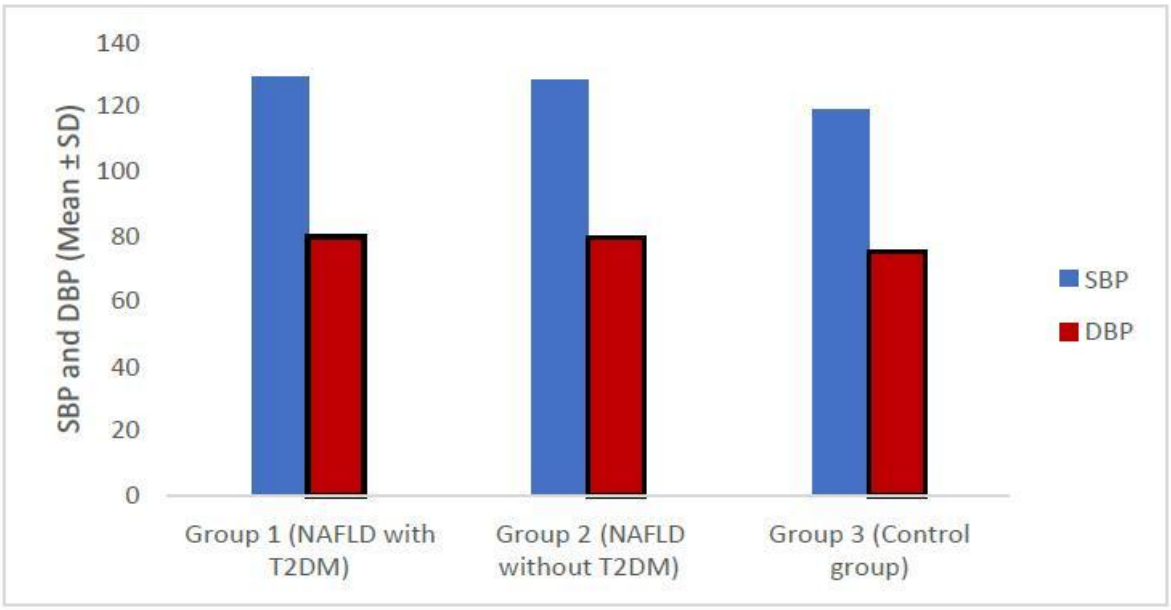

Figure (1): SBP and DBP in the three study groups.

No statistically significant difference was detected between the three groups as regards hemoglobin, platelets count, WBCs count, albumin, creatinine and urea. However, ALT, AST, uric acid, total cholesterol, LDL and fasting insulin levels were statistically significantly higher in groups 1 and 2 than in group 3, with no significant difference in between. TG and HOMA-IR were statistically significantly higher in groups 1 and 2 compared to group 3. Moreover, TG and HOMA-IR were significantly higher in group 1 than group 2. HDL was significantly lower in groups 1 and 2 than in group 3. HDL was significantly lower in group 1 than in group 2. The FBG and HbA1c were statistically significantly higher in group 1 than in groups 2 and 3 (Table 2).

Table (2): Comparison of the laboratory data in the study groups

Variables	Group 1 (NAFLD with T2DM) (n=46)	Group 2 (NAFLD without T2DM) (n=46)	Group 3 (Control group) (n=46)	Significance test	Intergroup Significance
Hemoglobin (gm/dL) [Mean ± SD]	13.55 ± 1.26	13.71 ± 1.24	13.80 ± 0.78	F = 1.665 P = 0.448	P1= 0.616 P2 = 0.580 P3= 0.764
PLTs(10^3/ml) [Mean ± SD]	291.57 ± 70.41	296.54 ± 65.63	295.80 ± 63.06	F = 0.075 P = 0.928	P1= 0.931 P2 = 0.950 P3= 0.998
WBCs (10^3/ml) [Mean ± SD]	5.97 ± 1.44	6.22 ± 1.41	5.88 ± 0.87	F = 0.866 P = 0.423	P1=0.613 P2 = 0.945 P3 = 0.417
ALT (U/L) [Mean ± SD]	35.11 ± 8.32	33.85 ± 8.56	20.46 ± 4.34	F = 37.015 **P <0.001** *	P1 = **0.782** **P2<0.001** * P3<**0.001** *
AST (U/L) [Mean ± SD]	22.20 ± 4.47	23.70 ± 4.28	17.11 ± 4.24	F = 17.916 **P <0.001** *	P1=**0.397** **P2<0.001** * P3<**0.001** *
Albumin (mg/dL) [Mean ± SD]	4.21 ± 0.38	4.19 ± 0.36	4.30 ± 0.41	F = 1.135 P = 0.325	P1= 0.943 P2 = 0.509 P3= 0.325
Creatinine (mg/dL) [Mean ± SD]	0.70 ± 0.13	0.74 ± 0.13	0.70 ± 0.05	F = 1.520 P = 0.342	P1= 0.230 P2 = 0.986 P3= 0.238
Urea (mg/dL) [Mean ± SD]	17.43 ± 3.67	16.72 ± 3.35	16.59 ± 2.66	F= 0.904 P = 0.407	P1= 0.543 P2 = 0.427 P3= 0.980
Uric acid (mg/dL) [Mean ± SD]	6.08 ± 1.06	5.77 ± 1.02	4.32 ± 0.75	F= 44.487 P <**0.001** *	P1=0.269 **P2<0.001** * P3<**0.001** *
Total cholesterol (mg/dL) [Mean ± SD]	261.54 ± 26.33	254.70 ± 27.16	119.22 ± 14.80	F= 38.984 P <**0.001** *	P1 = 0.344 **P2<0.001** * P3<**0.001** *
Triglycerides (mg/dL) [Mean ± SD]	164.04 ± 16.33	132.89 ± 17.03	84.33 ± 7.90	F = 59.845 **P <0.001** *	P1<**0.001** * **P2<0.001** * P3<**0.001** *
HDL (mg/dL) [Mean ± SD]	39.33 ± 5.99	49.33 ± 5.54	56.91 ± 3.67	F = 34.153 P <**0.001** *	P1<**0.001** * **P2<0.001** * P3<**0.001** *
LDL (mg/dL) [Mean ± SD]	120.41 ± 11.88	123.24 ± 13.78	92.74 ± 12.20	F = 18.947 P <**0.001** *	P1 = **0.533** **P2<0.001** * P3<**0.001** *
FBG (mg/dL) [Mean ± SD]	128.54 ± 21.68	87.50 ± 9.81	86 ± 9.15	F = 23.770 P <**0.001** *	P1<**0.001** * **P2<0.001** * P3= **0.877**
HbA1c (%) [Mean ± SD]	8.91 ± 1.42	5.11 ± 0.43	4.95 ± 0.42	F = 89.364 P <**0.001** *	P1<**0.001** * **P2<0.001** * P3= **0.649**
Fasting insulin (mIu/L) [Mean ± SD]	16.35 ± 4.03	17.30 ± 4.24	7.63 ± 1.84	F = 57.911 P <**0.001** *	P1=**0.600** **P2<0.001** * P3<**0.001** *
HOMA-IR [Median (range)]	4.95 (2.24-9.89)	3.52 (1.66-7.86)	1.57 (0.76-2.61)	KW = 89.84 P <**0.001** *	P1<**0.001** * **P2<0.001** * P3<**0.001** *

Median and range: non-parametric test. KW: Kruskal Wallis test, F: One-way ANOVA test, P: General intergroup significance, P1: Comparing between Group 1 (NAFLD with T2DM) and Group 2 (NAFLD without T2DM), P2: Comparing between Group 1 (NAFLD with T2DM) and Group 3 (Control group), P3: Comparing between Group 2 (NAFLD without T2DM) and Group 3 (Control group), *: Statistically significant ($p \leq 0.05$)

The median (range) of OPN level was 89 ng/ml (44 - 210), 57 ng/ml (29 - 94) and 20.5 ng/ml (10 - 36) in groups 1, 2 and 3 respectively.

The median OPN was statistically significantly higher in groups 1 and 2 than in group 3 (P <0.001). Also, the median OPN was statistically significantly higher in group 1 than in group 2 **(Figure 2).**

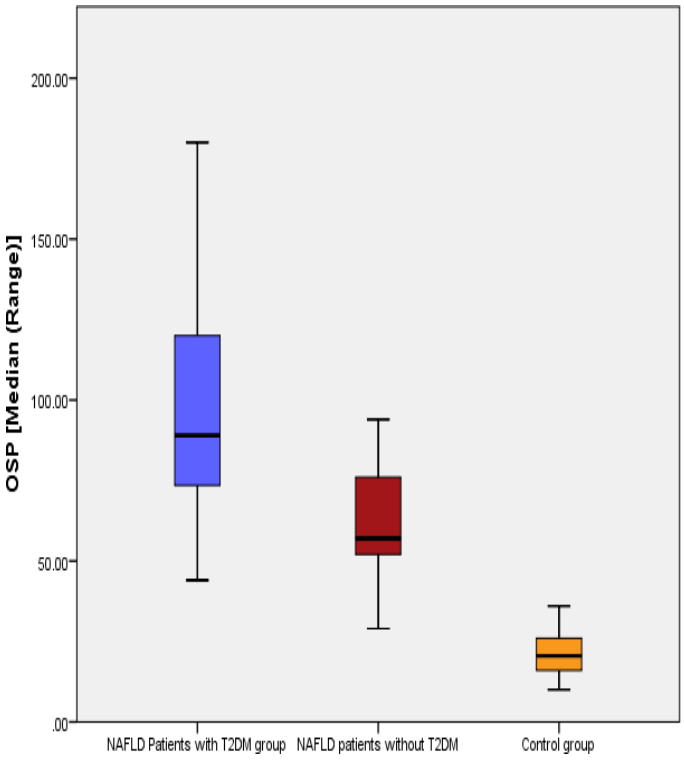

Figure (2): Box Blot OPN level among studied groups

In group 1, a significant positive relationship existed between OPN and BMI. Other variables didn't show statistically significant correlation with OPN. In group 2, there was a significant positive relationship between OPN and BMI, uric acid and HDL. On the other hand, there was a significant negative relationship between OPN and SBP. Other variables didn't show statistically significant correlation with OPN (Table 3).

Table (3): Relationship between OPN with other variables in group 1 (NAFLD with T2DM) and correlation between OPN with other variables in group 2 (NAFLD without T2DM)

		OPN with other variables in Group 1	**Correlation between OPN with other variables in Group 2 (NAFLD without T2DM)**
Age	rs	0.001	0.057
	P	0.992	0.707
BMI	rs	**0.811****	**0.615****
	P	**0.000**	**0.000**
SBP	rs	0.203	**-0.301***
	P	0.176	**0.042**
DBP	rs	0.012	-0.263
	P	0.938	0.077
HGB	rs	0.064	-0.035
	P	0.671	0.818
WBC	rs	-0.008	-0.238
	P	0.956	0.111
Platelet	rs	0.208	0.174
	P	0.166	0.247
ALT	rs	-0.066	-0.187
	P	0.663	0.214
AST	rs	-0.140	0.089
	P	0.353	0.558
Albumin	rs	0.053	0.148
	P	0.729	0.326
creatinine	rs	-0.063	-0.037
	P	0.678	0.807
urea	rs	-0.030	0.101
	P	0.845	0.504
UA	rs	0.112	**0.303***
	P	0.459	**0.041**
TC	rs	0.170	0.024
	P	0.258	0.872
TGs	rs	0.035	-0.087
	P	0.817	0.565
HDL	rs	-0.110	**0.296***
	P	0.468	**0.046**
LDL	rs	-0.290	-0.164
	P	0.051	0.276
FBS	rs	0.131	0.152
	P	0.386	0.315
HBA1C	rs	-0.027	0.084
	P	0.857	0.578
Fasting insulin	rs	0.082	-0.132
	P	0.590	0.382
HOMAIR	rs	0.154	-0.057
	P	0.307	0.709

The optimum cutoff point of OPN level to differentiate group 1 from group 2 was > 69.5 ng/ml, with high sensitivity (82.6%) and moderate specificity (69.6%) with an area under the curve (AUC) = 0.824 (Table 4 and figure 3**).** The optimum cutoff point of OPN level to differentiate between group 2 and group 3 was > 33 ng/ml, with high sensitivity (95.7%) and specificity (91.3%) with (AUC) = 0.992 (Table 4 and Figure 4).

Table (4): Predictive value of OPN to differentiate between Group 1 (NAFLD with T2DM) and Group 2 (NAFLD without T2DM) and Predictive value of OPN to differentiate between Group 2 (NAFLD without T2DM) and control group

	OPN to differentiate group 1 from group 2	**OPN to differentiate group 2 from controls**
AUC	0.824	0.992
Cut offpoint	**> 69.5**	**> 33**
Sensitivity	**82.6%**	**95.7%**
Specificity	**69.6%**	**91.3 %**
PPV	**78.2%**	**94.6%**
NPV	**67.3%**	**95.2%**
Accuracy	**76.2%**	**94.8%**
P	< 0.001*	< 0.001*

AUC: Area under curve, PPV: positive predictive value, NPV: Negative predictive value

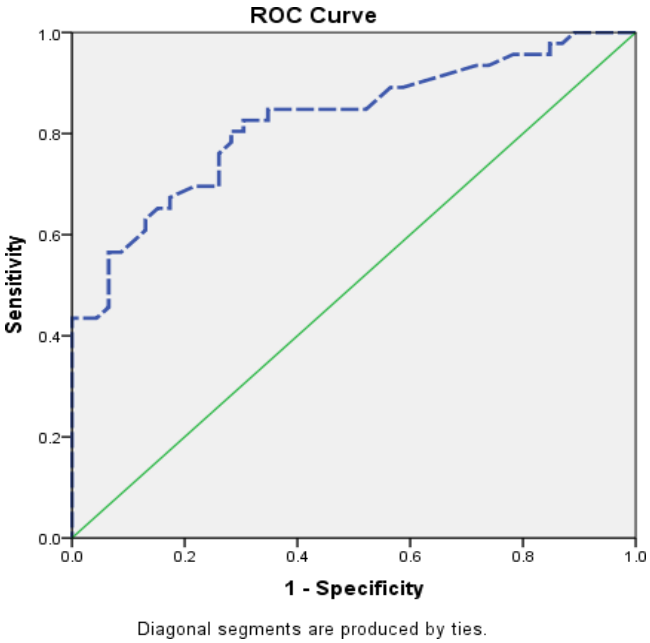

Figure (3): ROC curve of OPN in NAFLD and T2DM patients

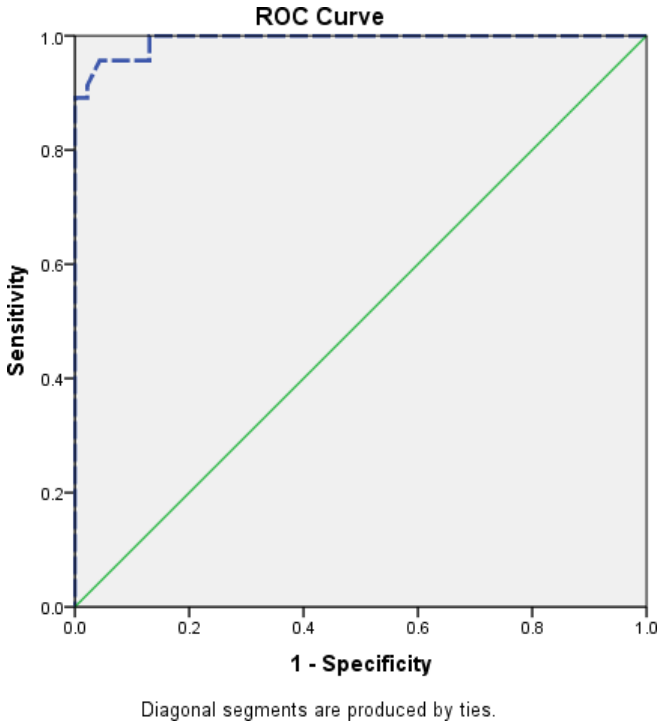

Figure (4): ROC curve of OPN in NAFLD patients

DISCUSSION

NAFLD stands as a prominent cause of CLD in developed nations and is a significant factor driving liver transplantation in the United States. The interplay of genetic, demographic, clinical, and environmental factors contribute to NAFLD's pathogenesis and associated economic strain on healthcare systems, necessitating effective risk factor identification and patient screening [(9)]. Diabetes independently heightens NAFLD risk, escalating its progression to advanced liver conditions, such as fibrosis, cirrhosis, and hepatocellular carcinoma, with approximately 70% prevalence in T2DM patients. Amid these challenges, the quest for a simple, cost-effective, and noninvasive diagnostic method for NAFLD becomes pivotal [(10)].

OPN, first identified in 1979, is a multifunctional protein that is expressed in the kidneys and bones in a healthy setting. However, in pathological settings affecting multiple organs, its presence has been associated to inflammation, angiogenesis, fibrosis, and carcinogenesis [(10)]. As a predictor of liver fibrosis in a variety of liver illnesses, including NASH, alcoholic liver disease, HBV, and HCV. OPN concentration in plasma has showed potential. OPN is a strong option for noninvasive evaluation and screening in the diagnosis and treatment of NAFLD because of this potential [(11)].

Our study aimed at to determination if there is a connection between the level of plasma OPN and the occurrence of NAFLD in people with T2DM.

In the current study, age, gender, and smoking prevalence did not significantly differ across the three groups under study. There was no discernible difference between the case groups, and groups 1 and 2 had considerably higher mean BMIs than the control

group. **Gorden *et al.*** [12] and **Petrovi *et al.*** [13] both reported findings that were similar. Higher BMI was strongly associated with an elevated risk of NAFLD/NASH in few additional investigations by **Loomis *et al.*** [14] and **Tang *et al.*** [15].

In our study, the analysed group's mean DBPs did not differ substantially from one another, while group 1 and group's 2 mean SBPs were considerably greater than group's 3. Researchers **Fan *et al.*** [16] and **Pasanta *et al.*** [17] found a link between NAFLD and hypertension. Groups 1 and 2 had considerably higher ALT and AST values than group 3 did. NAFLD is frequently diagnosed using the liver damage markers ALT and AST. However, **Vernon *et al.*** [18] found that individuals with NAFLD can have normal levels of liver enzymes, limiting their predictive value. A study by **Sanyal *et al.*** [19] showed a significant relationship between NAFLD and elevated ALT and AST levels, especially in diabetic cases.

In our study, the levels of uric acid were considerably greater in groups 1 and 2 compared to group 3, which is consistent with earlier studies by **Afzali *et al.*** [20] showing a connection between uric acid and liver conditions such NAFLD and NASH. Additionally, a research by **Jensen *et al.*** [21] showed that having high uric acid levels increases the chance of developing NAFLD. In line with the current study, Using 3362 participants from the Multi-Ethnic research of Atherosclerosis cohort, **DeFilippis *et al.*** [22] conducted a sizable research and discovered that those with NAFLD had higher triglyceride levels and lower levels of HDL-cholesterol, but no significant differences were detected in LDL-cholesterol. Our results on the lipid profile are consistent with various earlier investigations by **Tang *et al.*** [15] and **Feng *et al.*** [23]. Our findings, however, are in opposition to a **Nigam *et al.*** [24] research that reported no discernible variation in HDL levels between NAFLD patients and healthy controls.

Regarding glycemic state, NAFLD patients with diabetes had significantly higher FBG, HbA1c, fasting insulin, and HOMA-IR levels compared to NAFLD without diabetes and control groups. These results are in line with earlier researches by **Feng *et al.*** [15], and **Tang *et al.*** [23]. A meta-analysis of 15 studies done by **Sookoian and Pirola** [25] found a significant elevation in fasting blood sugar levels in lean and obese subjects with NAFLD compared to those without NAFLD. Previous studies by **Mashahit *et al.*** [8] and **Gutierrez-Buey *et al.*** [26] have also shown a significant association between insulin resistance (evaluated by HOMA-IR) and the presence of NAFLD.

In our study, OPN levels were substantially greater in groups 1 and 2 than in group 3, with group 1 having the highest values relative to group 2. The same results were reported by **Wang *et al.*** [27] who discovered a larger proportion of NAFLD and T2DM patients in higher OPN quartiles and hypothesised that OPN could be a viable diagnostic biomarker for predicting NAFLD and T2DM. A prior work by **Bertola *et al.*** [28] also demonstrated OPN's contribution to the development of NAFLD and NASH. Steatosis and insulin resistance in obese people have been linked to increased OPN expression in the liver. OPN may serve as a non-invasive biomarker for liver fibrosis, according to research by **Glass *et al.*** [29] that demonstrated a correlation between hepatic OPN expression and plasma OPN concentrations and liver fibrosis in NAFLD patients.

In our study in NAFLD patients with diabetes, there was a link between OPN and BMI that was positive, as well as positive correlations between OPN and BMI, uric acid, and HDL in NAFLD patients without diabetes. **Wang *et al.*** [27] also found elevated OPN levels in overweight/obese individuals and observed correlations between OPN and various metabolic parameters like uric acid and HDL. A study by **Wang *et al.*** [27] conducted ROC analysis and found that a combination of OPN, TG, and uric acid improved the diagnostic accuracy for NAFLD and NAFLD with T2DM, with higher sensitivity and specificity compared to OPN alone.

CONCLUSION

Patients having NAFLD with T2DM had higher plasma OPN levels than NAFLD patients without T2DM. Additionally, our study showed a favourable association between plasma OPN level and BMI, uric acid, and HDL, suggesting that plasma OPN may play a role in the adjustment of the metabolic state.

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Competing interests: Nil.

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