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Interrelationship between Serum Osteopontin and FGF-23 Levels with Carotid Artery Calcification among Diabetic Patients

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ABSTRACT

Background: Carotid artery calcification (CAC) is a recognized marker of vascular complications in type 2 diabetes mellitus (T2DM). Osteopontin (OPN) and fibroblast growth factor-23 (FGF-23) are key regulators of mineral metabolism and vascular function, but their specific contributions in newly diagnosed T2DM are not fully established.
Aims: This study aimed to investigate the interrelationship between serum OPN and FGF-23 levels and the presence of carotid artery calcification in patients with recent-onset T2DM.
Methods: A total of 86 participants were enrolled, including 45 patients with newly diagnosed T2DM (Group A) and 41 age- and sex-matched healthy controls (Group B). Biochemical parameters, including fasting serum glucose and glycated hemoglobin (HbA1c), were measured. Serum OPN and FGF-23 concentrations were quantified using immunoassays, while carotid ultrasonography was used to detect and characterize vascular calcification.
Results: Compared with controls, patients in Group A showed significantly higher fasting glucose, HbA1c, and serum FGF-23 levels, while OPN concentrations were significantly lower ($p < 0.05$). OPN exhibited a negative correlation with fasting glucose and HbA1c, suggesting that reduced OPN may reflect poorer glycemic control. In contrast, elevated FGF-23 was strongly associated with the presence of carotid artery calcification in diabetic patients, independent of renal function, highlighting its specific role in vascular pathology.
Conclusion: In newly diagnosed T2DM, OPN appears linked primarily to glycemic regulation, whereas FGF-23 is more directly associated with vascular calcification. FGF-23 may therefore serve as a potential early biomarker for vascular risk stratification in diabetic patients.

KEYWORDS: Osteopontin, FGF-23, Carotid Artery Calcification, Type 2 Diabetes Mellitus, Serum Calcium, Serum Phosphate.

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INTRODUCTION

Diabetes mellitus (DM) is a complex, long-term condition that requires ongoing medical care and multifaceted risk-reduction approaches beyond merely managing glucose. It is a metabolic disorder specified by persistent hyperglycemia with varying degrees of deterioration in the metabolism of carbohydrates, lipids, and proteins [1-2]. Diabetes self-management, education, and support are crucial for empowering individuals, preventing acute complications, and reducing the risk of long-term problems. There is strong evidence supporting various interventions that improve diabetes outcomes [2]. Poorly controlled glycemic concentration levels can be associated with the initiation and progression of gingivitis, periodontitis, and alveolar bone loss [3]. Long-term hyperglycemia damages and malfunctions many of the body's organs [4]. Diabetes mellitus morbidity and mortality are greatly enhanced by the vascular complications that arise from the disease [5]. Because of this, identifying diabetes complications is extremely important the main causes of metabolic abnormalities that result in a wide range of complications, such as cardiovascular diseases, nephropathy, neuropathy, and retinopathy [6]. Diabetes is characterized by high blood glucose levels, which can cause damage to the heart, blood vessels, eyes, kidneys, and nerves over time. More than 90% of diabetes cases are type 2 diabetes mellitus (T2DM), a condition marked by insufficient insulin production from pancreatic islet B-cells, tissue insulin resistance (IR), and an inadequate compensatory insulin response [7]. Patients with T2DM are at increased risk of developing atherosclerosis. While previous studies have explored the factors linked to diabetic macrovascular disease, it remains unclear whether these factors also apply to T2DM patients with carotid atherosclerosis [8]. Furthermore, hyperglycemia reduces bone density through multiple interconnected mechanisms, mainly affecting osteoblasts and osteoclasts. In people with T2DM, high blood glucose levels directly harm osteoblasts, lowering their number and function, which inhibits bone formation. At the same time, increased osteoclastic activity—partly due to higher expression of RANKL and proinflammatory cytokines like TNFα—leads to increased bone resorption, causing a net bone loss [9]. In parallel, vascular calcification in diabetes appears to mimic bone mineralization processes, including matrix vesicle release, osteogenic differentiation of vascular smooth muscle cells (VSMCs), and subsequent calcium-phosphate crystal deposition [10]. Under hyperglycemic conditions, extracellular pyrophosphate metabolism is disrupted—marked by decreased ectonucleotide pyrophosphatase activity and increased alkaline phosphatase—resulting in reduced inhibition of hydroxyapatite deposition and an increased tendency for calcification [9]. A key link between bone loss and vascular calcification involves the increased release of calcium and phosphate from the skeleton caused by osteoclast-mediated bone resorption [11]. These mineral ions—or their complexes—enter the bloodstream and can

deposit in arterial walls, forming initial niduses for calcification or locally increasing mineral levels enough to cause calcium-phosphate complex precipitation within the vascular matrix [8]. Vascular calcification involves transforming mesenchymal cells in the arterial wall, particularly smooth muscle cells (SMCs), into osteoblast-like phenotypes driven by various pathological factors. Additionally, this process leads to the abnormal buildup of calcium salts within the vascular wall, resulting in both intimal and medial calcification [12]. Cardiovascular disease is a leading cause of illness and death worldwide; glycaemic control plays a vital role in diabetic microvascular complications. It was shown that for each 1% reduction in the updated mean of HbA1c, there is a 37% reduction in microvascular complication risks [13]. Both microvascular and macrovascular complications are frequently associated with Type 2 DM. Vascular problems can be identified in the early stages of Type 2 DM or even in the Prediabetes stage, which makes it difficult to diagnose because the illness is frequently moderate or asymptomatic [14].

One main factor is changes in blood vessel structure, such as atherosclerosis and arteriosclerosis. Arteriosclerosis describes the hardening and calcification of the arterial media, while atherosclerosis involves lipid buildup and the formation of athermanous plaques in the arterial intima, often with secondary calcification. The calcification processes in both conditions are believed to involve shared underlying mechanisms in recent years; many studies have examined how hyperglycemia promotes vascular calcification [15-16]. Insulin stimulates the vascular endothelium to release NO, which then oxidizes lipoproteins, decreasing the intimal calcification rate and preventing its progression. When insulin resistance (IR) occurs, there is an increased production of VSMCs. Free fatty acids enter the liver, and the body responds by increasing hepatic absorption of triglycerides and the production and secretion of VLDL; consequently, the risk of vascular calcification also increases.(9)

The fibroblast growth factor (FGF) protein family plays a role in metabolic homeostasis. Certain members of the FGF-19 subfamily, including FGF-19, FGF-21, and FGF-23, have been widely studied for their endocrine functions. Fibroblast Growth Factor 23 (FGF23) is a circulating peptide hormone secreted by bone cells. Its main physiological role in healthy individuals is to maintain stable serum phosphorus levels [17]. FGF-23 is produced by many tissues, mainly by osteocytes and mature osteoblasts, and it acts on the kidneys and parathyroid glands, thereby affecting bone mineralization. Its role in vascular calcification, particularly concerning T2DM, is an increasingly researched topic due to its implications for cardiovascular health complications [18-19]. Osteopontin, a sialic acid-rich glycoprotein first isolated from bone, is a well-known noncollagenous protein in the bone matrix that regulates calcification. Similar to Matrix Gla Protein, osteopontin also influences calcification during bone development and remodeling. Osteopontin is a pro-fibrotic and pro-inflammatory cytokine that encourages inflammation by stimulating macrophage proliferation. Consequently, it plays a role in adipogenesis and metabolic-associated fatty liver disease, and it is upregulated in obesity and insulin resistance. It is a multifunctional glycoprotein highly expressed in atherosclerotic plaques, which has emerged as a potential biomarker for ASCVDs. OPN may act as an inflammatory mediator and/or a vascular calcification (VC) mediator, contributing to atherosclerosis progression and plaque formation destabilization [20-21].

MATERIALS AND METHODS

This case–control study was carried out at the Imam Hassan National Center for Diabetes Treatment and Research, Karbala, Iraq, between October 2024 and March 2025. Ethical approval was obtained from the Research Ethics Committee of the College of Pharmacy, University of Baghdad (Approval No. FECO42460A). Written informed consent was secured from all participants after they were provided with a clear explanation of the study objectives, procedures, and potential benefits prior to enrollment.

Subjects' Groups

Forty-five newly diagnosed T2DM patients (23 males and 22 females), aged 30–70 years with BMI 18.6–32 kg/m², and were recruited from the outpatient clinic of the Imam Hassan National Center for Diabetes Treatment under endocrinology supervision. Diagnosis was based on ADA 2024 criteria (FPG ≥126 mg/dL or HbA1c ≥6.5%). The control group included 41 healthy subjects (10 males and 31 females), aged 30–70 years, with BMI 20–30 kg/m², selected from the general population. After overnight fasting, venous blood samples were collected. Ethical approval was obtained from the Local Research Ethics Committee, and all participants gave written informed consent.

Biochemical and Hormonal Analysis

Serum urea, creatinine, and albumin were determined using Roche Diagnostics kits (Cobas C111, Germany) (22). Parathyroid hormone levels were measured with the Cobas® e411 system (23). Osteopontin and FGF-23 were quantified using sandwich ELISA kits (BT Lab, China) and analyzed with an ELISA plate reader (Beckman Coulter, Austria) [24-25].

Statistical Analysis

Data were entered into a coded data sheet, and accuracy was verified through double checking. Statistical analyses were performed using SPSS version 28.0 (IBM, Chicago, IL, USA) and the Real Statistics Resource Pack for Excel (Mac Release 7.2). Descriptive statistics were presented as frequencies and percentages for categorical variables, and as means with standard deviations for continuous data. Normality of distributions was assessed using the Shapiro–Wilk test. Associations between variables were evaluated using odds ratios (ORs) with 95% confidence intervals (CIs) via logistic regression. Group comparisons of categorical variables were conducted with appropriate analytical tests. Receiver operating characteristic (ROC) analysis was applied to determine cut-off values with optimal sensitivity and specificity. A two-tailed p-value <0.05 was considered statistically significant [26].

RESULTS

The (Table 1) summarizes the Sociodemographic and clinical characteristics of the study population, including 45 patients with newly diagnosed T2DM and 41 healthy controls. The mean age did not differ significantly between the groups, though distribution patterns varied. In the control group, most subjects were aged 30–40 years (n=18), while in the patient group the majority were 41–50 years (n=14), closely followed by 30–40 years (n=15). The smallest subgroup in both cohorts was aged 61–70 years (patients n=4; controls n=6). Body mass index (BMI) patterns indicated a higher prevalence of obesity in both groups, with slightly more cases among patients n=24 compared to controls n=22. Overweight status was also more frequent among patients (n=18 vs. 13), whereas normal weight was more common in controls (n=6 vs. 3). A notable difference was observed in family history, where 28 patients reported a positive history compared with only 2 controls. Smoking was also more prevalent in the patient group n=25 than in controls n=11, while non-smokers predominated in controls (n=30 vs. 20). Systemic diseases such as hypertension, dyslipidemia, and cardiovascular disorders were more frequently reported by patients n=9 compared with a single case in controls. Doppler ultrasonography findings for carotid calcification were confined to the patient group, with 7 showing positive results, while all controls and the remaining 38 patients had negative findings.

The (figure1) presents The Diabetes Mellitus group showed a higher mean HbA1c level of 9.4±2.3%, indicating poor long-term glycemic control, compared to the Healthy Control group, which had a mean HbA1c of 5.21±0.62%, within the non-diabetic range. Similarly, the Fasting Blood Sugar (FBS) levels were markedly elevated in the Diabetes Mellitus group, with a mean of 238.6±108.3 mg/dL. In contrast, the Healthy Control group maintained normal fasting glucose
levels, averaging 104.73±6.85 mg/dL.

Figure 1:

The (figure 2) presents the distribution of Fibroblast Growth Factor-23 (FGF23) and Osteopontin levels in the Diabetes Mellitus and Healthy Control groups. For Osteopontin, the Diabetes group had a mean concentration of 20.15±4.84 ng/mL. In contrast, the Healthy Control group showed a higher mean of 24.38±10.46 ng/mL. Regarding FGF23, the Diabetes group exhibited a mean level of 394.02±222.40 pg/mL. The Healthy Control group had a considerably lower mean of 243.12±51.54 pg/mL. Comparing the groups, the mean Osteopontin level was lower in the Diabetes Mellitus group (20.15±4.84 ng/mL) compared to the Healthy Control group (24.38±10.46 ng/mL), while the mean FGF23 level was substantially higher in the Diabetes Mellitus group (394.02±222.40 pg/mL) than in the Healthy Control group (243.12±51.54 pg/mL). Based on the provided t-test, there was a statistically significant difference ($p \leq 0.05$) observed in both Osteopontin and FGF23 levels between the Diabetes and Healthy Control groups.

Table 2:

The current study found no significant difference in the mean values of serum calcium levels among the various groups analyzed $p>0.05$, as shown in (Table 2). As shown in (Table 2), serum phosphate levels did not differ significantly between groups $p>0.05$, consistent with evidence that phosphate can contribute to vascular calcification even within normal ranges. This finding aligns with previous research indicating that higher circulating calcium levels, even within normal limits, are associated with carotid plaque thickening, an early indicator of cardiovascular disease. Similarly, the mean serum phosphate levels did not differ significantly among the groups studied $p>0.05$, as presented in (Table 2)

Correlation Analysis of Serum Level of Fibroblast Growth Factor-23 and Osteopontin with Other Biomarkers among Diabetes Patients

The (Table 3) presents Pearson's correlation coefficients (r) and p-values between FGF-23 and selected biomarkers in the diabetes group, with significance set at $p<0.05$. A strong positive correlation was found between FGF-23 and osteopontin ($r=0.80$, $p<0.001$), indicating that higher osteopontin levels are closely associated with elevated FGF-23. A weak positive correlation was also observed with albumin ($r=0.24$, $p=0.05$). No significant associations were detected between FGF-23 and glycemic markers (HbA1c, FBS), renal indices (urea, creatinine), or calcium–phosphate regulators (serum calcium, phosphate, PTH). These findings suggest a specific interaction between FGF-23 and osteopontin, with a modest link to albumin, independent of other metabolic parameters.

Table 3:

The (Table 4) presents correlation coefficients (r) and p-values between Osteopontin and selected biomarkers in the diabetes group (significance at $p < 0.05$). A strong positive correlation was found between Osteopontin and FGF-23 ($r = 0.80$, $p < 0.001$), indicating a close direct relationship. Weaker positive correlations were noted with urea ($r = 0.40$, $p = 0.034$) and albumin ($r = 0.30$, $p = 0.003$). No significant associations were observed with glycemic markers (HbA1c, FBS) or calcium–phosphate regulators (creatinine, serum phosphate, serum calcium, PTH).

Table 4:

A consistent finding across both correlation analyses was the strong positive relationship between FGF-23 and osteopontin ($r = 0.80$, $p < 0.001$), observed reciprocally, suggesting a close biological interaction or co-regulation in diabetes mellitus. Both markers also correlated significantly with albumin, with the association being slightly stronger for osteopontin, indicating that albumin may play a role in their regulation. Osteopontin, but not FGF-23, showed a moderate positive correlation with urea ($r = 0.40$, $p = 0.034$), highlighting a unique link with renal function.

Association of Factors between Groups

As shown in (Table 5), odds ratio (OR) analysis compared biomarkers between diabetic patients and healthy controls. FGF-23 demonstrated a significant positive association with diabetes, with an OR of 1.023 (95% CI: 1.011–1.036, $p < 0.001$), meaning each unit increase in FGF-23 raised diabetes odds by about 2.3%. Similarly, fasting blood sugar (FBS) showed a highly significant association (OR = 1.268, 95% CI: 1.104–1.456, $p = 0.001$), where each unit increase increased diabetes odds by 26.8%, underscoring its diagnostic value. In contrast, other biomarkers, including osteopontin, creatinine, urea, phosphate, calcium, albumin, and PTH, did not exhibit significant associations between groups.

Table 5

Receiver Operating Characteristic (ROC) Curve

ROC analysis was performed to evaluate the diagnostic performance of osteopontin and FGF-23 in patients with diabetes mellitus (Figure 3). For osteopontin, the area under the curve (AUC) was 0.64, with a sensitivity of 65% and specificity of 60.4% at the optimal cut-off value of <20.6 ng/mL. The p-value was 0.03, indicating statistical significance despite the modest diagnostic accuracy. In contrast, FGF-23 showed stronger diagnostic utility. The AUC was 0.80, reflecting good discriminative ability. At a cut-off value >275 pg/mL, the sensitivity was 71% and specificity 70.7%, correctly identifying most patients with and without the condition. The p-value was <0.001, confirming high statistical significance. Overall, the ROC analysis demonstrates that while osteopontin has limited diagnostic value, FGF-23 is a promising biomarker with substantially higher accuracy (Figure 4). Its strong AUC and significance suggest it can reliably differentiate between diabetic patients and controls, highlighting its potential role in risk assessment and clinical evaluation.

Figure 3

Figure 4

DISCUSSION

Persistent hyperglycemia, together with the accumulation of advanced glycation end products (AGEs), contributes to the osteogenic transdifferentiation of vascular smooth muscle cells (VSMCs) through the upregulation of transcriptional regulators such as Runx2 and bone morphogenetic protein-2 (BMP2). In addition, these pathological stimuli enhance extracellular matrix remodeling and suppress endogenous inhibitors of mineralization. Collectively, these mechanisms accelerate the nucleation and deposition of calcium–phosphate crystals within the arterial wall, even during the early stages of diabetes [27]. Recent studies have shown that serum fibroblast growth factor-23 (FGF-23) levels are significantly elevated in patients with type 2 diabetes mellitus $p<0.01$. This increase is attributed to disturbances in phosphate metabolism, renal dysfunction, inflammation, and hormonal imbalance, all of which contribute to diabetic complications [18]. A correlation has been identified between FGF-23 concentrations and bone mineral density (BMD) in patients with type 2 diabetes mellitus. Evidence from Mendelian randomization and clinical studies indicates that elevated FGF-23 is causally linked to reduce BMD at specific skeletal sites, particularly the femoral neck and heel. This site-specific effect suggests that FGF-23 may serve as a potential biomarker of altered bone metabolism in diabetes, although not all BMD locations appear equally affected [28]. FGF-23 promotes calcium conservation but suppresses vitamin D synthesis, a process that may facilitate vascular calcification. Elevated FGF-23 has been consistently associated with arterial stiffness, endothelial dysfunction, and greater cardiovascular risk, particularly in type 2 diabetes. Levels are significantly higher in diabetic patients with cardiovascular disease than in those without $p<0.01$. Evidence indicates a dose-dependent effect, with rising FGF-23 predicting future cardiovascular events and mortality, independent of kidney function. Its presence in vascular atherosclerotic lesions, even in individuals with normal renal function, supports a direct role in vascular pathology. Experimental data suggest these effects are largely mediated through vitamin D suppression, impairing vascular and cardiac health [28]. In this study, serum osteopontin (OPN) levels were significantly lower in patients with type 2 diabetes mellitus compared with healthy controls $p<0.01$. This decline may reflect impaired osteogenic signaling in vascular smooth muscle cells, dysregulated inflammatory pathways, and disrupted bone–vascular communication. Recent evidence in the literature supports these mechanisms, highlighting the role of reduced OPN in diabetic vascular pathology [29]. A 2025 cohort study reported that patients with type 2 diabetes mellitus exhibit reduced expression of matrix vesicle–producing osteogenic regulators, including osteopontin (OPN), in vascular smooth muscle cells. This reduction appears to be driven by chronic hyperglycemia–induced signaling deficiencies. The consequent decline in OPN expression may impair the regulation of mineralization and disrupt the dynamic interactions between vascular and bone tissues [30]. A 2025 clinical investigation reported that patients with type 2 diabetes mellitus who maintained well-controlled glycaemia exhibited significantly lower osteopontin (OPN) levels compared with hyperglycemic individuals. This reduction was attributed to decreased OPN expression, likely mediated by improved inflammatory profiles, particularly reduced interleukin-6 and TNF-α levels [31]. In type 2 diabetes mellitus (T2DM), multiple clinical and observational studies have demonstrated a generalized reduction in bone turnover markers (BTMs), encompassing both bone formation markers such as osteocalcin and bone resorption markers such as CTX. Given that osteopontin (OPN) is abundantly secreted by osteoblasts, osteocytes, and mineralized tissues, diminished osteoblast activity may underlie the observed decrease in circulating OPN. This mechanism is consistent with evidence from reviews and large cohort studies reporting lower BTMs in T2DM compared with healthy controls [29]. A 2025 meta-analysis highlighted that T2DM is associated with an overall reduction in bone turnover markers, including OPN, osteocalcin, and CTX. The study indicated that this widespread suppression of osteogenic activity may contribute to the decreased systemic OPN levels observed in diabetic patients [32].

Correlations between Fibroblast Growth Factor -23 and Osteopontin

Fibroblast growth factor-23 (FGF-23), a key regulator of phosphate metabolism, is elevated in patients with vascular calcification and strongly associated with arterial remodeling and cardiovascular risk. Evidence indicates that, even in type 2 diabetes mellitus (T2DM) patients with preserved renal function, higher serum FGF-23 levels are significantly correlated with carotid and systemic

vascular calcification. Mechanistically, FGF-23 influences vascular smooth muscle cells by inducing osteoclastic differentiation through ERK1/2 signaling and by disrupting calcium–phosphate homeostasis, thereby facilitating pathological mineral deposition within arterial walls [33]. FGF-23 expression is upregulated in calcified vascular tissue, whereas its co-receptor Klotho is downregulated, suggesting a complex regulatory interplay. Elevated FGF-23 has also been linked to increased arterial thickness and stiffness, both key indicators of vascular disease progression in type 2 diabetes mellitus. Moreover, FGF-23 may promote vascular inflammation and oxidative stress, thereby amplifying its detrimental impact on vascular structure and function [34]. Unlike FGF-23, OPN generally functions as an inhibitor of vascular calcification by preventing mineral buildup in blood vessels. Under normal conditions, OPN levels are low but essential for healthy arteries. In disease states, such as T2DM, OPN levels can increase in response to vascular damage and inflammation, where it aids in cell attachment, movement, and survival to support tissue repair [35].

Phosphorylation of osteopontin (OPN) is critical for its inhibitory role in vascular calcification, and impaired function promotes abnormal mineral deposition. In diabetes, elevated OPN has been linked to microvascular complications, while negative correlations with fasting glucose and HbA1c suggest its involvement in glycemic regulation. These findings emphasize OPN's dual role in controlling glucose metabolism and mitigating cardiovascular risk in type 2 diabetes mellitus [36]. In type 2 diabetes mellitus (T2DM), the interplay between fibroblast growth factor-23 (FGF-23) and osteopontin (OPN) in vascular calcification reflects a disrupted balance of pro- and anti-calcific signals. Elevated FGF-23 promotes vascular smooth muscle cell transformation and phosphate dysregulation, accelerating mineral deposition, whereas OPN counteracts these effects by inhibiting calcification and supporting vascular repair. Altered bone metabolism and impaired glucose regulation in T2DM disturb this equilibrium, enhancing vascular calcification and cardiovascular risk. Consequently, elevated FGF-23 and dysregulated OPN represent valuable biomarkers and potential therapeutic targets in T2DM-related vascular complications [32, 37].

Serum Calcium and Phosphate

Although serum calcium levels were within the normal reference range, they may still play a role in the development of vascular calcification [38]. This finding aligns with recent research showing that higher serum calcium levels, even when within the normal range, are linked to increase carotid plaque thickness, an early indicator of cardiovascular risk disease [39]. Elevated circulating calcium within normal limits has been linked to the progression of arterial stiffness and vascular calcification, both recognized risk factors for cardiovascular events. These associations persist even after adjusting for traditional cardiovascular risk factors, suggesting that serum calcium may directly affect vascular pathology beyond simple calcification [40]. The interaction between serum calcium and factors such as age and baseline arterial stiffness further highlights the potential influence of calcium on vascular health and cardiovascular risk, emphasizing the importance of monitoring calcium metabolism in preventing vascular issues disease [49]. They suggested that elevated serum phosphate, even within normal limits, might be a risk factor for coronary artery atherosclerosis, especially in healthy young individuals [42]. This process, driven by phosphate transporters like Pit-1, promotes vascular calcification even at normal phosphate levels. Higher serum phosphate levels are linked to increased calcium-phosphate product formation, contributing to coronary artery plaque and atherosclerosis. Furthermore, phosphate may work synergistically with calcium and other pathological stimuli to speed up vascular disease development [43]. Therefore, although no significant differences in mean serum phosphate levels were found among the groups in the study, phosphate's physiological effects on vascular calcification and cardiovascular risk are clear, highlighting its potential as an early biomarker and target for cardiovascular disease prevention [44]. Although serum calcium levels were within the normal reference range, it may still contribute to the development of vascular calcification. Despite remaining within the normal range, phosphate is believed to play a role in vascular calcification. This observation is supported by Foley et al., who, through multivariate analysis, found that phosphate levels were significantly correlated with coronary artery calcium. Elevated serum phosphate, even when within the normal range, may act as a risk factor for coronary artery atherosclerosis, especially in healthy young individuals. [45]

Mineral deposition in the vascular wall leading to plaque formation occurs when local ion concentrations, especially calcium and phosphate, exceed the solubility product of calcium salts, causing precipitation. This process changes the vascular microenvironment to resemble bone, encouraging calcified plaque development [46]. Mechanistically, vascular smooth muscle cells (VSMCs) undergo a phenotypic transformation into osteoblast-like cells that produce matrix vesicles serving as nucleation sites for calcium-phosphate crystals. These vesicles and apoptotic bodies initiate mineral deposition in the vessel wall. This pathological mineralization is not passive but a highly regulated, cell-mediated process that involves cellular stress, loss of calcification inhibitors, and altered calcium-phosphate homeostasis. Elevated local calcium levels promote this shift in VSMCs and support hydroxyapatite crystal formation, mimicking bone mineralization in the vasculature tissue [47]. Thus, the observed relationship between serum calcium and plaques demonstrates that these are calcified deposits formed through regulated cellular processes that cause calcium phosphate salts to precipitate in the vessel wall, transforming soft tissue into mineralized, bone-like material [48]. This explanation supports evidence that vascular calcification mimics bone formation, where excess local calcium surpasses solubility thresholds and triggers osteogenic transformation of vascular cells, leading to pathological mineral deposition within the arterial wall[9]. Phosphate, together with calcium, promotes calcification by inducing vascular smooth muscle cell (VSMC) transdifferentiation into osteoblast-like cells. These cells release matrix vesicles that act as nucleation sites for calcium–phosphate crystal deposition within the arterial wall, thereby driving vascular mineralization [43]. Multiple studies have shown that, in multivariate models, serum phosphate levels are independently associated with coronary artery calcium. Elevated phosphate, even within normal limits, is recognized as a risk factor for coronary atherosclerosis, particularly in young healthy individuals. Phosphate released during bone resorption, especially under chronic inflammatory states, can accumulate with calcium in the vascular wall, thereby accelerating mineralization and promoting early vascular calcification [42,44]. Phosphate enhances vascular calcification by entering vascular smooth muscle cells (VSMCs) through sodium-dependent transporters like Pit-1, activating PI3K/AKT and p38 MAPK pathways that induce RUNX2 and osteogenic differentiation. Even slight or transient

increases in serum phosphate, despite tight systemic regulation, can markedly elevate calcification risk [45]. This process effectively modifies the vascular microenvironment to encourage the deposition of calcium phosphate crystals, which is similar to bone formation [47]. Although there was no significant difference in average serum phosphate levels between groups, recent evidence indicates its role as a contributing factor in vascular calcification, even within normal ranges, by linking phosphate levels to coronary artery calcium and atherosclerosis risk [49].

CONCLUSIONS

This study shows that, among patients with type 2 diabetes mellitus and preserved renal function, higher fibroblast growth factor-23 (FGF-23) and lower osteopontin (OPN) are independently associated with carotid artery calcification, reflecting perturbations in bone–vascular–glucose crosstalk. Both biomarkers demonstrated clinically meaningful discriminative performance on ROC analysis, supporting their potential utility for cardiovascular risk stratification in this population.

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Tables

Table 1: Subject's Characteristics

Variable	Groups	(Group B) Control N=41	(Group A) Patients N=45	P-Value
Age Groups (Years)	30-40	18(43.9%)	15(33.3%)	0.543[NS]
	41-50	9(22.0%)	14(31.1%)	
	51-60	8(19.5%)	12(26.7%)	
	61-70	6(14.6%)	4(8.9%)	
BMI Groups (Kg/M^2)	Normal weight (≤25)	6(14.6%)	3(6.7%)	0.425[NS}
	Overweight (25-30)	13(31.7%)	18(40.0%)	
	Obese (≥ 30)	22(53.7%)	24(53.3%)	
Sex	Male	10(24.4%)	23(51.1%)	0.011[S]
	Female	31(75.6%)	22(48.9%)	
Family History for DM	Yes	2(4.9%)	28(62.2%)	<0.001[S]
	No	39(95.1%)	17(37.8%)	
Smoking	Yes ≥10/day	11(26.8%)	25(55.6%)	0.007[S]
	No	30(73.2%)	20(44.4%)	
Systemic Disease (HTN, Dyslipidemia, CVA)	Yes	1(2.4%)	9(20.0%)	0.011[S]
	No	40(97.6%)	36(80.0%)	
Doppler Examination*	Positive	0(0.0%)	7(15.6%)	0.008[S]
	Negative	41(100.0%)	38(84.4%)	

*Examination for carotid artery calcification. HTN: Hypertension; CVA: Cardiovascular disease. Data are presented as number and percentage within each group. Chi-square test *p≤0.05, N: number; S: significant; NS: non-significant.*

Table 2: Biochemical Evaluation (Mean ± SD) of Serum Calcium, Phosphate, and PTH among Diabetes Mellitus and Healthy Control Groups

Biomarkers	Patients group (N=45)	Control group (N=45)	P-Value
Serum Phosphate (mg/dl)	3.71±0.37	3.65±0.37	0.654[NS]
Serum Calcium (mg/dl)	9.32±0.46	9.43±0.45	0.216[NS]
PTH (mg/dl)	36.48±16.43	37.3±15.6	0.053[NS]

T test was *: significant at $p \leq 0.05$, PTH: Parathyroid Hormone, N: number of cases; SD: standard deviation; S: significant; NS: Non-significant

Table 3: Pearson's Correlation Coefficients between Fibroblast Growth Factor-23 and Studied Biomarkers among Diabetes Mellitus Patients

Biomarkers	FGF23	
	Correlation coefficient (r)	P value
Osteopontin	0.8	<0.001[S]
HbA1c	0.1	0.858[NS]
FBS	-0.2	0. 386 [NS]
Creatinine	0.3	0.126[NS]
Urea	0.4	0.034[S]
Serum Phosphate	0.1	0.575[NS]
Serum Calcium	0.1	0.453[NS]
Albumin	0.3	0.003[S]
PTH	-0.1	0.743[NS]

p<0.05 considered significantly different, [S] = Significant, [NS] = Non-significant, r: Correlation Coefficient

Table 4: Pearson's Correlation Coefficients between Osteopontin and Studied Biomarkers among Diabetic Patients

Biomarkers	Osteopontin	
	Correlation coefficient (r)	P value
FGF23	0.8	<0.001[S]
HbA1c	0.1	0. 972 [NS]
FBS	-0.2	0. 438 [NS]
Creatinine	0.2	0.230[NS]
Urea	0.1	0.690[NS]

Serum Phosphate	0.1	0.643[NS]
Serum Calcium	0.1	0.444[NS]
Albumin	0.24	0.05[S]
PTH	0.1	0.975[NS]

p<0.05 considered significantly different, [S] = Significant, [NS] = Non-significant, r: Correlation Coefficient

Table 5: Estimation of the Association of the Analyzed Factors among Diabetes Mellitus and Healthy Control as a Reference

Variable	OR (Lower-Upper)	P value
Osteopontin	0.964 (0.915-1.015)	0.161 [NS]
FGF23	1.023 (1.011-1.036)	<0.001 [S]
FBS	1.268 (1.104-1.456)	0.001[S]
Creatinine	4.801 (0.532-14.342)	0.162 [NS]
Urea	0.958 (0.901-1.020)	0.179 [NS]
Serum Phosphate	1.514 (0.472-4.851)	0.485 [NS]
Serum Calcium	0.622 (0.241-1.608)	0.327 [NS]
Albumin	1.884 (0.332-10.690)	0.475 [NS]
PTH	0.996 (0.969-1.023)	0.755 [NS]

p<0.05 considered significantly different- [S] = Significant, [NS] = Non significant, OR= odd ratio

Figures with their Legends

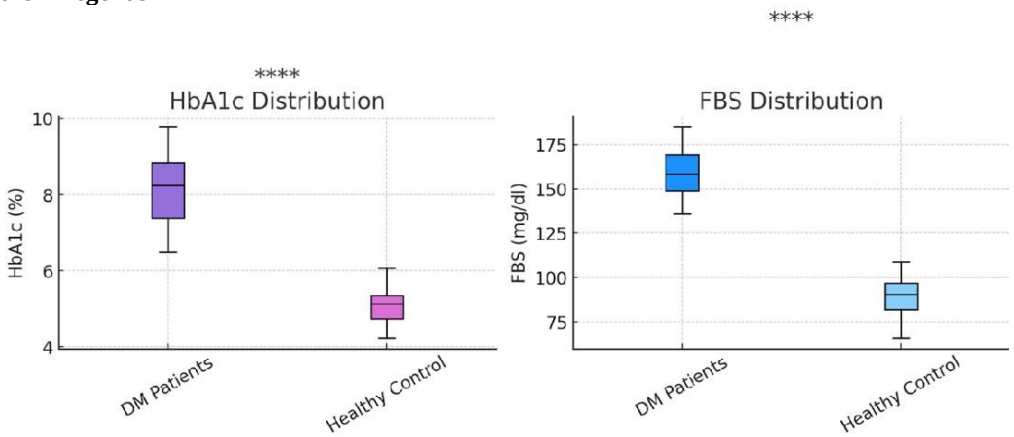

Figure 1: Evaluation of HbA1c (%) and FBS (mg/dl) among Diabetes Mellitus and Healthy control groups (t- test was *: significant at p≤0.05)

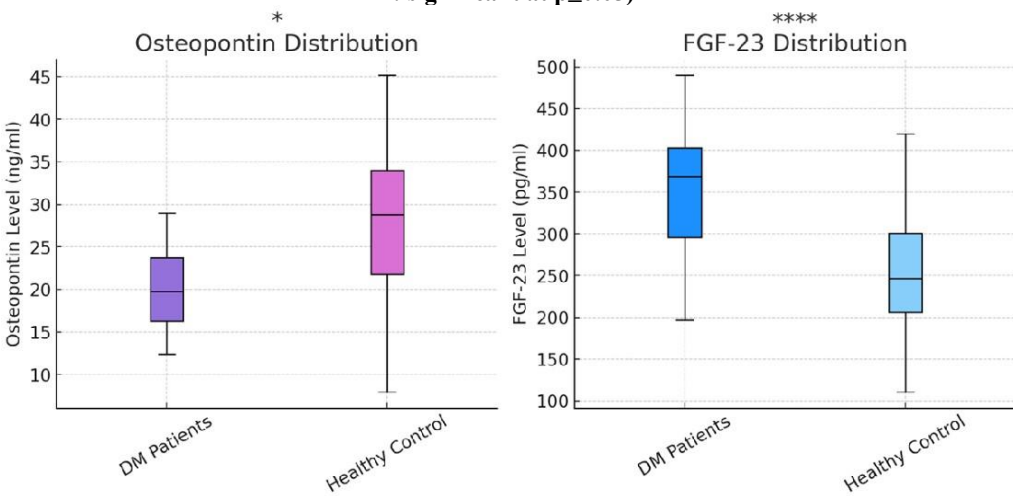

Figure 2: Evaluation of Serum Fibroblast Growth Factor-23 (pg/ml) and Osteopontin (ng/ml) among Diabetes Mellitus and Healthy Control Groups (t- test was *: significant at p≤0.05, **: significant at p≤0.0001)**

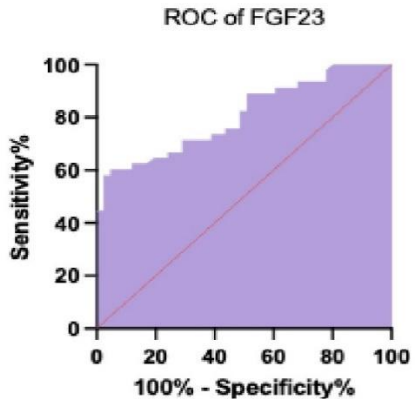

Figure 3: ROC curve of FGF23 in *DM* patients (Total and Stratification levels) (*FGF23*: AUC 0.80, 71% sensitivity, 70.7% specificity, cut-off > 275, P value was< 0.001

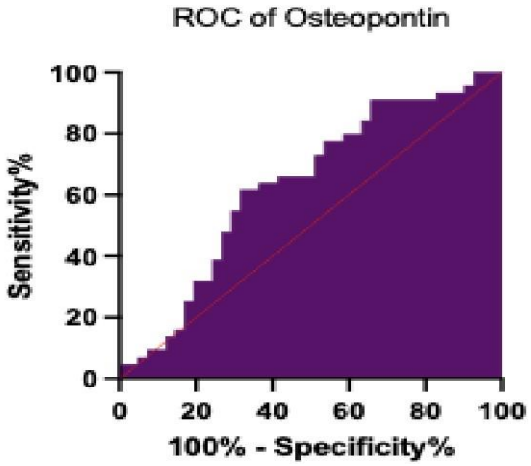

Figure 4: ROC curve of Osteopontin in *DM* patients/ (Total and Stratification levels) (*Osteopontin*: AUC 0.64, 65% sensitivity, 60.4% specificity, cut-off <20.6, *P* value was 0.03