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The Role of Osteopontin Level in Development of Non-alcoholic Fatty Liver Disease in Patients with Type 2 Diabetes mellitus

Qassim Khalf Khadom[1], Manal Kamal Rasheed[2], Khalid Abdulla Jasim Alkazraj[3]

[1] *Department of Biochemistry, College of medicine-University of Baghdad, Iraq.*
Email: qasem.khalf1209f@comed.uobaghdad.edu.iq
[2] *Clinical Biochemistry, Department of Biochemistry, College of Medicine, University of Baghdad, Iraq.*
Email: dr.manalkamal@comed.uobaghdad.edu.iq
[3] *Specialist Binoculars of the digestive system/ Baghdad Hospital /Al-Tayeb City, Iraq.*
Email: drkhalid1959git@comed.uobaghdad.edu.iq

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Abstract

Type 2 diabetes mellitus (T2DM), one of the most common metabolic disorders, is caused by a combination of two primary factors: defective insulin secretion by pancreatic β-cells and the inability of insulin-sensitive tissues to respond appropriately to insulin.

The results of the present investigation show a considerable rise in circulating OPN in patients with T2DM and NAFLD. More importantly, the results of the current study indicate that OPN is a single predictor of NAFLD and T2DM and may help the metabolic condition get worse. To determine how OPN affects those with more severe metabolic abnormalities, such as NAFLD and T2DM, more research will need to be done.

In pathogenic and physiologically healthy conditions, the secreted protein osteopontin (OPN) has a range of roles in processes like biomineralization, tissue remodeling, and chronic inflammation.

Aim of study

The goal of the current investigation was to determine the clinical correlates of osteopontin levels in this cohort and the relationship between serum OPN concentration and the prevalence of non-alcoholic fatty liver disease in individuals with type 2 diabetes mellitus.

Method

Two groups of 80 Iraqi volunteers, aged 35 to 67 (46 men, 34 women), were formed: group (A) included 40 patients with type 2 diabetes and non-alcoholic fatty liver, while group (B) included 40 healthy controls. After an 8 to 12-hour fast, the patients' veins had approximately 5 ml of blood. Two portions of each blood sample were separated.

A. The initial 2 ml of whole blood were kept in EDTA tubes to be used for the NYCOCARDTM reader II's measurement of glycated hemoglobin (HbA1C).
B. the subsequent Three milliliters of blood were centrifuged at 3000 revolutions per minute for ten minutes to separate the blood into two aliquots that were then placed in Eppendorf tubes for the automated assays of lipid profile, FBS, GPT, GOT, albumin, and alkaline phosphate.

Following osteopontin and insulin enzyme-linked immunosorbent assay (ELISA) measurements, equation

calculations of insulin resistance (IR) values for each sample and BMI measurements for each patient were performed.

Result

The case group (A) in this study had significantly higher mean levels of FBS, HbA1c, insulin, HOMA-IR, AST, ALT, triglycerides, LDL, and VLDL than the controls group (B) did, according to the comparison of biochemical parameters between the two groups (A, B).
($P \leqslant 0.05$). At the same time, the case group's mean HDL level was noticeably lower than the controls. Osteopontin level in groups A higher than group B (control) means that OPN Markers to (NAFLD) and OPN are greatly increased in (NAFLD), which can be used to diagnose Diabetes Mellitus type 2 with non-alcoholic fatty liver disease (NAFLD) and OPN. No significant difference ($P \geqslant 0.05$) was found in the mean levels of cholesterol, LDLc, and ALP between the studied groups.

Conclusion

All indicators were within the increased in the group of individuals with Type 2 diabetes mellitus and nonalcoholic fatty liver disease. It may be argued that those measures serve as diagnostic signs for disease because the levels of (FBS, HbA1c, insulin, HOMA-IR, cholesterol, triglyceride (TG), LDL-c, VLDL-c, ALT, AST, and ALP) were higher than in the control group. The ideal Osteopontin cut-off value for the detection of non-alcoholic fatty acid liver disease and type 2 diabetes mellitus was 17.48 ng/ml. Osteopontin levels above 17.48 ng/ml are indicative of type 2 diabetes and non-alcoholic fatty liver disease. Patients with diabetes mellitus had significantly lower HDL-ch levels than those in good health. These results convince us that low HDL-ch levels are a significant factor in illness risk. Age may raise the likelihood of developing diabetes mellitus, according to the results of the osteopontin, insulin, and HOMA-IR study).

Keywords

Osteopontin, Type 2 diabetes mellitus, Non-alcoholic fatty liver.

The interplay of two essential causes leads to Type 2 diabetes mellitus (T2DM), one of the most prevalent metabolic diseases: reduced insulin production by pancreatic beta cells and improper insulin response in organs sensitive to insulin. (1). In 2021, there will be 2 million people in Iraq between the ages of 20 - 79 who have diabetes mellitus, making about 13–9% of the nation's population, according to the International Diabetes Federation (IDF). Osteopontin is ahighy phosphorylated glycophosphoprotein with acidic characteristics and 300 amino acids, osteopontin also contains O- and N-linked oligosaccharides. It is also quite high in aspartic acid. (2) Since "pontin" is a derivative of the Latin word "pons," which means "bridge," it is implied that osteopontin functions as a linking protein. "Osteo" in the protein's name denotes that it is expressed in bone. (3) The human gene is responsible for the production of the bone-forming protein 44 K BPP (bone phosphoprotein), also known as osteopontin, bone sialoprotein I (BSPI), early T-lymphocyte activation (ETA-I), urinary stone protein, nephropontin, uropontin secreted phosphoprotein 1, and rickettisia resistance (Ric). About 300 amino acid residues, 30 carbohydrate residues, and 10 sialic acid residues are joined to the extracellular structural protein of osteopontin. (4). a significant contributor to plaque formation and calcification as well as a marker of chronic coronary artery disease (5) adherence, may contribute to significant variations in glycemic control and adiposity markers of type 2 diabetes (T2DM) patients.(6). the liver enzymes level of(alkaline phosphatase, alanine transaminase, aspartate transaminase gama glutaminase transferase) is significantly higher in obese diabetic patients than non –obese diabetic patients and control group(7).

The **aims** of the present study were to investigate the relationship between serum OPN concentration and the presence of non-alcoholic fatty liver disease in individuals with type 2 diabetes mellitus and to determine the clinical correlates of osteopontin levels in this population.

Subjects, Materials And Methods

40 patients with type 2 diabetes and nonalcoholic fatty liver disease were included in group A of the case-control study, which included 80 Iraqi subjects with ages ranging from 35 to 67 years (both males and females), and 40 healthy controls in group B. From October 2022 to February 30th, 2023, samples were collected at the Baghdad Teaching Hospital in medicine city Baghdad Iraq of medicine. The college sought authorization to conduct the study in consultation with the diabetic unit at Baghdad Teaching Hospital, the biochemistry laboratory, and Al Zahraa Teaching Hospital in Kut.Draw In this study, participants (control and patients) who had fasted for 8–12 hours had their veins sampled for about 5 ml of blood. Two portions of each blood sample were separated. A - The first 2 ml of whole blood is kept in EDTA tubes for glycated hemoglobin (HbA1C) measurement using the NYCOCARDTM reader II.
B- Three milliliters of blood, the second component, were centrifuged at 3000 rpm for ten minutes. For further investigation, the aspirated blood was separated into two aliquots and put in Eppendorf tubes. Using an automated process and the Abbott Architect 4000, the following parameters were assessed instantly: FBS , GPT, GOT, albumin, alkaline phosphate, and lipid profile.

Enzyme-linked immunosorbent assay (ELISA) measurements of osteopontin and insulin were followed by the calculation of insulin resistance (IR) values for each sample using an equation and a BMI measurement.

Statistical Analysis

The Statistical Package for Social Sciences (SPSS) version 25 was utilized to conduct the analysis. The mean, SD, and ranges of the data were reported. Using the Pearson correlation approach, two quantitative variables were correlated, and a t-test was utilized to determine the significance of the connection at the 0.05 level.

Result

There was no statistically different that was identified. ($P \geqslant 0.05$) between the two groups (healthy control group B and patient Diabetes Mellitus Type 2 with nonalcoholic fatty liver group A in terms of age, gender, and BMI (Table1).

Table 1: Comparison of age, gender, and BMI between A,B groups

Patients Characteristics	Study Groups		P-Value*
	Groups A n=40	Groups B n=40	
Age(years)			
35-44	8 (20.0)	11(27.5)	
45 - 54	14 (35.0)	21 (52.5)	
⩾ 55	18 (45.0)	8 (20.0)	0.734
Gender			
Male	25 (62.5)	21 (52.5)	0.640
Female	15 (35.5)	19 (47.5)	
BMI			
Normal	13 (32.5)	11 (27.5)	0.636
Overweight	16 (40.0)	20 (50.0)	
Obese	11 (27.5)	9 (22.5)	

Student-test results indicating no difference between the two independent means 0.05 level ($p \geqslant 0.05$).

Comparison of biochemical parameters

Between group A and group B

A comparison of biochemical markers between the two groups revealed that group A had significantly higher mean levels of FBS, HbA1c, insulin, HOMA-IR, AST, ALT, triglycerides, LDL, and VLDL than the control group. ($P < 0.05$) Although group A's mean HDL level was much higher than group B's, it was still significantly lower in group A. no significant difference ($P \geqslant 0.05$) was seen between the two groups in the mean levels of ALP and cholesterol. (Table 2).

Table 2: Comparing the mean levels of the control group's iochemical parameters to those of group A

Parameters	Study Groups		P - Value*
	Group A Mean ± SD	Control Group Mean ± SD	
FBS (mg/dl)	157.8 ± 27.9	89.4 ± 7.54	0.001
HbA1c (mmol/mol)	7.92 ± 0.97	5.03 ± 0.48	0.001
Insulin (ng/ml)	29.29 ± 5.17	9.68 ± 3.80	0.001
HOMA-IR	9.93 ± 1.99	1.79 ± 0.31	0.001
AST (U/L)	86.16 ± 40.1	20.52 ± 8.53	0.001
ALT (U/L)	107.8 ± 54.2	18.43 ± 8.67	0.001
ALP (U/L)	4.14 ± 1.82	4.05 ± 0.38	0.916
Cholesterol (mg/dl)	168.9 ± 24.7	155.8 ± 34.9	0.057
Triglyceride (mg/dl)	212.7 ± 34.8	118.5 ± 31.7	0.011
HDL (mg/dl)	26.80 ± 8.54	47.93 ± 9.72	0.001
LDL (mg/dl)	99.61 ± 24.67	75.57 ± 30.82	0.011
VLDL (mg/dl)	42.51 ± 6.93	23.38 ± 6.27	0.001

* There is a considerable difference between two independent means when using a Student's test. 0.05 level($p \geqslant 0.05$).

Osteopontin level

A statistically significant difference was discovered in this investigation. ($P \leqslant 0.05$) the osteopontin mean values between two groups. (Table3).

Table 3: Comparison of Osteopontin level between the study groups

Group A had higher levels of osteopontin than group B because group A has type 2 diabetes along with non-alcoholic fatty liver disease (NAFLD) and OPN indicators, which are markedly elevated in (NAFLD).

Osteopontin ng/ml	Group A Mean ± SD	Group B Mean ± SD	P- Value
	23.66 ± 4.95	5.43 ± 1.67	0.001

* Significant difference between two independent means using Student-test at 0.05 level ($p \leqslant 0.05$)

To corroborate the variations in the mean Osteopontin levels between the research groups, post hoc tests (LSD) were performed. Group A and Group B had significantly greater amounts of osteopontin than the control group. (23.66 and 15.65 ng/ml vs 5.43 ng/ml, $P \leqslant 0.001$) (Table 4).

Cut-off value of Osteopontin

The osteopontin level was created for the Receiver Operating Characteristic (ROC) curve analysis for the diagnosis of type 2 diabetes mellitus and non-alcoholic fatty acid liver disease. The optimal osteopontin cut-off value for the identification of type 2 diabetes mellitus and non-alcoholic fatty liver disease was 17.48 ng/ml. Consequently, osteopontin levels > 17.48 ng/ml is a predictor for non-alcoholic fatty acid liver disease and type 2 diabetes mellitus. This cut-off value was extremely accurate, with a sensitivity and specificity of 95% and 77.5%, respectively, and an accuracy of 86.3%. The positive and negative predictive values for osteopontin were 80.9% and 93.9%, respectively. (Figure2)(Table4)

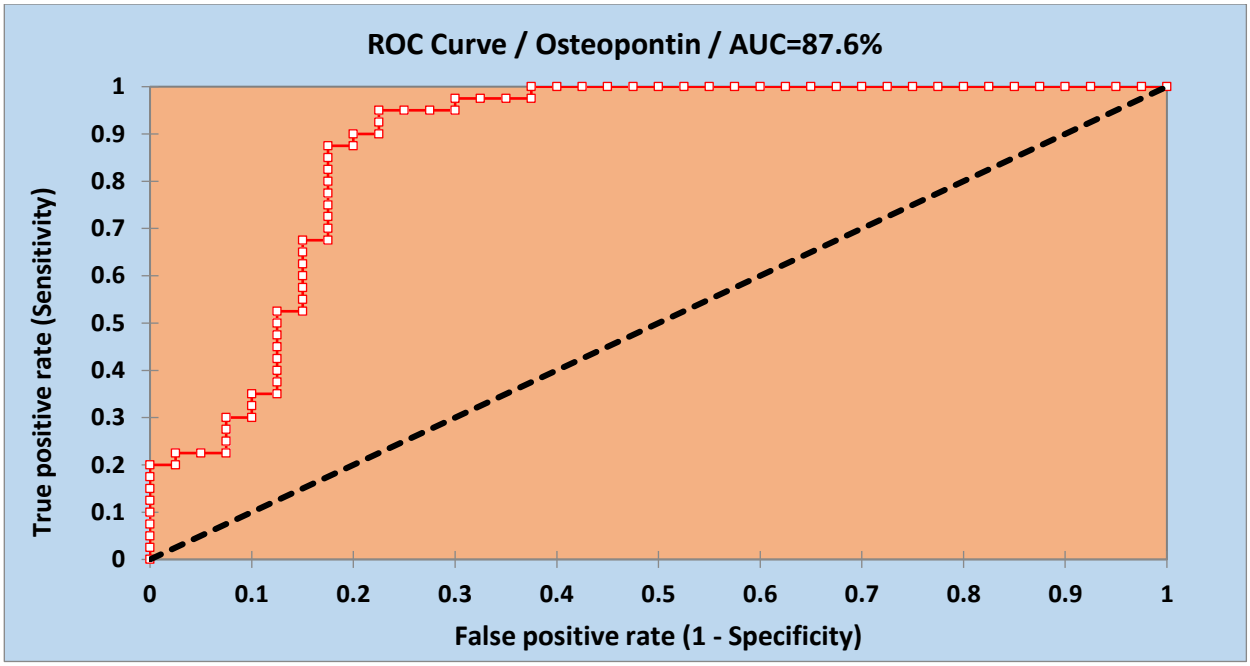

Figure 2: ROC curve for osteopontin in the identification of type 2 diabetes mellitus and non-alcoholic fatty liver disease with control.

Table 4. Cutoff value , sensitivity and specificity of Osteopontin

Clinical Parameter	Cut-off value	SN	SP	PPV	NPV	Accuracy
Osteopontin (ng/ml)	17.48	95%	77.5%	80.9%	93.9%	86.3%

Correlation between Osteopontin level and biochemical parameters of study groups

In the Pearson correlation analysis, there was a significant positive correlation between Osteopontin levels and BMI (r= 0.397, P⩽0.001), FBS (r= 0.701, P⩽0.001), HbA1c (r= 0.679, P⩽0.001), insulin (r= 0.675, P⩽0.001), HOMA-IR (r= 0.784, P⩽0.001), AST (r= 0.386, P⩽ 0.001), ALT (r= 0.578, P⩽0.001), triglycerides (r= 0.580, P⩽0.001), and VLDL (r= 0.588, P⩽0.001). On the other hand, Osteopontin level was negatively correlated with HDL (r= - 0.578, P⩽0.001), while it was not significantly correlated (P ⩾ 0.05) with cholesterol ,ALP and LDL (Table 5).

Table 5: Correlation of Osteopontin levels with biochemical parameters.

Parameters	Osteopontin (ng/ml)	
	r	P - Value*
BMI (kg/m^2)	0.397	0.001
FBS (mg/dl)	0.701	0.001
HbA1c (mmol/mol)	0.679	0.001
Insulin (ng/ml)	0.675	0.001
HOMA-IR	0.784	0.001
AST (U/L)	0.386	0.001
ALT (U/L)	0.578	0.001
ALP (U/L)	0.007	0.943
Cholesterol (mg/dl)	0.096	0.297
Triglyceride (mg/dl)	0.580	0.001
HDL (mg/dl)	- 0.578	0.001
LDL (mg/dl)	0.149	0.105
VLDL (mg/dl)	0.588	0.001

*Correlation is significant at the 0.05 level p⩽0.05.

Discussion

The findings of this research It has been found that insulin resistance, which causes lipid dysregulation and accumulation in people with NAFLD or beta cell malfunction in people with T2DM, is the primary contributing factor to the link between NAFLD and T2DM (6). In both obese and lean patients with NAFLD, it has been shown that a 10% decrease in body fat mass is adequate to correct steatosis, NAFLD, and hepatic insulin sensitivity (7). Losing weight has been demonstrated to be quite advantageous in this regard.

Furthermore, because mitochondrial activity degrades with aging, the prevalence of fatty liver will rise if it is associated with diabetes or other metabolic diseases. Due to increased TNF production by Kupffer cells, gut leakiness hastens the progression of steatohepatitis from fatty liver(8). Oxidative stress is made worse by increased Reaction Oxygen Species (ROS) generation and a compromised oxidation defense mechanism.

In times of inflammation, osteopontin promotes T-cell proliferation by increasing the expression of T-helper type-1 cytokines in macrophages and T-cells. It additionally encourages tissue fibrosis and causes an accumulation of extracellular matrix by sticking to type-I collagen, fibronectin, and osteocalcin (9). Studies on both humans and animals discovered that obese patients, mice, and rats had considerably increased osteopontin plasma concentrations and adipose content. Antibodies suppressed osteopontin function, which decreased inflammation brought on by obesity-related adipose tissue swelling. Signal transduction related to insulin resistance and glucose balance was turned around in different mouse species. According to current logic and strong data, osteopontin might provide a unique therapeutic approach to avoid obesity-related issues like NAFLD and T2DM (10).

Osteopontin levels were found to be positively correlated with BMI, FBS, HbA1c, insulin, HOMA-IR, AST, ALT, triglycerides, and VLDL in this investigation (P0.05) (10). This study and other investigations demonstrated a positive association between plasma osteopontin, neutrophils, and hsCRP, suggesting that OPN may be involved in inflammation brought on by metabolism. Block transcription factor, hepatic Akt, and insulin receptor substrate-2 (IRS-2) are some of the possible routes of osteopontin in glucose homeostasis and insulin sensitivity that may be significantly increased by osteopontin deficiency. Gluconeogenic target genes of Fork Head Box O1 include phosphoeno pyruvate carboxyl kinase (PEPCK) and glucose-6 phosphatase (G6P). OPN suppresses the hepatic STAT3 signal transducer and activator of transcription, according to another study (11). Triglycerides (TGs), in particular, are known to build up in the liver in NAFLD, albeit this is generally accepted. Furthermore, research has shown a link between hepatic osteopontin levels and liver TG levels (10). On the other hand, researchers observed that osteopontin, in addition to existing as an extracellular matrix molecule that is immobilized, is an attractive potential tumor marker because it may be found in bodily fluids like plasma (12).

Post hoc tests (LSD) were carried out to confirm the differences in the mean Osteopontin levels between the research groups. Group A has significantly higher osteopontin levels than group B (23.66 ng/ml versus 5.43 ng/ml, P=0.005). Because the serum OPN level was elevated in those with NAFLD and T2DM and was even higher when the two diseases were combined, these results imply that the serum concentration of OPN may be a potential diagnostic biomarker for the prediction of NAFLD and T2DM as well as may predict the deterioration of the metabolic state. Particularly nonalcoholic steatohepatitis (NASH) is thought to be related to the metabolic syndrome, which also includes T2DM. OPN was found to be increased in the transition from simple steatosis to nonalcoholic steatohepatitis and fibrosis(9) and to be expressed mostly in hepatocytes and inflammatory cells(13). Both the expression of OPN and the neutralization of OPN by antibodies were demonstrated by Kwon HJ and coworkers.

Type 2 diabetes is characterized by insulin resistance in the target organs and dysfunction of the pancreatic beta cells (14).

According to the findings of the current study, patients with T2DM and NAFLD have significantly higher levels of circulating OPN. More importantly, the current study's findings show that OPN is a single predictor of NAFLD and T2DM and may contribute to the worsening of the metabolic disorder. More research will be required to understand how OPN impacts

people with more severe metabolic disorders, such as NAFLD and T2DM.

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