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Fetuin-A as A Marker of Vascular Calcification in Chronic Kidney Disease

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ABSTRACT

Multiple factors contribute to vascular calcification in chronic kidney disease. Fetuin-A is known for its potent inhibitory effects on ectopic calcification **Objectives:** To determine the association between fetuin-A levels and vascular calcification in chronic kidney disease patients. **Methods:** 90 samples were collected from patients admitted to the Nephrology ward of Shahida Islam Medical Complex, Lodhran due to renal disease, and 90 samples were collected from normal healthy subjects. Patients with congestive heart failure, use of hormonal contraceptives or hormone replacement therapy, malignancy, pregnancy and with a history of trauma or surgery within a month were excluded. Mann-Whitney was applied to test Serum Fetuin A between cases and controls keeping $p<0.05$ as statistically significant. **Results:** Of 90 cases and 90 controls, 63 male (70%) and 27 female (30%) were in cases and 45 (50%) male and female were in the control group. the cases exhibited lower levels of Fetuin-A (0.4416 ± 0.17 g/L) compared to the controls (0.752 ± 0.176 g/L). The clustering revealed a possible association between the severity of chronic kidney disease and decreased Fetuin-A levels. The values ranged from as low as 0.034 to a peak at 2.132 g/L, with several outliers distributed across the chronic kidney disease stage. **Conclusions:** It was concluded that comparing fetuin-A levels in chronic kidney disease patients to controls revealed a significant correlation. Patients had lower levels of fetuin-A compared to controls.

INTRODUCTION

Chronic kidney disease (CKD) is a significant healthcare burden the world over, affecting millions [1]. CKD is characteristically denoted by progressive loss of kidney function, posing substantial challenges, such as being associated with multiple complications, like cardiovascular disease (CVD) [2]. Amongst the most concerning cardiovascular complications is vascular calcification, which is marked by the deposition of calcium phosphate crystals in vascular tissues [3]. The pathological calcification leads to an increase in arterial stiffness, elevation of pulse pressure, and heightened risk of adverse cardiovascular events, which are leading causes of mortality and morbidity among CKD patients [4]. In CKD, Vascular calcification is multifactorial which involves complex interactions in-between mineral imbalances, inflammatory processes, and metabolic disturbances. One of the key biomarkers in CKD pathophysiology is fetuin-A, a glycoprotein predominantly synthesized in the liver [5]. Fetuin-A is recognized for its potent inhibitory effects on ectopic calcification. The function of Fetuin-A acts via binding to calcium and phosphate ions and prevents their precipitation and subsequent deposition in vascular tissues. This resultantly causes fetuin-A to serve as a crucial regulator of mineral metabolism and thus a potential marker for vascular calcification [6]. According to published research, CKD is associated with lower levels of fetuin-A, which are correlated with the degree of arterial calcification and can result in severe cardiovascular events

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[7]. This progressive decline in renal function observed in CKD disrupts the homeostasis of calcium and phosphate, and so promotes an environment conducive to vascular calcification [8]. The concomitant reduction in fetuin-A exacerbates this process, underscoring its significance as both a protective factor and a biomarker [9]. Researchers have shown that lower serum fetuin-A levels are linked with increased calcification scores and arterial stiffness in CKD patients, especially. This inverse relationship suggests that monitoring fetuin-A levels could offer valuable insights into the extent of vascular calcification and overall cardiovascular health in this population, especially in hypertension [10]. Moreover, fetuin-A's role extends beyond a mere biomarker; it also represents a potential therapeutic target [11]. Enhancing fetuin-A levels or mimicking its inhibitory effects on calcification could pave the way for novel interventions aimed at mitigating vascular calcification and improving cardiovascular outcomes in CKD [12]. Despite the promising implications, the precise mechanisms by which fetuin-A modulates vascular calcification in CKD remain incompletely understood [13]. Ongoing research aims to elucidate the pathways involved, with a focus on the interplay between fetuin-A, mineral metabolism, and inflammatory mediators. Understanding these mechanisms is crucial for developing targeted therapies and refining clinical strategies to manage vascular calcification in CKD patients [14]. Fetuin-A emerges as a pivotal marker of vascular calcification in chronic kidney disease, reflecting its broader role in mineral metabolism and cardiovascular health. As CKD continues to pose significant health challenges, leveraging biomarkers like fetuin-A for early detection, risk stratification, and therapeutic intervention holds promise for improving patient outcomes [15]. Since the burden of CKD is high in the local population along with its associated complications, investigating the potential biomarker (Feutin-A) for early detection of vascular calcification in CKD is vital for Pakistani patients.

This study aims to determine the association between fetuin-A levels and vascular calcification in CKD patients.

METHODS

This case-control study was done at Shahida Islam Medical Complex, Lodhran for six months after approval of the research proposal from the Institutional Review Board Committee (IRB) letter no. SIMC/ET.C/10012/23. The study was done from February 2023 to July 2023. The sample size for the study was calculated using open epi online software for sample size calculation. Keeping the prevalence of chronic kidney disease in Pakistan at 12.5% as reported in local research and a 95% confidence level, with precision at 5% the sample size came out to be 169. However, 180 samples were taken from patients (accounting for loss to follow-up and a 5 % margin of error). admitted to the Nephrology Department of Shahida Islam Teaching Hospital due to any renal illness, and 90 samples were collected from otherwise healthy people as controls. Informed consent was taken from all subjects. Out of 180 samples, 90 were cases of renal disease between 18 and 75 years of age, whilst 90 were standard demographically compared to healthy controls. Patients with congestive heart failure, use of hormonal contraceptives or hormone replacement therapy, malignancy, pregnancy, and a history of trauma or surgery within a month were excluded from the study. The sampling method used was non-probability convenience sampling. The demographical data included age, weight, and status of diabetes while laboratory data included Feutin-A, fasting glucose, urea, creatinine, and creatinine clearance; serum calcium and phosphorus were assessed after a 12-hour fast wherein serum was obtained after centrifugation of blood at 3000 rpm. For Fetuin A, an ELISA kit was used for testing in blood serum. This kit is for Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre-coated using human FETU-A antibodies. The FETU-A from the sample was added, and it interacted with the antibodies on the boreholes by binding to them. The subsequent addition of biotin-conjugated human FETU-A antibody causes it to adhere to the sample's FETU-A. Streptavidin-HRP is then added, which binds the biotinylated FETU-A antibody. Post incubation, unattached Streptavidin-HRP was eliminated through washing. After that, a substrate mixture was introduced. As additional Human FETU-A is included, the color gradually changes. When an acidic stop solution was introduced to stop the reaction, absorbance at 450 nm was measured. For data analysis, SPSS version 23.0 was used. Mean and standard deviation were reported for numerical variables while frequency and percentage for categorical variables. Stratification of data was performed according to age. Mann-Whitney was applied to test Serum Fetuin A between cases and controls keeping $p<0.05$ as statistically significant.

RESULTS

The key variables from the 90 cases and 90 controls are contrasted. The mean age of the cases was 44.55 ± 15.95 years, while the controls had a similar mean age of 45.5 years ± 15.95 years. BMI was slightly higher among cases ($27.33 \pm 5.31\ kg/m^2$) compared to controls ($25.5 \pm 5.13\ kg/m^2$). The distribution of gender among the participants was notably skewed in the cases group, with 63 male (70%) and 27 female (30%). In contrast, the control group had an equal gender distribution, with 50% male and 50% female. Regarding comorbidities, diabetes was present in 30 % of the cases compared to 7.8% of the controls. Hypertension was also more prevalent among the cases (38.9%) compared to the controls (10%). Biochemically, the patients had lower Fetuin-A levels (0.4416 ± 0.17 g/L) than the controls (0.752 ± 0.176 g/L). Furthermore, the cases showed altered glucose levels (104.67 ± 23.01 mg/dL) compared to the controls (92.05 ± 97.57 mg/dL). Renal function indicators, including urea and creatinine, were highly elevated in the cases, with urea levels at 100.95 ± 28.73 mg/dL and creatinine at 2.07 ± 3.15 mg/dL, compared

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to controls who had urea levels at 23.92 ± 3.44 mg/dL and creatinine at 0.94 ± 0.01 mg/dL. The mean Glomerular Filtration Rate (GFR) (mL/min/1.73 m2) in cases was 42.47 ± 16.72 mL/min/1.73 m2 while in controls was 98.39 ± 11.29 mL/min/1.73 m2. Serum calcium in cases was 12.65 ± 0.72 mg/dl while in controls was 9.81 ± 0.5 mg/dl. Serum cholesterol in cases was 237 ± 37.22 mg/dl while in controls was 173.34 ± 23.95 mg/dl (Table 1).

Table 1: Demographics of Patients Included in the Study (n=180)

Variables		Cases n=90	Controls n=90
Age (Years)		44.55 ± 15.95	45.5 ± 15.95
BMI (kg/m^2)		27.33 ± 5.31	25.5 ± 5.13
Gender	Male	63 (70 %)	45 (50 %)
	Female	27 (30 %)	45 (50 %)
Co-morbidities	Diabetes	27 (30 %)	07 (7.8 %)
	Hypertension	35 (38.9 %)	09 (10 %)
Fetuin-A (g/l)		0.4416 ± 0.17	0.752 ± 0.176
Glucose (mg/dl)		104.67 ± 23.01	92.05 ± 97.57
Urea (mg/dl)		100.95 ± 28.73	23.92 ± 3.44
Creatinine (mg/dl)		2.07 ± 3.15	0.94 ± 0.01
Creatinine Clearance (ml/min)		33.99 ± 22.24	97.48 ± 8.51
Glomerular Filtration Rate (mL/min/1.73 m^2)		42.47 ± 16.72	98.39 ± 11.29
Calcium (mg/dl)		12.65 ± 0.72	9.81 ± 0.5
Phosphorus (mg/dl)		5.54 ± 1.1	4.41 ± 0.37
Serum Cholesterol (mg/dl)		237 ± 37.22	173.34 ± 23.95

A graphical representation of the stages of kidney disease among the cases (n=90) was analyzed. The distribution indicates a progression of the disease, with a significant proportion of cases (54%) in Stage V, which represents end-stage kidney disease. The earlier stages of kidney disease, such as Stage 0, were less prevalent, comprising only 13% of the cases, while intermediate stages show a gradual increase in prevalence, with 20% in Stage IV, 8% in Stage III, 3% in Stage II, and 2% in Stage I (Figure 1).

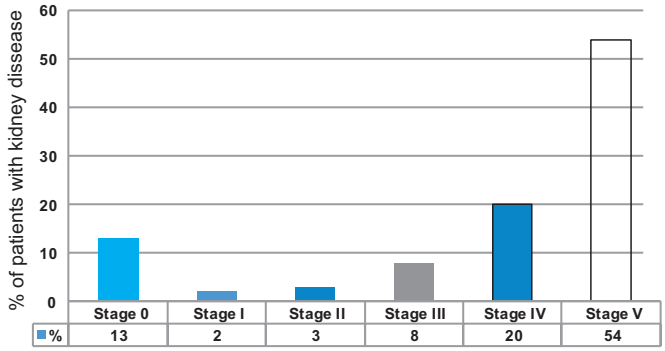

Figure 1: Graphical Representation of Stages of Kidney Disease in Cases (n=90)

The relationship between serum Fetuin-A levels and age in both patients and controls is examined. Serum Fetuin-A levels showed a notable age-related decrease in cases across all age categories. The mean Fetuin-A level in subjects aged 18 to 30 was 0.7390 ± 0.22111 g/L for controls and 0.6440 ± 0.11871 g/L for cases, with a significant p-value of 0.007. In all age categories, there was a consistent pattern of reduced Fetuin-A levels in cases relative to controls, which became more noticeable as people aged. For instance, in the 51-60 years age group, cases had a mean Fetuin-A level of 0.3936 ± 0.11879 g/L compared to 0.7598 ± 0.18164 g/L in controls, with a highly significant p-value of 0.0001. The oldest age group (61-75 years) showed the lowest mean Fetuin-A levels, with cases at 0.3667 ± 0.15254 g/L versus controls at 0.6916 ± 0.16181 g/L, again with a significant p-value of 0.0001 (Table 2).

Table 2: Association of Serum Fetuin-A Levels According to Age among Cases Vs Controls (n=180)

Year	Serum Fetuin-A (g/L)		p-value
	Cases	Control	
18-30 Years	0.6440 ± 0.11871	0.7390 ± 0.22111	p=0.007*
31-40 Years	0.4648 ± 0.15180	0.7532 ± 0.19389	p=0.0001**
41-50 Years	0.4893 ± 0.18718	0.7932 ± 0.16947	p=0.0001**
51-60 Years	0.3936 ± 0.11879	0.7598 ± 0.18164	p=0.0001**
61-75 Years	0.3667 ± 0.15254	0.6916 ± 0.16181	p=0.0001**

A scatter plot illustrating the distribution of Fetuin-A levels among cases with different stages of kidney disease (n=90). The plot displays a wide range of Fetuin-A levels, with a notable clustering at lower levels, particularly in advanced stages of kidney disease. This clustering suggested a potential correlation between lower Fetuin-A levels and the severity of kidney disease. The values range from as low as 0.034 to a peak at 2.132 g/L, with several outliers distributed across the stages. There was variability between Fetuin-A levels and kidney disease progression (Figure 2).

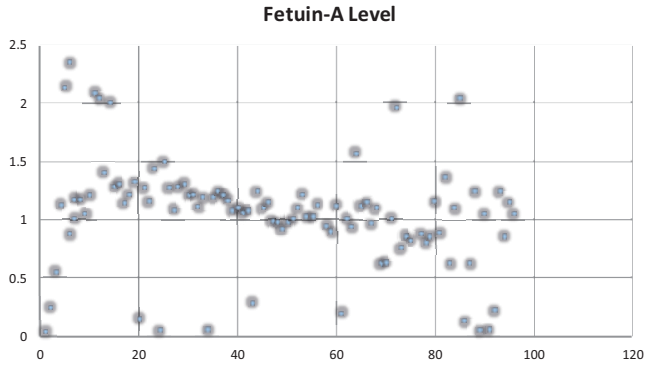

Figure 2: Scattered Diagram of Different Fetuin-A Levels in Kidney Disease Cases (n=90)

DISCUSSION

The relationship between fetuin-A levels and vascular calcification in patients with chronic kidney disease was investigated in this study. Fetuin-A was noted to be a biomarker for the progression of renal illness for the assessment of baseline kidney failure. It was seen that even in subjects with slight to severe kidney weakening, fetuin-A had a significant diagnostic value. Also, we found that serum concentrations of creatinine at baseline were

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excellent indicators of CKD disease progression. This is consistent with previous studies findings [16-18]. Serum fetuin-A concentrations were considerably lower in patients with renal disease (Mean=0.452 0.16 g/L) compared to the control group (mean=0.765 0.17 g/L; p=0.001). Additionally, it was shown that fetuin-A levels decreased gradually from stage 2 renal failure (CrCl=60-90 ml/min) to stage 5 renal failures (CrCl=15 ml/min). This demonstrates that decreases in serum fetuin-A quantity occur rather early in the course of renal illness. These results are comparable to a study done by Caglar *et al.*, which exposed all levels of CKD but Stage 1 had a decline in serum fetuin-A levels [19]. Another study by Makulska *et al.*, reported lesser levels of serum fetuin-A in children having vascular calcifications in CKD [20]. Serum Calcium and Serum cholesterol levels have been attributed to vascular calcification. In the current study, both serum calcium and cholesterol were found to be within normal limits in the control group (having higher fetuin-A levels while the cases showed higher than normal levels of serum calcium and cholesterol with low fetuin-A levels, denoting vascular calcification. The mean serum fetuin-A level in CKD cases in this study was 0.45 0.16 g/L. These findings are consistent with research by Cottone *et al.*, who discovered that CKD patients had an average fetuin-A level of 0.53 0.17 g/L. Furthermore, as compared to controls, this study discovered that the amount of serum fetuin-A was significantly lower in both sexes and at all ages, indicating that serum fetuin-A levels are unaffected by age or sex. Synthesis of fetuin-A was down-regulated in the chronic inflammatory state of CKD [21].

CONCLUSIONS

It was concluded that when compared to controls, this study showed a strong correlation between fetuin-A levels and CKD. Lower levels of fetuin-A were observed in cases as compared with controls. Estimating these levels may help with early intervention in CKD for managing vascular calcification. The advancement of cardiovascular disease in CKD may be slowed down by early and quick control of the pro-inflammatory factors and the use of fetuin-A.

Authors Contribution

Conceptualization: RS
Methodology: SUH, AZ
Formal analysis: JM, IMB
Writing review and editing: SUH, JM, IMB, GP, AZ

All authors have read and agreed to the published version of the manuscript

Conflicts of Interest

All the authors declare no conflict of interest.

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