https://doi.org/10.48047/AFJBS.6.2.2024.4464-4476

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

Genetic Insights into Fetuin-A Polymorphism: Implications for Cardiovascular Disease and Bone Health

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Article History

Volume 6, Issue 2, Apr-Aug 2024

Received: 5 July 2024

Accepted: 20 July 2024

Published: 25 July 2024

Doi ; *10.48047/AFJBS.6.2.2024.4464-4476*

Abstract: Background: Fetuin-A, a glycoprotein primarily produced by the liver, plays a crucial role in regulating calcium and phosphate metabolism. Genetic polymorphisms in the Fetuin-A gene have been linked to alterations in its expression and function, potentially contributing to cardiovascular disease by influencing vascular calcification and impacting bone mineral density. Understanding these genetic variations can provide insights into shared pathways between cardiovascular and skeletal health, paving the way for targeted therapeutic strategies. **Aim:** To asses possible correlation between Fetuin-A levels with cardiovascular disease risk and bone mineral density among diabetic nephropathy patients compared to controls. **Methods:** This case control study was conducted at Nephrology unit and Internal Medicine Department of Zagazig University Hospitals and Nephrology department of Theodor Biharz research institute. This study included 80 participants of both sexes across different age groups, all enrolled after providing written informed consent. The participants were divided into four groups: 20 healthy controls, 20 diabetic patients with normoalbuminuria (urinary albumin/creatinine <30 mg/gm), 20 diabetic patients with microalbuminuria (30–300 mg/gm), and 20 diabetic patients with macroalbuminuria (>300 mg/gm). All subjects underwent measurements of the common carotid artery intima-media thickness (IMT) using high-resolution real-time B-mode ultrasonography with a 7.5-MHz linear transducer (GE Logic F8 Expert). Serum Fetuin-A levels were assessed using an enzyme-linked immunosorbent assay (ELISA) kit, following the manufacturer's protocols. **Results:** There is statistically significant difference between the studied groups regarding serum Fetuin A, it was found significantly higher among cases compared to controls ($p<0.001$). There is statistically significant difference between the studied groups regarding diastolic dysfunction, diagnosis, and EF. On doing posthoc test, difference is significant between macroalbuminuric group and both control and microalbuminuric groups. ($P<0.001$). There is statistically significant positive relation between higher level of serum Fetuin-A and presence of Regional wall motion abnormalities (RWMA), diastolic dysfunction, aortic valve and mitral annular calcification, while there is significant negative correlation between serum Fetuin-A and ejection fraction (EF) ($p<0.05$). There is a statistically significant difference ($p < 0.001$**) in CIMT values across the studied groups, the macroalbuminuric group showed the highest CIMT value (1.54 ± 0.08), followed by the microalbuminuric group (1.32 ± 0.06), while the control (0.95 ± 0.1) and normoalbuminuric groups (0.91 ± 0.28) had the lowest values. Post-hoc analysis revealed significant differences between each pair of groups, except between the control and normoalbuminuric groups ($p = 0.875$), where there is no observed significant difference. **Conclusions:** the current study supports the growing body of evidence linking serum Fetuin-A levels to cardiovascular disease and bone mineral density deterioration in diabetic patients. Elevated serum Fetuin-A levels were strongly associated with vascular calcification, reduced cardiac function, and declining bone density.

Keywords: *Fetuin-A, Cardiovascular Disease, Bone Mineral Density*

Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. It is classified mainly into type 1 diabetes, resulting from autoimmune destruction of pancreatic β-cells, and type 2 diabetes, characterized by insulin resistance and relative insulin deficiency. DM is a global health concern, with increasing prevalence contributing to significant morbidity and mortality worldwide [1]. Chronic hyperglycemia in diabetes leads to long-term damage and dysfunction of various organs, including the eyes, kidneys, nerves, heart, and blood vessels. Cardiovascular disease (CVD) and diabetic nephropathy (DN) are among the most common and severe complications of diabetes, significantly increasing healthcare burdens and reducing patients' quality of life [2].

Diabetic nephropathy (DN) is one of the most serious microvascular complications of diabetes and remains the leading cause of end-stage renal disease (ESRD) worldwide [3]. It is characterized by persistent albuminuria, declining glomerular filtration rate (GFR), and increased blood pressure. The progression of DN is strongly associated with poor glycemic control, hypertension, and genetic predisposition. DN also plays a pivotal role in increasing cardiovascular risk in diabetic patients, as kidney dysfunction is closely linked to vascular calcification and arterial stiffness [4]. Recent evidence highlights the role of biochemical markers, such as Fetuin-A, in understanding the pathophysiology of DN and its association with cardiovascular health and bone metabolism [5].

Fetuin-A, also known as α2-Heremans-Schmid glycoprotein (AHSG), is a glycoprotein predominantly synthesized in the liver and secreted into the bloodstream. It serves as an essential regulator of calcium and phosphate homeostasis by inhibiting ectopic calcification and promoting proper bone mineralization [6]. Fetuin-A binds to calcium-phosphate crystals, preventing their deposition in vascular and soft tissues. Additionally, it has been linked to insulin resistance, adiposity, and inflammation, suggesting its role in metabolic syndrome [7]. Changes in Fetuin-A levels have been associated with chronic diseases, including diabetes, cardiovascular disease, and bone disorders.

Fetuin-A plays a paradoxical role in cardiovascular health. Low levels of Fetuin-A are associated with increased vascular calcification, arterial stiffness, and cardiovascular morbidity, especially in patients with chronic kidney disease (CKD) and diabetes mellitus [8]. On the other hand, elevated levels of Fetuin-A have been linked to insulin resistance and metabolic syndrome, suggesting a dual role depending on metabolic context. In diabetic patients, abnormal Fetuin-A levels contribute to endothelial dysfunction, oxidative stress, and chronic inflammation, leading to increased cardiovascular risk [9]. Therefore, Fetuin-A is considered both a protective and pathological biomarker for cardiovascular health.

Fetuin-A also plays a critical role in bone mineralization by modulating calcium and phosphate homeostasis. It prevents pathological mineralization in soft tissues while promoting physiological mineral deposition in bones [10]. Studies have shown that lower Fetuin-A levels are associated with decreased bone mineral density (BMD) and increased risk of osteoporosis, particularly in diabetic and CKD patients [5]. Conversely, excessively high levels of Fetuin-A may impair bone mineralization, suggesting a finely tuned balance is required for optimal skeletal health.

The association between Fetuin-A, cardiovascular disease, and bone density highlights the concept of the **bone-vascular axis**, where shared regulatory pathways affect both vascular calcification and bone health [11]. In pathological conditions such as diabetes and CKD, altered Fetuin-A levels can simultaneously promote vascular calcification while impairing bone mineralization. This interplay suggests that vascular and skeletal systems are not independent entities but rather interconnected through common molecular regulators like Fetuin-A.

Understanding the association between Fetuin-A levels, cardiovascular disease, and bone mineral density provides valuable insights into potential therapeutic targets. Modulating Fetuin-A levels through pharmacological or lifestyle interventions could offer dual benefits for cardiovascular and skeletal health, particularly in diabetic and CKD populations [6]. Moreover, Fetuin-A could serve as a biomarker for early detection and risk stratification of patients prone to vascular calcification and bone loss, improving clinical outcomes through targeted interventions.

Fetuin-A is a multifunctional glycoprotein with significant roles in cardiovascular health and bone metabolism. Its dual impact on vascular calcification and bone mineralization underscores its importance in understanding the complex pathophysiology of diabetes, diabetic nephropathy, and related complications. Further research is needed to clarify the molecular mechanisms governing Fetuin-A regulation and its potential as a therapeutic target. Integrating Fetuin-A assessment into clinical practice could improve the management of cardiovascular and skeletal complications in high-risk patients.

This study aimed to asses possible correlation between Fetuin-A levels with cardiovascular disease risk, and bone mineral density among diabetic nephropathy patients compared to controls.

Patients and Methods

This case-control study was conducted at the Nephrology Unit and Internal Medicine Department, Zagazig University Hospitals, and the Nephrology Department of Theodor Bilharz Research Institute from April 2023 to April 2024, focusing on type 2 diabetic patients. The study included 80 participants aged 30–70 years, with a mean age of 50 years, comprising both males and females. All participants provided written informed consent, and the study was approved by the hospital's ethics committee. The participants were divided into four groups based on albuminuria levels: 20 healthy control subjects, 20 diabetic patients with normoalbuminuria (*urinary albumin/creatinine <30 mg/gm*), 20 diabetic patients with microalbuminuria (*urinary albumin/creatinine 30–300 mg/gm*), and 20 diabetic patients with macroalbuminuria (*urinary albumin/creatinine >300 mg/gm*).

Eligible participants included adults aged 30–70 years with type 2 diabetes mellitus who provided informed consent. Patients with acute metabolic disturbances, such as ketoacidosis or hyperosmolar states, ongoing infections, or inflammatory conditions, were excluded. Additionally, those with end-stage renal disease or undergoing hemodialysis, autoimmune diseases, malignancies, recent use of systemic steroids, acute cardiovascular or cerebrovascular diseases, and type 1 diabetes mellitus were not considered. Furthermore, participants with abnormal renal ultrasound findings beyond diabetic nephropathy were excluded from the study.

The sample size was determined based on an assumed mean Fetuin-A level of 528.7±299 in diabetic cases compared to controls. Using 80% power and a 95% confidence interval, the sample size was calculated as 80 subjects, with 20 subjects in each group. This calculation was performed using the EPI program, ensuring statistical robustness and adequate power to detect significant differences between groups.

Data collection included a comprehensive medical history, including details about diabetes duration, hypertension, and diabetic retinopathy. Each participant underwent a full clinical examination, including measurements of height, weight, and arterial blood pressure (ABP). Fasting routine laboratory investigations were performed, including complete blood count (CBC), liver and kidney function tests, serum uric acid, fasting blood glucose, 2-hour postprandial blood glucose, HbA1c, lipid profile, urinary albumin/creatinine ratio, and estimated glomerular filtration rate (eGFR) calculated using the CKD-EPI formula.

Radiological assessments included pelvi-abdominal ultrasound and common carotid artery intima-media thickness (CIMT) measurements. CIMT was assessed using high-resolution B-mode ultrasonography with a 7.5-MHz linear transducer (GE Logic F8 Expert). Measurements were taken bilaterally in longitudinal projections, focusing on approximately 2–3 cm segments of the common carotid artery just below the carotid bulb. The intima-media thickness (IMT) was measured in plaque-free arterial segments, and four measurements from each side were averaged to obtain the mean CIMT. All ultrasound studies were performed by a single blinded investigator, ensuring consistency, with an intraobserver coefficient of variation of 8%.

Specific laboratory investigations included fasting serum Fetuin-A measurement, performed using an enzyme-linked immunosorbent assay (ELISA) kit following the manufacturer's protocols. The assay utilized a double-antibody sandwich technique, where serum samples reacted with pre-coated Fetuin-A antibodies and labeled secondary antibodies. Results were measured at a 450 nm wavelength, and concentrations were determined using a standard curve ranging from 0.468 to 30 ng/mL.

To investigate AHSG Thr265Ser (rs4918) gene polymorphisms, polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was performed. Genomic DNA was extracted using the Genaid Genomic DNA Purification Kit, and the target sequence was amplified using validated primers. The PCR conditions included initial denaturation at 94°C for 3 minutes, followed by 45 cycles of denaturation, annealing at 56°C, and extension at 72°C. The final extension step was conducted at 72°C for 7 minutes. The amplified product was digested overnight at 37°C with the SacI restriction enzyme, and the resulting fragments were analyzed on a 1.5% agarose gel. The c.766C allele remained undigested, while the c.766G allele yielded fragments of 193 bp and 212 bp.

Urinary albumin/creatinine ratio was assessed using turbidimetry (DADE BEHRING) at a 600 nm wavelength after incubation at 37°C for 6 minutes. Microalbuminuria was defined as urinary albumin excretion of 30–300 mg per 24 hours or a spot urine collection ratio of 30–300 μg/mg creatinine. Positive cases were re-examined after 3 months to exclude transient albuminuria.

Blood samples were collected under sterile conditions and processed appropriately. Plasma and serum samples were stored at -80°C until analysis. Serum Fetuin-A concentrations were determined using ELISA, and the results were interpreted using a standard curve or calculated using regression equations derived from standard OD values.

This study's methodological rigor, including standardized assessments and blinded analysis, ensured reliable results regarding the association between Fetuin-A levels, cardiovascular disease, and bone mineral density in diabetic patients across varying degrees of albuminuria.

Results

In this study, demographic data, including gender, age, and body mass index (BMI), were compared across the four groups: control, normoalbuminuric, microalbuminuric, and macroalbuminuric groups. Regarding gender, all groups had an equal distribution of males and females, with 50% males and 50% females in each group ($\chi^2 = 0$, $p > 0.999$). This indicates no statistically significant difference in gender distribution across the groups.

In terms of age, a significant difference was observed between the groups ($F = 50.502$, $p < 0.001$*)*. The control group had a mean age of 48.45 ± 2.21 years, while the normoalbuminuric group had a mean age of 44.5 ± 3.53 years, the microalbuminuric group had a mean age of 51.5 ± 3.72 years, and the macroalbuminuric group had the highest mean age at 56.8 ± 3.38 years. Post-hoc analysis revealed significant differences between each pair of groups, including control vs normoalbuminuric ($p < 0.001$), control vs microalbuminuric ($p = 0.004$), and control vs macroalbuminuric ($p < 0.001$), emphasizing a progressive increase in age with the severity of albuminuria. For BMI, there was also a statistically significant difference across the groups ($F = 7.381$, $p < 0.001$*)*. The control group had a mean BMI of 27.05 ± 5.26 kg/m^2, the normoalbuminuric group had a mean BMI of 29.1 ± 3.64 kg/m^2, the microalbuminuric group had a mean BMI of 30.1 ± 2.71 kg/m^2, and the macroalbuminuric group had the highest BMI at 32.3 ± 1.84 kg/m^2. Post-hoc analysis showed significant differences between the control group and both the microalbuminuric ($p = 0.009$) and macroalbuminuric groups ($p < 0.001$). Additionally, a significant difference was noted between the normoalbuminuric and macroalbuminuric groups ($p = 0.006$).

Serum Fetuin-A levels were compared across the four study groups: control, normoalbuminuric, microalbuminuric, and macroalbuminuric groups. The analysis revealed a statistically significant difference in Fetuin-A levels among the groups (*KW = 70.47, p < 0.001**)*, highlighting a progressive increase in Fetuin-A concentration with the severity of albuminuria.

In the control group, the median Fetuin-A level was 7.1 ng/mL (IQR: 5.73–8.1), while in the normoalbuminuric group, it increased to 13.9 ng/mL (IQR: 12.8–14.4). In the microalbuminuric group, Fetuin-A levels rose further to 22.85 ng/mL (IQR: 17.28–25.13). The macroalbuminuric group exhibited the highest Fetuin-A levels, with a median of 74.45 ng/mL (IQR: 66.2–81.1).

Post-hoc pairwise comparisons confirmed statistically significant differences between most group pairs. The difference between the control and normoalbuminuric groups ($p = 0.002$), control and microalbuminuric

groups ($p < 0.001$), and control and macroalbuminuric groups ($p < 0.001$) was significant. Additionally, significant differences were observed between the normoalbuminuric and microalbuminuric groups ($p = 0.045$), as well as between the microalbuminuric and macroalbuminuric groups ($p = 0.002$).

In conclusion, serum Fetuin-A levels increased progressively from the control group to the macroalbuminuric group, indicating a strong association between higher Fetuin-A concentrations and increased albuminuria severity. These findings suggest that Fetuin-A could serve as a potential biomarker for disease progression in diabetic patients with varying degrees of kidney involvement.

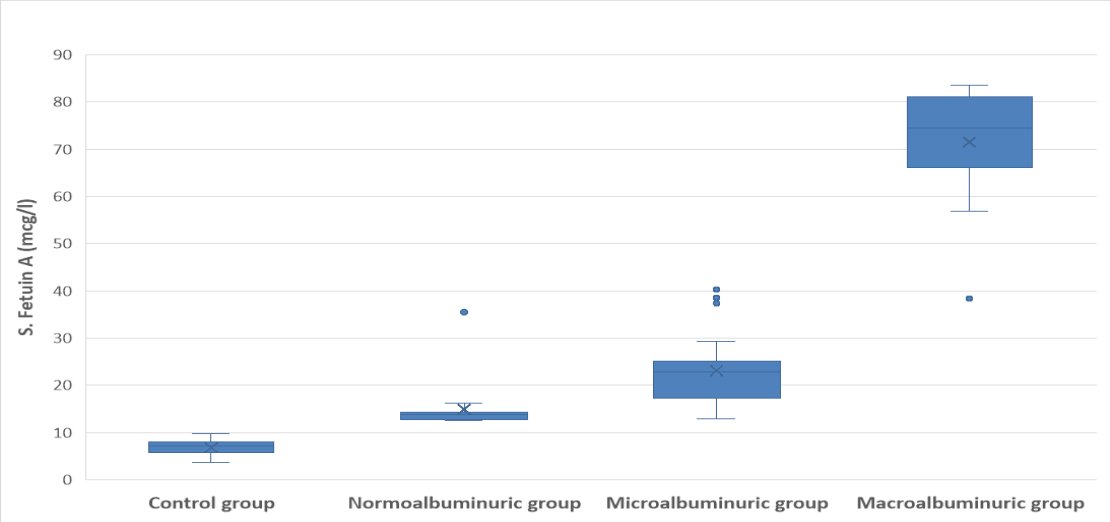

Figure (1): Boxplot showing comparison between the studied groups regarding serum Fetuin A

A significant association was observed between AHSG genotypes and the risk of developing microalbuminuria, with the CG genotype presenting the highest risk. Although differences in allele frequencies were not statistically significant overall, the G allele showed a notable trend toward increased risk in microalbuminuric patients. These findings suggest a potential genetic predisposition to the progression of diabetic nephropathy linked to AHSG gene polymorphisms.

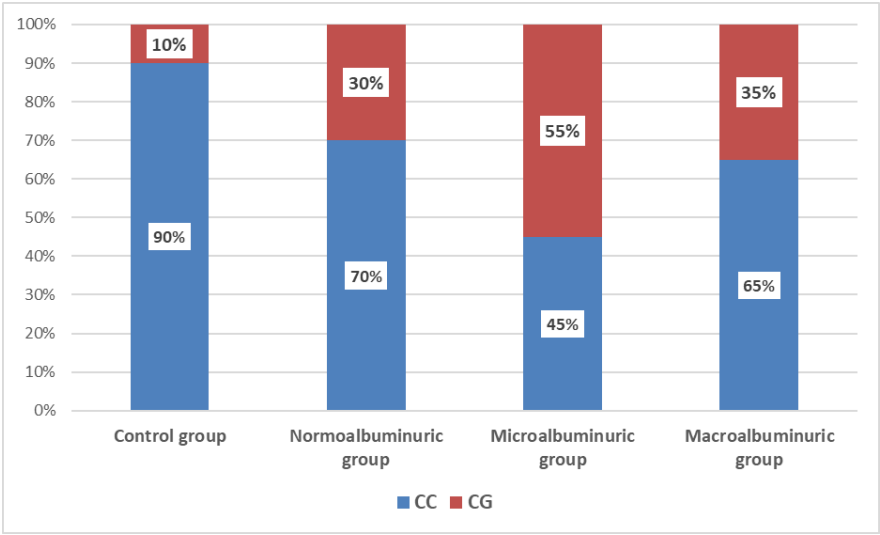

Figure (2): Multiple bar chart showing Comparison between the studied groups regarding AHSG PCR genotypes

Echocardiographic parameters, including regional wall motion abnormalities (RWMA), diastolic dysfunction, mitral annular calcification, aortic valve calcification, diagnosis, ejection fraction (EF), interventricular septal

thickness (IVST), and carotid intima-media thickness (CIMT), were compared among the control, normoalbuminuric, microalbuminuric, and macroalbuminuric groups. **Diastolic dysfunction** showed a statistically significant difference across the groups ($\chi^2 = 12.33, p = 0.006$). In the control and normoalbuminuric groups, 10% of participants had diastolic dysfunction, which increased to 20% in the microalbuminuric group and further rose to 50% in the macroalbuminuric group. This trend indicates a clear progression of diastolic dysfunction severity with worsening albuminuria.

Ejection fraction (EF) showed a statistically significant difference across the groups ($F = 4.459, p = 0.006$). The control group had a mean EF of 64.55 ± 5.37%, decreasing progressively in the normoalbuminuric group (63.15 ± 4.28%), the microalbuminuric group (60.7 ± 8.52%), and reaching the lowest value in the macroalbuminuric group (55.6 ± 12.58%). Post-hoc analysis revealed significant differences between the macroalbuminuric group and both the control group (p = 0.006) and the microalbuminuric group (p = 0.027).

In contrast, carotid intima-media thickness (CIMT) displayed a highly significant difference across the groups ($F = 75.081, p < 0.001$*)*. The control group had the lowest CIMT value (0.95 ± 0.1 mm), while the macroalbuminuric group had the highest (1.54 ± 0.08 mm). Post-hoc analysis confirmed significant differences between each group pair, except between the control and normoalbuminuric groups.

Table (1) :Comparison between the studied groups regarding echocardiographic data:

	Control group	**Normoalbuminuric group**	**Microalbuminuric group**	**Macroalbuminuric group**	χ^2	**p**
	n=20 (%)	**n=20 (%)**	**n=20 (%)**	**n=20 (%)**		
RWMA						
NAD	19 (95%)	17 (80%)	18 (95%)	13 (70%)		
Global hypokinesia	0 (0%)	0 (0%)	0 (0%)	1 (5%)		
LAD, LCX territory	0 (0%)	1 (5%)	0 (0%)	1 (5%)	15.529	0.625
LAD, RCA territory	0 (0%)	0 (0%)	0 (0%)	1 (5%)		
LAD territory	1 (5%)	1 (5%)	1 (5%)	2 (10%)		
LCX territory	0 (0%)	0 (0%)	0 (0%)	1 (5%)		
RCA territory	0 (0%)	1 (5%)	1 (5%)	1 (5%)		
Diastolic dysfunction						
Absent	18 (90%)	18 (90%)	16 (80%)	10 (50%)	12.33	0.006*
Present	2 (10%)	2 (10%)	4 (20%)	10 (50%)		
Mitral annular calcification						
Absent	19 (95%)	18 (90%)	17 (85%)	14 (70%)	5.49	0.139
Present	1 (5%)	2 (10%)	3 (15%)	6 (30%)		
Aortic valve calcification						
Absent	19 (95%)	17 (85%)	17 (85%)	13 (65%)	6.58	0.087
Present	1 (5%)	3 (15%)	3 (15%)	7 (35%)		
Diagnosis						
Normal	18 (90%)	13 (65%)	13 (65%)	6 (30%)		
AS	1 (5%)	1 (5%)	0 (0%)	2 (10%)	33.002	0.017*
IHD	1 (5%)	2 (10%)	2 (10%)	2 (10%)		
LVH	0 (0%)	1 (5%)	3 (15%)	0 (0%)		
Hypertensive heart disease	0 (0%)	0 (0%)	2 (10%)	3 (15%)		
ICM	0 (0%)	2 (10%)	0 (0%)	4 (20%)		
MS	0 (0%)	1 (5%)	0 (0%)	3 (15%)		
	Mean ± SD	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	F	p
EF	64.55 ± 5.37	63.15 ± 4.28	60.7 ± 8.52	55.6 ± 12.58	4.459	0.006*
LSD	P_1 0.951	P_2 0.789	P_3 0.222	P_4 0.466	P_5 0.006*	P_6 0.027*
IVST	10.15 ± 1.5	10.0 ± 1.75	10.05 ± 2.21	9.8 ± 0.83	0.159	0.923
CIMT	0.95 ± 0.1	0.91 ± 0.28	1.32 ± 0.06	1.54 ± 0.08	75.081	<0.001**

RWMA(Regional wall motion abnormalities),NAD(*No Abnormality Detected),LAD(*Left anterior descending artery),LCX(circumflex branch of the left coronary artery),RCA(right coronary artery),AS(AORTIC STENOSIS),IHD(ISCHEMIC HEART DISEASES),LVH(left ventricular hypertrophy),ICM(ischemic cardiomyopathy),MS(mitral stenosis),EF(ejection fraction),IVST(INTERVENTRICULAR SEPTAL THICKNESS),CIMT(carotid intimal medial thickness)

Patients without RWMA had a median serum Fetuin-A level of 14.05 ng/mL (IQR: 8.35–33.95), whereas those with RWMA showed significantly higher levels at 64.7 ng/mL (IQR: 14.83–73.08) (Z = -2.635, p = 0.008). Similarly, patients without diastolic dysfunction had a median Fetuin-A level of 13.95 ng/mL (IQR: 8.18–23.3), while those with diastolic dysfunction exhibited markedly elevated levels at 58.05 ng/mL (IQR: 18.23–76.33) (Z = -3.238, p = 0.001*)*. Regarding mitral annular calcification, patients without calcification had lower serum Fetuin-A levels at 14.15 ng/mL (IQR: 8.8–28.35) compared to those with calcification, who had significantly higher levels at 56.65 ng/mL (IQR: 17.05–81.2) (Z = -2.891, p = 0.004). Similarly, aortic valve calcification was associated with elevated serum Fetuin-A levels, with 14.05 ng/mL (IQR: 8.65–24.38) in those without calcification and 47.65 ng/mL (IQR: 21.3–76.65) in those with calcification (Z = -2.78, p = 0.005).

In terms of overall cardiac diagnosis, patients with normal echocardiographic findings had significantly lower serum Fetuin-A levels at 13.7 ng/mL (IQR: 8.1–22.7) compared to those with abnormal findings, who had markedly elevated levels at 40.3 ng/mL (IQR: 14.25–77) (Z = -4.174, p = 0.001*)*. Additionally, a significant negative correlation was observed between serum Fetuin-A levels and ejection fraction (EF) (r = -0.343, p = 0.002), suggesting that higher serum Fetuin-A levels are associated with reduced cardiac function. However, no significant correlation was found between serum Fetuin-A and interventricular septal thickness (IVST) (r = -0.019, p = 0.866).

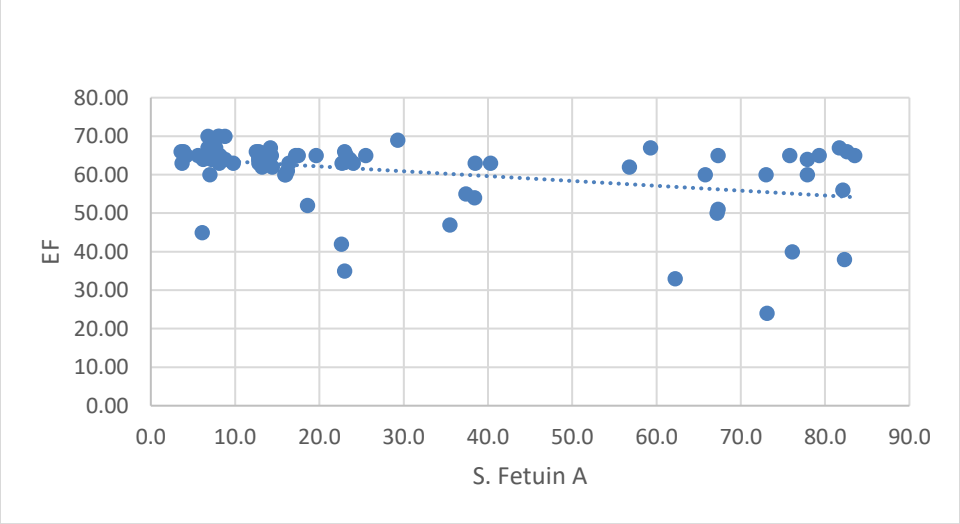

Figure (3): Scatter dot plot showing significant positive correlation between serum Fetuin and EF

Additionally, a positive correlation was identified between serum Fetuin-A levels and CIMT (r = 0.768, p < 0.001*)*. This correlation suggests that as serum Fetuin-A levels increase, CIMT values also rise, highlighting a potential role of Fetuin-A in the progression of vascular calcification and arterial wall thickening in diabetic patients. CIMT increases significantly with worsening albuminuria levels, reflecting progressive vascular structural changes associated with diabetic nephropathy. Furthermore, the strong positive correlation between serum Fetuin-A and CIMT indicates that elevated Fetuin-A levels may contribute to, or at least reflect, the severity of vascular calcification and arterial stiffness in this population. These findings reinforce the importance of monitoring CIMT and Fetuin-A levels as potential biomarkers for cardiovascular risk in diabetic patients

Table (2): Comparison between the studied groups(control and diabetic) regarding CIMT:

	Control group	Normoalbuminuric group	Microalbuminuric group	Macroalbuminuric group	F	P
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD		
CIMT	0.95 ± 0.1	0.91 ± 0.28	1.32 ± 0.06	1.54 ± 0.08	75.081	<0.001**
LSD	P_1 0.875	P_2 <0.001**	P_3 <0.001**	P_4 <0.001**	P_5 <0.001**	P_6 <0.001**

Correlation between Serum Fetuin A and CIMT

	r	P
CIMT	**0.768**	**<0.001****

There is no statistically significant relationship between AHSG genotypes (CC and CG) and bone density or vascular calcification. These results suggest that genetic variations in the AHSG gene may not play a direct role in determining bone health or the presence of vascular calcification in diabetic patients.

Table (3): Relation between AHSG genotypes and bone density in diabetic patients:

	CC	CG	χ^2	P
	N=54 (%)	N=26 (%)		
Bone density				
Normal	31 (57.4%)	15 (57.7%)		
Mild osteopenia	15 (27.8%)	8 (30.8%)	0.869	0.833
Moderate osteopenia	5 (9.3%)	1 (3.8%)		
Marked osteopenia	3 (5.6%)	2 (7.7%)		
Calcification				
Absent	46 (85.2%)	23 (88.5%)	Fisher	>0.999
Present	8 (14.8%)	3 (11.5%)		

α2-Heremans-Schmid glycoprotein (AHSG)

The relationship between serum Fetuin-A levels, bone density, and vascular calcification was analyzed, revealing statistically significant associations across these parameters (*KW = 23.058, p < 0.001**; Z = -3.899, p = 0.001**)*.

In terms of bone density, serum Fetuin-A levels increased progressively with worsening bone health. Patients with normal bone density had the lowest median serum Fetuin-A levels at 13 ng/mL (IQR: 8.1–19.65). Those with mild osteopenia demonstrated moderately elevated levels at 23 ng/mL (IQR: 13.8–67.2). A significant rise was observed in the moderate osteopenia group, where median levels reached 75.95 ng/mL (IQR: 51.88–78.85). The highest levels of serum Fetuin-A were recorded in the marked osteopenia group, with a median value of 82.1 ng/mL (IQR: 52.85–83.05). Post-hoc analysis confirmed that the differences in serum Fetuin-A levels were statistically significant between normal and abnormal bone density groups and between mild and marked osteopenia, indicating a clear correlation between increasing Fetuin-A levels and worsening bone density.

For vascular calcification, a statistically significant difference in serum Fetuin-A levels was noted between patients with and without calcification (*Z = -3.899, p = 0.001**)*. Patients without calcification had a median serum Fetuin-A level of 14.1 ng/mL (IQR: 8.5–23.8), while those with calcification exhibited significantly elevated levels at 73 ng/mL (IQR: 59.3–77.9). This finding highlights a strong association between elevated serum Fetuin-A levels and the presence of vascular calcification, serum Fetuin-A levels increase significantly in patients with worsening bone density and the presence of vascular calcification. The observed patterns suggest a potential dual role for Fetuin-A in both bone demineralization and vascular calcification, emphasizing its value as a potential biomarker for skeletal fragility and vascular complications in diabetic patients.

Table (4): Relation between ECHO data and serum Fetuin A in diabetic patients:

	Serum fetuin A	KW	P
	Median (IQR)		
Bone density			
Normal	13(8.1 – 19.65) [1,2, 3]		
Mild osteopenia	23(13.8 – 67.2) [3]	23.058	<0.001**
Moderate osteopenia	75.95(51.88 – 78.85)		
Marked osteopenia	82.1(52.85 – 83.05)		
Calcification			
Absent	14.1(8.5 – 23.8)	-3.899	0.001**
Present	73(59.3 – 77.9)		

KW Kruskal Wallis test Z Mann Whitney test **p≤0.001 is statistically highly significant

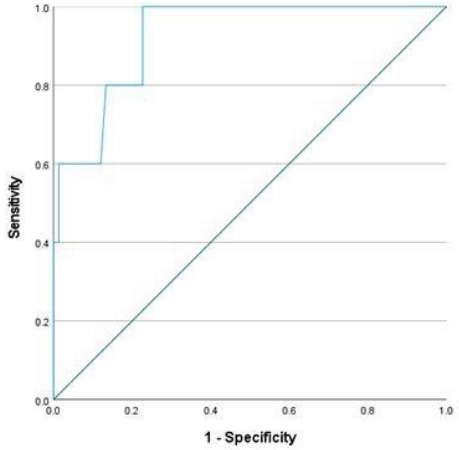

Figure (4): ROC curve showing performance of serum Fetuin-A in diagnosis of marked osteopenia among studied groups

The best cutoff of Fetuin A in diagnosis of marked osteopenia is ≥37.9 µg/L with area under curve 0.927, with sensitivity 100%, specificity 77.3%, positive predictive value 22.7%, negative predictive value 100% and overall accuracy 78.8% (p<0.001)

Discussion

Diabetes mellitus (DM) is defined by persistent hyperglycemia, accompanied by disruptions in carbohydrate, lipid, and protein metabolism, primarily caused by reduced insulin secretion, impaired insulin action, or both. Prolonged hyperglycemia in diabetes contributes to long-term damage, dysfunction, and failure of multiple organs, including the kidneys, eyes, nerves, blood vessels, and heart [12].

The increasing prevalence of DM, coupled with a rising incidence of chronic complications stemming from both microvascular (e.g., nephropathy, retinopathy) and macrovascular (e.g., stroke, coronary artery disease, peripheral artery disease) pathologies, highlights the significant challenges this condition poses to global healthcare systems in the 21st century [13].

Diabetic kidney disease (DKD) represents the most common cause of end-stage renal disease (ESRD) globally, affecting approximately 20–40% of diabetic patients [14]. Historically referred to as diabetic nephropathy, DKD now encompasses not only diabetic nephropathy but also ischemic nephropathy, atheroembolic disease, and interstitial fibrosis, all of which are directly linked to diabetes [15].

Research has consistently shown that several factors, including genetic predisposition, chronic inflammation, hyperglycemia, dyslipidemia, and familial clustering, play pivotal roles in increasing susceptibility to diabetic nephropathy [16].

Our findings revealed a statistically significant difference in age and body mass index (BMI) across the studied groups, particularly when comparing patients with diabetic nephropathy to the control group ($p = 0.009$ and $p < 0.001$*, respectively). These results align with previous studies that reported similar findings regarding the association between age, BMI, and diabetic nephropathy [17,18,19].

Fetuin-A is a 62-kDa glycoprotein synthesized in the liver, classified within the cystatin family of proteinase inhibitors. It plays a critical role in inhibiting insulin receptor tyrosine kinase activity and is directly linked to insulin resistance and dyslipidemia [20]. Elevated Fetuin-A levels have been observed in cases of insulin resistance, and it is recognized as an independent predictor of type 2 diabetes mellitus (T2DM) [21].

Research suggests that increased serum Fetuin-A levels are associated with impaired insulin sensitivity, leading to metabolic comorbidities such as hypertriglyceridemia, obesity, impaired glucose tolerance, T2DM, non-alcoholic fatty liver disease (NAFLD), and early-stage chronic kidney disease (CKD) [22].

In our current study, we observed that Fetuin-A levels begin to rise early in diabetic patients, even in those with a disease duration of less than five years and normoalbuminuria, with a progressive increase corresponding to the severity of nephropathy.

This finding aligns with previous studies that reported elevated serum Fetuin-A levels in diabetic nephropathy (DN) patients, highlighting its sensitivity as a biomarker for early microalbuminuria [23-28]. These studies confirmed that serum Fetuin-A levels were significantly higher in DN patients compared to healthy controls and non-nephropathic T2DM patients.

Furthermore, earlier research demonstrated an inverse relationship between serum Fetuin-A levels and urinary albumin excretion, indicating lower Fetuin-A concentrations are associated with microvascular complications in early diabetic nephropathy. Additionally, a positive association between Fetuin-A levels and glomerular filtration rate (GFR) was noted, underscoring its potential role as a marker for renal function preservation in diabetic patients [29,30].

The current study demonstrated that serum Fetuin-A levels were significantly elevated in patients with cardiovascular abnormalities, including regional wall motion abnormalities (RWMA), diastolic dysfunction, mitral annular calcification, aortic valve calcification, and overall abnormal echocardiographic findings. Additionally, a positive correlation between serum Fetuin-A levels and carotid intima-media thickness (CIMT) ($r = 0.768, p < 0.001$*) and a negative correlation with ejection fraction (EF) ($r = -0.343, p = 0.002$) were observed. These findings indicate that higher serum Fetuin-A levels are associated with increased vascular calcification, arterial stiffness, and reduced cardiac function. Similar results were reported by Lorant et al., who found elevated serum Fetuin-A levels in patients with type 2 diabetes mellitus (T2DM) and peripheral arterial disease, suggesting a strong link with vascular calcification and impaired cardiac function [31]. Likewise, Ju et al. demonstrated a positive association between Fetuin-A levels and vascular complications in patients with diabetic nephropathy [32]. Furthermore, Song et al. reported that elevated Fetuin-A levels contribute to impaired insulin sensitivity and vascular calcification, highlighting its predictive value in cardiovascular complications associated with diabetes [33]. These findings collectively support the current study's results, emphasizing Fetuin-A's role as a biomarker for cardiovascular calcification and dysfunction in diabetic populations.

The current study also observed a progressive increase in serum Fetuin-A levels with worsening bone density. Median Fetuin-A levels were 13 ng/mL (IQR: 8.1–19.65) in patients with normal bone density, rising to 23 ng/mL (IQR: 13.8–67.2) in mild osteopenia, 75.95 ng/mL (IQR: 51.88–78.85) in moderate osteopenia, and peaking at 82.1 ng/mL (IQR: 52.85–83.05) in patients with marked osteopenia. Post-hoc analysis revealed significant differences between normal and abnormal bone density groups and between mild and marked osteopenia, indicating a clear association between rising Fetuin-A levels and deteriorating bone density. These findings are consistent with results from Al-Said et al., who reported an association between elevated serum Fetuin-A levels and decreased bone mineral density (BMD) in T2DM patients with nephropathy [34]. Similarly, Madani et al. demonstrated increased serum Fetuin-A levels in diabetic patients with osteopenia and

osteoporosis, emphasizing its role in impaired bone mineralization [35]. Additionally, El-Batch et al. highlighted the dual regulatory role of Fetuin-A in mineral metabolism, linking it to both vascular calcification and bone density abnormalities [36]. The current study's results align with these observations, reinforcing the role of Fetuin-A as a potential biomarker for bone fragility in diabetes-related complications.

In terms of vascular calcification, the current study found that patients with calcification had significantly elevated serum Fetuin-A levels compared to those without calcification (*73 ng/mL vs. 14.1 ng/mL, p = 0.001**)*. This suggests a strong association between elevated Fetuin-A levels and the presence of vascular calcification. These findings are supported by Roos et al., who described a correlation between high serum Fetuin-A levels and early vascular calcification in diabetic nephropathy patients [37]. Similarly, Ismail et al. reported an inverse relationship between serum Fetuin-A and albuminuria, indicating its role in vascular pathology among diabetic patients [38]. Furthermore, Chekol Abebe et al. described Fetuin-A as a key inhibitor of vascular mineralization, with elevated levels reflecting ongoing calcification processes [39]. The current study's findings align with this evidence, highlighting Fetuin-A's dual involvement in both vascular calcification and skeletal fragility.

Interestingly, the current study found no significant association between AHSG genotypes (CC and CG) and bone density or vascular calcification (*p = 0.833, p > 0.999*). This suggests that AHSG genetic polymorphisms may not directly influence bone mineral density or vascular calcification outcomes in diabetic patients. These results are consistent with findings by Ma et al., who reported no direct association between AHSG gene polymorphisms and bone density, suggesting that the effects might be mediated through serum Fetuin-A levels [40]. Similarly, Siddiq et al. noted no significant correlation between AHSG genotypes and vascular calcification, proposing that epigenetic or environmental factors might play a modifying role [41]. Additionally, Osawa et al. emphasized that the impact of AHSG gene polymorphisms on bone and vascular health could be influenced by population-specific or environmental factors [42]. These findings are consistent with the current study, indicating that while AHSG genetic polymorphisms may not directly affect bone density or vascular calcification, their downstream effects via serum Fetuin-A levels warrant further investigation.

In conclusion, the current study supports the growing body of evidence linking serum Fetuin-A levels to cardiovascular disease and bone mineral density deterioration in diabetic patients. Elevated serum Fetuin-A levels were strongly associated with vascular calcification, arterial stiffness, reduced cardiac function, and declining bone density. However, no significant association was observed between AHSG genetic polymorphisms (CC and CG) and bone density or vascular calcification, suggesting a more complex interaction influenced by serum Fetuin-A levels and environmental factors. These findings emphasize the potential utility of Fetuin-A as a dual biomarker for assessing cardiovascular and skeletal health in diabetes mellitus.

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