AMERICAN JOURNAL OF BIOMEDICINE AND PHARMACY
https://biojournals.us/index.php/AJBP
ISSN: 2997-7177

Article

# Association of Fetuin-A with Mineral Metabolism and Cardiovascular Risk Markers in Chronic Kidney Disease: A Cross-Sectional Study from Iraq

**Mudhar Mahdi**[*1]
1. College of Science, University of Baghdad, Baghdad, Iraq
*Correspondence: mudhar.n@sc.uobaghdad.edu.iq

**Citation:** Mahdi M. Association of Fetuin-A with Mineral Metabolism and Cardiovascular Risk Markers in Chronic Kidney Disease: A Cross-Sectional Study from Iraq. American Journal of Biomedicine and Pharmacy 2026, 3(10), 71-84.

Received: 20th Aug 2026
Revised: 10th Sep 2026
Accepted: 30th Sep 2026
Published: 21st Oct 2026

**Copyright:** © 2026 by the authors. Submitted for open access publication under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/)

**Abstract:** Chronic kidney disease (CKD) is linked with gradual renal dysfunction and metabolic dysfunctions. The liver-derived glycoprotein fetuin-A is suggested as a biomarker of biochemical changes in CKD. This study aimed at investigating the level of serum Fetuin-A and its correlation with some clinical and biochemical parameters in patients with chronic kidney disease (CKD) in Iraq. 50 participants were recruited, including 20 stage 3-4 CKD conservative therapy, 20 maintenance hemodialysis (HD), and 10 healthy controls. ELISA was used to measure Serum Fetuin-A and correlations and ROC analysis was used to determine the associations of Fetuin-A with laboratory parameters. The levels of serum Fetuin-A were significantly lower in the HD group (378 ± 50 ng/mL) as compared to conservatively treated CKD patients (490 ± 73 ng/mL) and controls (498 ± 58 ng/mL) ($p < 0.001$). There was a correlation between fetuin-A and markers of renal function, calcium and lipid profile parameters. Good discrimination was observed between CKD patients and controls by using the ROC analysis (AUC = 0.82). Low serum Fetuin-A levels are linked to renal dysfunction and metabolic abnormalities, suggesting that it may be a biomarker for biochemical events in CKD.

**Keywords:** Blood Urea, Chronic Kidney Disease (CKD), Estimated Glomerular Filtration Rate (EGFR), Hemodialysis, Renal Biomarkers

## Introduction

Chronic kidney disease (CKD) is a progressive condition in which there is an alteration of kidney structure or function that persists for three months or longer and is associated with significant morbidity and mortality worldwide [1]. The prevalence of diabetes mellitus, hypertension, ageing and other metabolic diseases are the major contributors to the ever-growing burden of CKD around the globe, particularly in resource poor countries [2]. From a clinical point of view, the most frequent findings of CKD are a decrease in glomerular filtration rate, buildup of uremic toxins, abnormalities in mineral metabolism, anemia, inflammation and increase in metabolic complications [3]. Thus, detection of circulating biomarkers linked to renal dysfunction and biochemical disturbances could aid in the understanding of pathophysiological changes occurring in CKD [4].

Fetuin-A (alpha-2 Heremans-Schmid glycoprotein) is a glycoprotein secreted from the liver that has been shown to play a role in mineral metabolism, inflammation, and metabolic regulation [5], [6]. Fetuin-A is involved in the transport of calcium and phosphate ions, and plays a role in making soluble

calciprotein particles that help keep mineral solubility in the circulation [7]. This function may be of special significance in CKD as calcium, phosphate and renal function parameters are commonly disturbed [8].

Lower concentrations of fetuin-A have been previously reported to be associated with advanced renal failure, hemodialysis, abnormalities of mineral metabolism, inflammation, and poor clinical status [9], [10]. But because most available information is observational this information is interpreted as a correlation and not proof of causation. Fetuin-A should also be thought of as a potential biomarker, not a direct measure of vascular stiffness or cardiovascular disease [11], [12].

Besides its role in mineral metabolism, fetuin-A has been shown to be associated with biochemical markers of cardiometabolic risk factors, such as insulin signaling and lipid metabolism, which may account for its association with those biochemical markers [10]. Negative correlation of serum fetuin-A with renal dysfunction markers like blood urea and serum creatinine and positive correlation with estimated glomerular filtration rate (eGFR) have been reported by some studies [13]. However, the clinical significance of fetuin-A as a biomarker linked to renal impairment, mineral metabolism and lipid profile alteration has yet to be sufficiently clarified particularly in under-represented populations.

Information on serum fetuin-A levels in patients with CKD from Middle Eastern countries, such as Iraq, is still scarce. This population may be affected by factors such as environmental, genetic, nutritional, inflammatory and healthcare-related factors that can affect the trajectory of CKD and the patterns of biomarkers. Furthermore, there has been a paucity of studies assessing fetuin-A levels in relation to renal function, mineral metabolism and lipid profile parameters in both pre-dialysis and in hemodialysis patients.

The present cross-sectional study was therefore undertaken to determine the level of fetuin-A in the blood of Iraqi patients with CKD and its correlation with renal function parameters, mineral metabolism markers, lipid profile variables and some biochemical indicators in patients who were not receiving dialysis and hemodialysis patients on maintenance dialysis. As the design of the study is cross sectional and no direct vascular measures were undertaken, the results presented are considered only associations and not evidence of causality or direct cardiovascular impact.

## Materials and Methods

### *Study design and population*

This was a cross-sectional study which was performed a group of Iraqi patients with chronic kidney disease (CKD). The total number of participants were 50, 40 were patients of CKD who were taken from the hemodialysis units and outpatient nephrology clinics in Iraqi hospitals and 10 apparently healthy individuals as controls.

The participants were divided into three groups:

Group I (Pre-dialysis CKD patients): 20 patients with a stage 3-4 CKD who were under conservative (non-dialysis) treatment, comprising 11 males and nine females.

Group II (Hemodialysis patients): 20 patients with CKD who were undergoing regular maintenance hemodialysis (three times per week for more than nine months), 12 males and 8 females.

Group III (Control group): Ten healthy volunteers (5 male and 5 female) who do not have any renal or systemic disease in their known history.

Patients who fulfilled the following criteria were included: patients who had clinical and laboratory characteristics suggestive of CKD. Patients with acute kidney injury, congenital renal abnormalities, other inflammatory disorders, chronic liver disease, malignancy or pregnancy were excluded from the study, as were other systemic diseases likely to affect biochemical parameters.

All patients were carefully clinically assessed with regard to demographic features, duration of the disease, co-morbidity, dialysis related data, physical examination, and laboratory investigations.

As this was an exploratory biomarker study, there was not a formal sample size calculation conducted before recruiting. The relatively small number of respondents is recognized as a limitation and the results should be viewed with caution.

***Ethical Considerations***

The study was carried out following the ethical recommendations of Declaration of Helsinki. Informed written consent was given before enrolment in the study.

The study protocol was reviewed and approved by the Ethics and Scientific Committee of Baghdad Teaching Hospital, Baghdad, Iraq (Approval No. BTH-2023-117; approved on 15 June 2023). All participant information was treated confidentially and data were anonymised prior to statistical analysis to maintain confidentiality and privacy throughout the study.

***Sample Collection and Processing***

Venous blood was aseptically collected from each participant in an amount of about 5 mL in a sterile disposable syringe. The blood samples were left to clot at room temperature and then centrifuged at 1000 × g for 10 minutes and the serum was collected.

Aliquots of the serum were placed in sterile microtubes. Samples for routine biochemical analyses were processed immediately while samples for Fetuin-A determination were stored at −20°C until analyses.

***Laboratory investigations***

*Hematological analysis*

The parameters of complete blood count (CBC) such as hemoglobin (Hb), hematocrit (HCT), white blood cell count (WBC), and platelet count were assessed with an automated hematology analyzer as per the standard laboratory procedures.

*Renal function assessment*

The parameters of renal functions such as blood urea and serum creatinine were estimated using an automated clinical chemistry analyzer that relied on enzymatic techniques.

The estimated glomerular filtration rate (eGFR) was determined based on the CockcroftGault equation and was in the form of [14]:

$$eCCr = \frac{(140-\text{age})\times\text{body weight (kg)}}{72\times\text{serum creatinine (mg/dL)}} \quad (1)$$

For female patients, the result was multiplied by a correction factor of 0.85.

*Biochemical and Electrolyte measurements*

Depending on the specifications of the analyzer, serum electrolytes, such as sodium ($Na^-$), potassium ($K^-$), calcium (Ca2+) and phosphorus (PO43-) were analyzed by ion-selective electrode or colorimetric analysis.

Fasting blood glucose (FBG) and two-hour postprandial blood glucose (2hPPBS) were measured and used as the glycemic status using enzymatic glucose oxidase-based assays.

The enzymatic colorimetric parameters of lipid profile parameters such as total cholesterol and triglycerides were established using an automated chemistry analyzer.

*Liver function tests*

Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin, serum albumin, and prothrombin time (PT) were determined by commercially available diagnostic kits following the manufacturer's instructions.

*Serum Fetuin-A Measurement.*

Serum Fetuin-A level was determined with a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Bioassay Technology Laboratory, Shanghai, China; Catalog No. E3164Hu).

The detection range was 50-1500 ng/mL, intra-assay CV was ≤8 % and inter-assay CV was ≤10 % with a sensitivity of 12.5 ng/mL.

The assay was carried out following the manufacturer's protocol. Fetuin-A specific monoclonal antibodies were briefly added to the wells of a microplate pre-coated with the monoclonal antibodies. Following incubation and washings, the enzyme conjugated secondary antibodies were applied. The reaction was terminated with sulphuric acid and colour development was done with a chromogenic substrate. The microplate reader was used to measure the optical density at 450 nm. The concentration of fetuin-A was determined by measuring the standard calibration curve generated by adding known standard provided in the kit.

***Statistical analysis***

The statistical analysis was carried out in SPSS version 26.0 (IBM Corp., Armonk, NY, USA) and Microsoft Excel 2016.

Continuous variables were reported as mean ± standard deviation (SD) and categorical variables were reported as frequencies and percentages.

Data distribution was assessed and appropriate parametric statistical tests used for group comparisons. Pearson correlation was used to test the correlation of Fetuin-A level in serum with laboratory parameters.

Serum Fetuin-A concentration was analyzed for its ability to discriminate between CKD and healthy controls by Receiver Operating Characteristic (ROC) curve analysis. The area under the curve (AUC) and 95% confidence intervals (CIs) were determined. The Youden Index was used to find the best cut-off value and its sensitivity and specificity were presented.

Due to the exploratory nature of the study and the limited sample size, no adjustment for multiple comparisons was done. Thus, caution needs to be taken and statistical significance should be viewed as a hypothesis-generating result. A p-value < 0.05 was considered statistically significant.

## Results

***Clinical Characteristics of the Study Participants***

Table 1 shows the clinical data of the study population. The mean age was significantly different between the three groups ($p < 0.05$), the conservative-treatment CKD group having the highest mean age. Body weight also showed significant differences in each group ($p < 0.05$), being the lowest in the hemodialysis group. The duration of the disease was significantly longer in the conservative treatment CKD group compared to a hemodialysis group ($p < 0.001$). Patients receiving hemodialysis had more hypertension than those receiving conservative treatment ($p < 0.01$). The sex distribution, frequency of diabetes, and blood pressure (systolic and diastolic) did not differ significantly between groups.

**Table 1.** Clinical characteristics of the study participants.

| Parameter | Conservative (n=20) | Hemodialysis (n=20) | Control (n=10) | F | p-value |
|---|---|---|---|---|---|
| Age (years) | 49.2 ± 9.4 (26–64) | 45.3 ± 8.1 (30–61) | 40.1 ± 6.1 (30–50) | 4.1 | <0.05* |
| Sex (M/F) | 10/10 (50%/50%) | 12/8 (60%/40%) | 7/3 (70%/30%) | 0.7 | >0.05 |
| Weight (kg) | 81.2 ± 8.3 (67–96) | 75.0 ± 9.7 (60–94) | 82.7 ± 7.8 (71–92) | 4.0 | <0.05* |
| Diabetic (%) | 6 (30%) | 5 (25%) | — | 0.2 | >0.05 |
| Hypertensive (%) | 10 (50%) | 18 (90%) | — | 11.1 | <0.01** |
| Disease Duration (years) | 6.2 ± 1.6 (3–9.5) | 3.8 ± 1.1 (2.2–6.2) | — | 25.4 | <0.001*** |
| HD Duration (years) | — | 4.3 ± 1.6 (1.6–7.1) | — | — | — |

| | | | | | |
|---|---|---|---|---|---|
| SBP (mmHg) | 133.8 ± 32.5 (92–188) | 146.3 ± 9.1 (132–161) | 124.7 ± 4.7 (121–130) | 2.8 | >0.05 |
| DBP (mmHg) | 80.7 ± 18.9 (62–108) | 86.4 ± 9.0 (72–104) | 75.1 ± 5.3 (66–81) | 2.3 | >0.05 |

*Data are presented as mean ± SD (range) or number (%). $p < 0.05$, ** $p < 0.01$, *** $p < 0.0$

***Hematological Parameters***

The results of hematological parameters among the study subjects are presented in Table 2. The groups showed a significant difference in hemoglobin levels ($F = 77.5$, $p < 0.001$) with the lowest mean level in the hemodialysis group. The hematocrit also varied significantly between groups ($F = 85.0$, $P < 0.001$) with a lower value in the CKD patients than in the controls. The mean value of white blood cell counts was significantly different between the groups ($F = 7.1$, $p < 0.01$). White blood cell count was significantly different among groups with the hemodialysis group having a higher mean value. There were no significant differences in the platelet count and blood sugar levels (fasting and post two-hour meal) between the groups.

**Table 2.** Hematological characteristics of the study participants.

| Parameter | Conservative (n=20) | Hemodialysis (n=20) | Control (n=10) | F | p-value |
|---|---|---|---|---|---|
| Hemoglobin (g/dL) | 10.3 ± 1.1 (8.6–12.1) | 9.7 ± 1.0 (7.7–11.0) | 14.3 ± 0.6 (13.8–15.2) | 77.5 | <0.001*** |
| Hematocrit (%) | 33.2 ± 3.4 (28.5–38.7) | 30.2 ± 1.6 (26.9–37.9) | 45.1 ± 4.9 (38.3–51.5) | 85.0 | <0.001*** |
| Platelet count ($\times 10^9$/L) | 246 ± 44 (152–318) | 233 ± 55 (151–342) | 241 ± 54 (163–349) | 0.26 | >0.05 |
| White blood cells ($\times 10^3/\mu L$) | 6.74 ± 1.3 (5.2–9.1) | 7.92 ± 1.5 (5.1–10.9) | 6.09 ± 1.1 (4.1–7.9) | 7.1 | <0.01** |
| Fasting blood glucose (mg/dL) | 110 ± 35 (67–207) | 107 ± 60 (66–240) | 67 ± 4 (62–76) | 3.0 | >0.05 |
| 2-hour postprandial blood glucose (mg/dL) | 157 ± 51 (111–298) | 176 ± 74 (112–328) | 130 ± 6 (121–139) | 1.95 | >0.05 |

***Renal Function and Electrolyte Parameters***

Renal function and electrolyte parameters are listed in table 3. The BUN values of the groups were significantly different ($F = 30.7$; $p < 0.001$), and the mean value of BUN was highest in the hemodialysis group. Hemodialysis group also had significantly higher SC level than the conservative-treatment and control groups ($F = 423.0$, $p < 0.001$). On the other hand, estimated glomerular filtration rate was significantly reduced in CKD patients especially in the hemodialysis group compared to controls ($F = 437.5$, $p < 0.001$).

Serum potassium was significantly different among the groups ($F = 11.6$, $p < 0.001$) with the highest mean value in the hemodialysis group. There was also significant difference in serum calcium level between the groups ($F = 59.0$, $p < 0.001$); the level in the conservative-treatment CKD group was lower than that in the hemodialysis group and the control group. The levels of the serum phosphorus were significantly elevated in patients with CKD than the controls ($F = 42.3$, $p<0.001$). There was no significant difference in serum sodium and uric acid among the groups.

**Table 3.** Renal function and electrolyte parameters of the study participants.

| Parameter | Conservative (n=20) | Hemodialysis (n=20) | Control (n=10) | F | p-value |
|---|---|---|---|---|---|
| Blood urea (mg/dL) | 89 ± 36 (44–146) | 132 ± 37 (80–213) | 32 ± 5 (25–40) | 30.7 | <0.001*** |
| Serum creatinine (mg/dL) | 2.65 ± 1.02 (1.5–4.6) | 9.60 ± 0.96 (8.0–11.9) | 0.96 ± 0.14 (0.70–1.10) | 423.0 | <0.001*** |
| eGFR (mL/min) | 35.5 ± 14.6 (17.5–63.0) | 9.8 ± 0.98 (7.8–11.5) | 118 ± 4.9 (107–123) | 437.5 | <0.001*** |
| Sodium (mmol/L) | 140 ± 3 (136–145) | 142 ± 3 (135–146) | 139 ± 2 (137–142) | 1.85 | >0.05 |
| Potassium (mmol/L) | 4.4 ± 0.5 (3.6–5.2) | 4.9 ± 0.4 (4.3–5.4) | 4.1 ± 0.4 (3.5–4.8) | 11.6 | <0.001*** |
| Calcium (mg/dL) | 7.5 ± 0.7 (6.0–8.3) | 9.0 ± 0.4 (8.1–9.7) | 9.4 ± 0.5 (8.5–10.0) | 59.0 | <0.001*** |
| Phosphorus (mg/dL) | 5.1 ± 0.6 (4.0–6.3) | 5.4 ± 0.5 (4.5–6.3) | 3.4 ± 0.5 (2.9–4.0) | 42.3 | <0.001*** |
| Serum uric acid (mg/dL) | 5.6 ± 1.7 (3.2–8.0) | 6.4 ± 1.3 (4.7–8.9) | 5.6 ± 0.6 (4.1–6.6) | 1.8 | >0.05 |

***Lipid Profile and Liver Function Parameters***

The lipid profile parameters are presented in Table 4. There was significant difference between the groups in the mean of total cholesterol ($F = 9.6$, $p < 0.001$), with higher total cholesterol in the CKD patients than in the controls. The triglyceride level also was significantly different among groups ($F = 7.3$, $p < 0.01$), being higher in CKD patients. The hemodialysis group showed significantly higher LDL cholesterol level than the conservative-treatment CKD group ($F = 5.4$, $p < 0.01$) and the control group ($F = 8.8$, $p < 0.001$), while the HDL cholesterol level was significantly higher in the control group than in the conservative-treatment CKD group ($F = 10.4$, $p < 0.001$) and the hemodialysis group ($F = 7.5$, $p < 0.001$).

**Table 4.** Lipid profile parameters of the study participants.

| Parameter | Conservative (n=20) | Hemodialysis (n=20) | Control (n=10) | F | p-value |
|---|---|---|---|---|---|
| Total cholesterol (mg/dL) | 213 ± 25 (173–252) | 229 ± 35 (181–299) | 182 ± 11 (163–200) | 9.6 | <0.001*** |
| Triglycerides (mg/dL) | 149 ± 17 (121–197) | 156 ± 18 (131–182) | 130 ± 13 (113–149) | 7.3 | <0.01** |
| LDL cholesterol (mg/dL) | 94 ± 22 (60–132) | 118 ± 23 (67–148) | 100 ± 19 (79–129) | 5.4 | <0.01** |
| HDL cholesterol (mg/dL) | 42 ± 7 (30–57) | 44 ± 6 (32–56) | 54 ± 6 (43–62) | 10.4 | <0.001*** |
| Total bilirubin (mg/dL) | 0.9 ± 0.2 (0.5–1.2) | 0.8 ± 0.2 (0.5–1.2) | 0.7 ± 0.3 (0.4–1.2) | 1.6 | >0.05 |

Liver function-related parameters are given in Table 5. There were no significant differences in ALT, AST and prothrombin time between the groups. The alkaline phosphatase was significantly different between the groups ($F = 10.5$, $p < 0.001$). The mean value of serum albumin also varied significantly between the groups ($F = 5.5$, $p < 0.01$), and was lower in the CKD patients than the controls.

**Table 5.** Liver function parameters of the study participants.

| Parameter | Conservative (n=20) | Hemodialysis (n=20) | Control (n=10) | F | p-value |
|---|---|---|---|---|---|
| ALT (U/L) | 20 ± 6 (12–31) | 22 ± 9 (11–40) | 19 ± 5 (12–30) | 0.6 | >0.05 |
| AST (U/L) | 22 ± 8 (12–41) | 26 ± 11 (12–46) | 18 ± 8 (11–39) | 1.7 | >0.05 |
| ALP (U/L) | 78 ± 22 (46–112) | 105 ± 29 (45–149) | 113 ± 22 (80–146) | 10.5 | <0.001*** |
| Albumin (g/dL) | 3.8 ± 0.3 (3.4–4.3) | 3.7 ± 0.4 (3.0–4.5) | 4.1 ± 0.5 (3.5–4.9) | 5.5 | <0.01** |
| Prothrombin Time (sec) | 19 ± 26 (11–133) | 12 ± 1 (11–14) | 12 ± 1 (11–13) | 0.9 | >0.05 |

***Serum Fetuin-A Levels***

The mean serum Fetuin-A level for each group of the study is reported in the Table 6. There was significant difference in serum Fetuin-A levels between the three groups ($F = 10.9$, $p<0.001$). The lowest mean serum concentration of Fetuin-A was seen in the hemodialysis group (378 ± 50 ng/mL), the higher concentrations were seen in the conservative treatment CKD group (490 ± 73 ng/mL) and the control group (498 ± 58 ng/mL).

**Table 6.** Serum Fetuin-A concentrations among the study participants.

| Parameter | Conservative (n=20) | Hemodialysis (n=20) | Control (n=10) | F | p-value |
|---|---|---|---|---|---|
| Serum Fetuin-A (ng/mL) | 490 ± 73 (391–621) | 378 ± 50 (222–561) | 498 ± 58 (400–580) | 10.9 | <0.001*** |

***Distribution of Participants According to Serum Fetuin-A Concentration Categories***

The distribution of participants by serum Fetuin-A concentration categories as defined by the pre-defined cutoff for the exploratory analysis (≤475 ng/mL) is provided in Table 7. The low Fetuin-A serum level was seen in 11 participants (55.0%) in the conservative-treatment CKD group, 17 participants (85.0%) in the hemodialysis group, and 3 participants (30.0%) in the control group. A higher percentage of low levels of serum Fetuin-A was found in the hemodialysis group, but the difference was not statistically significant between the three groups ($\chi^2 = 2.9$; $p > 0.05$).

**Table 7.** Distribution of participants according to serum Fetuin-A concentration categories.

| Parameter | Conservative (n=20) | Hemodialysis (n=20) | Control (n=10) | $\chi^2$ | p-value |
|---|---|---|---|---|---|
| Serum Fetuin-A ≤ 475 ng/mL | 11 (55.0%) | 17 (85.0%) | 3 (30.0%) | 2.9 | >0.05 |

***Clinical Characteristics of CKD Patients Stratified by Serum Fetuin-A Category***

The clinical features of the CKD patients with low levels of serum Fetuin-A (≤475 ng/mL) are summarized in Table 8. There was a significant difference ($p = 0.013$) in the prevalence of hypertension between patients on hemodialysis and conservative treatment CKD patients. The disease duration was significantly longer in conservative treatment group than in the hemodialysis group ($p < 0.001$). The age, sex distribution, body weight and the frequency of diabetes were not statistically significantly different between the two groups.

**Table 8.** Clinical characteristics of CKD patients with low serum Fetuin-A concentrations (≤475 ng/mL).

| Parameter | Conservative (n=16) | Hemodialysis (n=11) | Test Statistic | p-value |
|---|---|---|---|---|
| Age (years) | 50.1 ± 9.8 (24.0–63.0) | 45.0 ± 5.7 (33.0–54.0) | t = 1.7 | 0.103 |
| Male, n (%) | 8 (50.0%) | 7 (63.6%) | $\chi^2 = 0.5$ | 0.484 |
| Female, n (%) | 8 (50.0%) | 4 (36.4%) | — | — |
| Weight (kg) | 80.1 ± 9.7 (65.0–95.0) | 74.5 ± 10.2 (57.0–95.0) | t = 0.7 | 0.467 |
| Diabetic, n (%) | 6 (37.5%) | 3 (27.3%) | $\chi^2 = 0.3$ | 0.580 |
| Hypertensive, n (%) | 7 (43.8%) | 10 (90.9%) | $\chi^2 = 6.2$ | 0.013* |
| Disease duration (years) | 6.1 ± 1.4 (4.0–9.0) | 3.2 ± 0.9 (2.0–5.0) | t = 6.5 | <0.001*** |

***Correlation Between Serum Fetuin-A and Laboratory Variables in the Conservative-Treatment CKD Group***

Laboratory variables and serum Fetuin-A levels in the conservative-treatment CKD group are correlated in Table 9. The serum Fetuin-A showed a significant negative correlation with the blood urea ($r = -0.74$, $p = 0.002$) and serum creatinine ($r = -0.80$, $p < 0.001$). However, a significant positive correlation was seen between serum Fetuin-A and eGFR ($r = 0.78$, $p < 0.001$).

Serum Fetuin-A levels did not correlate with any of the hematological parameters (all $p>0.05$), glucose levels, or electrolyte levels, and no correlation with lipid profile parameters, liver function tests, serum albumin or prothrombin time was found (all $p>0.05$).

**Table 9.** Correlation between serum Fetuin-A and laboratory variables in the conservative-treatment CKD group (n = 20).

| Parameter | Correlation coefficient (r) | p-value |
|---|---|---|
| Hemoglobin (g/dL) | 0.08 | 0.80 |
| Hematocrit (%) | 0.19 | 0.50 |
| Platelet count ($\times 10^9$/L) | -0.15 | 0.53 |
| White blood cells ($\times 10^3/\mu$L) | 0.12 | 0.69 |
| Fasting blood glucose (mg/dL) | -0.32 | 0.22 |
| 2-hour postprandial blood glucose (mg/dL) | -0.40 | 0.11 |
| Blood urea (mg/dL) | -0.74 | 0.002** |
| Serum creatinine (mg/dL) | -0.80 | <0.001*** |
| eGFR (mL/min) | 0.78 | <0.001*** |
| Sodium (mmol/L) | 0.46 | 0.08 |
| Potassium (mmol/L) | -0.36 | 0.16 |
| Calcium (mg/dL) | -0.26 | 0.35 |
| Phosphorus (mg/dL) | -0.28 | 0.31 |
| Serum uric acid (mg/dL) | 0.25 | 0.37 |
| Total cholesterol (mg/dL) | -0.02 | 0.94 |
| Triglycerides (mg/dL) | -0.14 | 0.65 |
| Total bilirubin (mg/dL) | -0.10 | 0.77 |
| ALT (U/L) | 0.11 | 0.72 |
| AST (U/L) | -0.08 | 0.81 |
| ALP (U/L) | -0.27 | 0.30 |

| | | |
|---|---|---|
| Albumin (g/dL) | -0.18 | 0.52 |
| Prothrombin time (sec) | -0.16 | 0.58 |

*Correlation Between Serum Fetuin-A and Laboratory Variables in the Hemodialysis Group*

The correlation between serum Fetuin-A levels and laboratory parameters in the hemodialysis group is shown in Table 10. Serum Fetuin-A showed a significant negative correlation with serum calcium ($r = -0.45$, $p = 0.042$), total cholesterol ($r = -0.64$, $p = 0.002$), and triglycerides ($r = -0.52$, $p = 0.031$).

There were no significant relationships between the serum Fetuin-A concentration and hemoglobin, hematocrit, platelet count, two-hour postprandial blood glucose, blood urea, serum creatinine, eGFR, sodium, potassium, phosphorus, serum uric acid, total bilirubin, ALT, AST, ALP, albumin, or prothrombin time levels (all $p > 0.05$).

**Table 10.** Correlation between serum Fetuin-A and laboratory variables in the hemodialysis group (n = 20).

| Parameter | Correlation coefficient (r) | p-value |
|---|---|---|
| Hemoglobin (g/dL) | 0.27 | 0.30 |
| Hematocrit (%) | -0.23 | 0.35 |
| Platelet count ($\times 10^9$/L) | 0.15 | 0.56 |
| White blood cells ($\times 10^3/\mu$L) | -0.20 | 0.40 |
| Fasting blood glucose (mg/dL) | -0.03 | 0.91 |
| 2-hour postprandial blood glucose (mg/dL) | -0.07 | 0.80 |
| Blood urea (mg/dL) | -0.41 | 0.085 |
| Serum creatinine (mg/dL) | -0.37 | 0.10 |
| eGFR (mL/min) | 0.01 | 0.96 |
| Sodium (mmol/L) | -0.30 | 0.22 |
| Potassium (mmol/L) | -0.08 | 0.75 |
| Calcium (mg/dL) | -0.45 | 0.042* |
| Phosphorus (mg/dL) | 0.19 | 0.46 |
| Serum uric acid (mg/dL) | 0.20 | 0.44 |
| Total cholesterol (mg/dL) | -0.64 | 0.002** |
| Triglycerides (mg/dL) | -0.52 | 0.031* |
| Total bilirubin (mg/dL) | -0.24 | 0.30 |
| ALT (U/L) | -0.42 | 0.077 |
| AST (U/L) | -0.46 | 0.058 |
| ALP (U/L) | 0.42 | 0.076 |
| Albumin (g/dL) | -0.14 | 0.52 |
| Prothrombin time (sec) | -0.18 | 0.48 |

*Diagnostic Performance of Serum Fetuin-A*

To assess the capacity of Serum Fetuin-A to discriminate between CKD patients and healthy control, receiver operating characteristic (ROC) curve analysis was carried out. Moderate discriminatory power was observed for serum Fetuin-A (AUC: 0.82 (95% CI: 0.700-0.940, $p<0.001$)). The best cut-off value was 490 ng/mL with a sensitivity of 93% and a specificity of 81%.

The results of this ROC analysis should be viewed with some caution, as it involved patients with known CKD and healthy controls. The study population's discriminatory ability demonstrated in

the analysis does not imply clinical diagnostic utility, prediction of disease progression or cardiovascular risk assessment.

Figure 1. The discriminatory ability of serum Fetuin-A in distinguishing chronic kidney disease (CKD) patients (n = 40) from healthy controls (n = 10) was depicted as receiver operating characteristics (ROC) curve. The results of this analysis should be treated as exploratory, as patients with established CKD were compared with healthy individuals, and are not intended to provide evidence of clinical diagnostic utility.

Abbreviations: ALT - Alanine Aminotransferase; AST - Aspartate Aminotransferase; ALP - Alkaline Phosphatase; ALB - Albumin; PT - Prothrombin Time; T. - Total.BIL, total bilirubin; FETA, serum Fetuin-A; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol. Summarized in (Table 11).

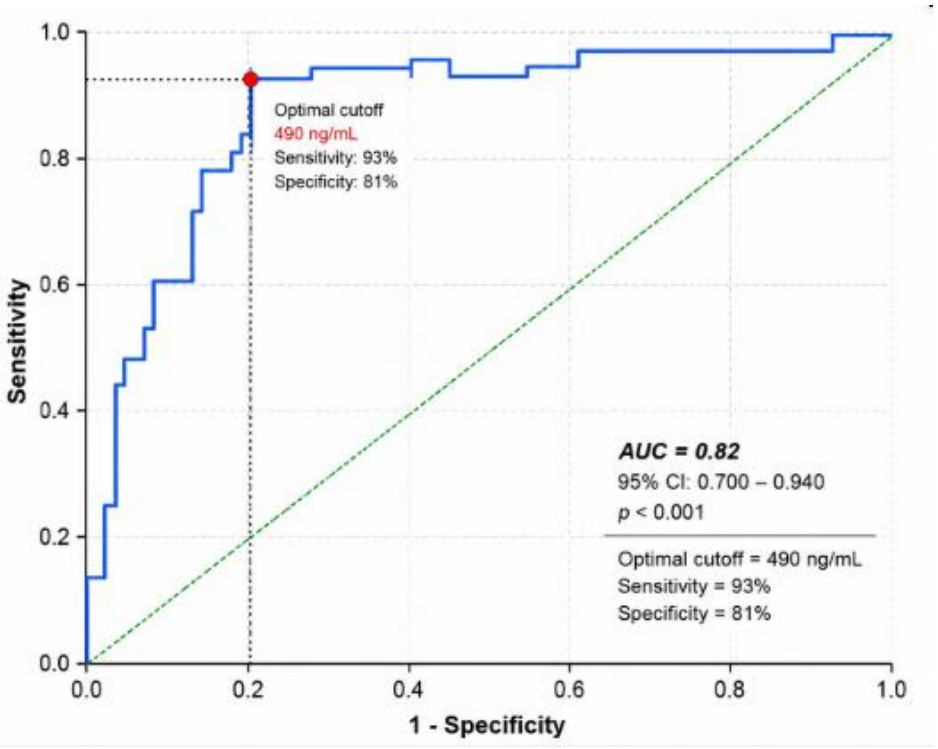


**Figure 1.** Receiver operating characteristic (ROC) curve of serum Fetuin-A for discriminating CKD patients from healthy controls.

**Table 11.** List of abbreviations used in the manuscript.

| Abbreviation | Definition |
|---|---|
| ALT | Alanine aminotransferase |
| AST | Aspartate aminotransferase |
| ALP | Alkaline phosphatase |
| ALB | Albumin |
| PT | Prothrombin time |
| T.BIL | Total bilirubin |
| FETA | Serum Fetuin-A |
| eGFR | Estimated glomerular filtration rate |
| HDL | High-density lipoprotein cholesterol |
| LDL | Low-density lipoprotein cholesterol |

**Discussion**

CKD is a growing public health issue, driven primarily by the growing prevalence of diabetes mellitus, hypertension, and an older population [15]. The results of this cross-sectional study showed that fetuin-A levels in the HD patients were significantly lower than those of the pre-dialysis CKD patients and healthy controls. Levels of serum fetuin-A were also correlated with some renal and biochemical markers such as blood urea, serum creatinine, eGFR, calcium, total cholesterol, and triglycerides. According to this result, fetuin-A might be linked to the renal dysfunction, mineral metabolism, and metabolic changes of CKD patients [11], [16].

Fetuin-A is a circulating glycoprotein which acts as systemic inhibitor of calcium-phosphate precipitation by forming soluble calciprotein particles [17]. Previous clinical and experimental studies have found links between decreased fetuin-A levels and mineral metabolism abnormalities in CKD and end-stages renal disease (ESRD) [10], [18]. However, it is important to emphasize that the present study did not directly measure vascular calcification, arterial stiffness, carotid intima-media thickness, pulse wave velocity, or cardiovascular outcomes. Thus, the results of this study need to be interpreted as biochemical associations only and not as associated vascular disease or cardiovascular risk prediction[19].

The present study showed that the serum fetuin-A level was significantly lower in patients with hemodialysis than in conservative-treatment CKD patients and control group. This observation is in line with the previous reports that the levels of fetuin-A were lower in patients with hemodialysis than in control groups [20], [21]. There are several reasons that have been proposed in the literature, such as modified hepatic production, inflammation, exposure to uremic toxins, and the consumption of fetuin-A in calcium-phosphate complexes [17], [22]. The cross-sectional nature of the present study does not allow for conclusions on whether lower levels of fetuin-A are a result of advanced ckd, dialysis-related factors, inflammation or other metabolic disorders.

In the conservative-treatment CKD group, there were highly significant negative correlations between serum fetuin-A and blood urea and serum creatinine, and a positive correlation between serum fetuin-A and eGFR. These results show that the renal function markers were lower with lower fetuin-A level in this group. Previous observational studies have also shown associations between markers of renal dysfunction and fetuin-A [13]. But at the present design it is not possible to say if this association is positive or negative. Whether impaired kidney function decreases fetuin-A or whether it is a marker of general metabolic and inflammatory changes of CKD is not known.

In the hemodialysis group, there was a significant negative correlation between fetuin-A and calcium in serum. Association should be viewed with caution. The present study did not evaluate vascular calcification or calcium deposition in tissues, although fetuin-A is involved in the biological processes of calcium-phosphate handling and calciprotein particle formation. Thus, the correlation observed between fetuin-A and calcium cannot be considered to be vascular calcification. Other causes may be changes in minerals due to dialysis, dietary intake, medication, secondary hyperparathyroidism or differences in calcium/phosphate balance between hemodialysis patients [23], [24].

Biochemical changes were also significant in the group of hemodialysis patients, as compared to the controls, such as elevated blood urea, serum creatinine, potassium, phosphorus and the dyslipidemic changes. In dialysis patients, the common complications include hyperkalemia and mineral metabolism disturbances [25]. Other factors such as secondary hyperparathyroidism, vitamin D abnormalities and phosphate retention also play a role in mineral imbalance in CKD [26], [27]. The findings of this study were consistent with the biochemical profile for advanced CKD and hemodialysis patients.

The present study also revealed there were significant inverse correlation between serum fetuin-A and total cholesterol and triglycerides in the hemodialysis group. The relationship of fetuin-A to lipid metabolism, insulin resistance and cardiometabolic profiles have been suggested previously [10], [28]. Results from different studies, however, are not always consistent and the relationship between fetuin-A and lipid metabolism could differ by the stage of CKD, diabetes status, inflammation, nutrition

status, and dialysis-related factors. The lipid results from the present study are thus exploratory [29], [30].

Hemoglobin, hematocrit levels were significantly lower in the hemodialysis group than in the controls. This aligns with the burden of anemia in the hemodialysis and CKD patients. Several factors can contribute to anemia in CKD, including decreased production of erythropoietin, chronic inflammation, loss of blood during dialysis, a reduced life span of red blood cells, and disturbances in iron metabolism [31], [32]. Anemia is a part of the clinical burden of CKD, but the present study did not reveal significant correlations between fetuin-A and hemoglobin or hematocrit.

The ROC analysis revealed moderate discrimination between CKD patients and controls for serum fetuin-A. This result must be viewed with caution, however, as both patient and healthy groups had established CKD and normal renal function, respectively. Thus, the finding of differences between these groups is not uncommon and does not necessarily indicate clinical diagnostic value. Future analyses should be more clinically applicable, comparing pre-dialysis and hemodialysis patients, various stages of CKD, or patients grouped by objective measurements of vascular or cardiovascular outcomes.

There are some restrictions which should be noted. First, the study was small-scale, especially in the control group, which decreases the statistical power and generalizability. Second, because of the cross-sectional design, it is impossible to determine causality. Thirdly, several correlations were tested and some significant correlations might have been found due to chance, thus these associations should be regarded as hypothesis-generating. Fourth, the presence of vascular calcification, arterial stiffness, CIMT, pulse wave velocity, cardiovascular events or mortality were not assessed. Finally, renal function was estimated using the Cockcroft–Gault equation, which provides an approximation of creatinine clearance, as opposed to the more recent preferred equation, the CKD-EPI. Yet another shortcoming of the present study is that the control group was younger than the CKD groups. Residual confounding cannot be ruled out due to a possible age-related difference in circulating Fetuin-A concentrations. Larger age-matched control groups should be recruited in future studies to minimize this potential source of bias. A limitation of the present study is the relatively small sample size which could limit statistical power for analyses of subgroups and correlations. These results must be considered exploratory and should be confirmed in larger multicenter studies.

Finally, the present study showed a decrease in the fetuin-A level in HD patients, as well as the relationship between this parameter with some renal, mineral and lipid parameters in Iraqi CKD patients. These results suggest that fetuin-A might be a biomarker related to biochemical changes in CKD. To better understand the clinical role of fetuin-A in CKD, larger prospective studies with direct vascular outcome measures and vascular measurements are needed.

**Conclusion**

In the present study, results showed a significant decrease in the serum fetuin-A level in Iraqi patients with chronic kidney disease who have hemodialysis than in pre-dialysis CKD patients and in healthy controls. Fetuin-A levels were significantly correlated with blood urea and serum creatinine, both negatively, and with estimated glomerular filtration rate (eGFR), positively, in all patients who were treated conservatively suggesting a relationship between the level of fetuin-A and renal function status.

Hemodialysis patients had significantly lower levels of fetuin-A in their serum levels than those of total cholesterol, serum calcium, and serum triglyceride levels. These findings suggest there may be a relationship between fetuin-A and disturbances in mineral metabolism and metabolic changes typical of advanced CKD.

The findings from this study reinforce the validity of serum fetuin-A as a disease severity and biochemical marker, in patients with CKD. However, due to the design of the cross-section and the small number of samples, it is not possible to draw any causal conclusions. In addition, vascular calcification, arterial stiffness, and cardiovascular outcome were not directly evaluated in this study and cannot be inferred in the examined population.

Large scale prospective studies with direct vascular assessment and cardiovascular outcome measures should be performed to further establish the clinical role of fetuin-A in chronic kidney disease.

This is one of the first studies assessing the relation between serum Fetuin-A, renal function, mineral metabolism and lipid profile parameters in Iraqi patients with chronic kidney disease and maintenance hemodialysis to the best of our knowledge. There is limited data available on Fetuin-A in Middle Eastern CKD populations, and there may be geographic variability in the genetic makeup and environmental exposures, nutrition, and healthcare services of population that affect biomarker profiles. Thus, the current study aims to give preliminary regional data about the clinical associations of Fetuin-A in Iraqi patients suffering from CKD.

**REFERENCES**

[1] J. R. Schelling, "Tubular atrophy in the pathogenesis of chronic kidney disease progression," Pediatr. Nephrol., vol. 31, no. 5, pp. 693–706, 2016.

[2] D. G. Matsell, "Congenital urinary tract obstruction—Diagnosis and management in the fetus," in Nephrology and Fluid/Electrolyte Physiology, Elsevier, pp. 391–409, 2019.

[3] W. Ju, V. Nair, S. Smith, L. Zhu, K. Shedden, P. X. Song, et al., "Tissue transcriptome-driven identification of epidermal growth factor as a chronic kidney disease biomarker," Sci. Transl. Med., vol. 7, no. 316, Art. no. 316ra193, 2015.

[4] P. Jirak, L. Stechemesser, E. Moré, M. Franzen, A. Topf, M. Mirna, et al., "Clinical implications of fetuin-A," Adv. Clin. Chem., vol. 89, pp. 79–130, 2019.

[5] P. Aghagolzadeh, M. Bachtler, R. Bijarnia, C. Jackson, E. R. Smith, A. Odermatt, et al., "Calcification of vascular smooth muscle cells is induced by secondary calciprotein particles and enhanced by tumor necrosis factor-$\alpha$," Atherosclerosis, vol. 251, pp. 404–414, 2016.

[6] K. Benz, I. Varga, D. Neureiter, V. Campean, C. Daniel, and K. Amann, "Vascular calcification in chronic kidney disease: The role of inflammation," Int. J. Nephrol., 2018, Art. ID 4310379.

[7] J. A. Leopold, "Vascular calcification: Mechanisms of vascular smooth muscle cell calcification," Trends Cardiovasc. Med., vol. 25, no. 4, pp. 267–274, 2015.

[8] L. Dai, E. Golembiewska, B. Lindholm, and P. Stenvinkel, "End-stage renal disease, inflammation and cardiovascular outcomes," Contrib. Nephrol., vol. 191, pp. 32–43, 2017.

[9] M. Fasshauer and M. Blüher, "Adipokines in health and disease," Trends Pharmacol. Sci., vol. 36, no. 7, pp. 461–470, 2015.

[10] Z. A. Massy and T. B. Drüeke, "Magnesium and outcomes in patients with chronic kidney disease: Focus on vascular calcification, atherosclerosis and survival," Clin. Kidney J., vol. 5, Suppl. 1, pp. i52–i61, 2012.

[11] S. Ossareh, A. Alaei, and D. Saedi, "Carotid intima-media thickness in maintenance hemodialysis patients," Nephrourol. Mon., vol. 3, pp. 138–143, 2011.

[12] M. Sato, Y. Kamada, Y. Takeda, S. Kida, Y. Ohara, H. Fujii, et al., "Fetuin-A negatively correlates with liver and vascular fibrosis in nonalcoholic fatty liver disease subjects," Liver Int., vol. 35, no. 3, pp. 925–935, 2015.

[13] M. Kanbay, D. Goldsmith, M. E. Uyar, F. Turgut, and A. Covic, "Magnesium in chronic kidney disease: Challenges and opportunities," Blood Purif., vol. 29, no. 3, pp. 280–292, 2010.

[14] K. Okasha, A. El Bendary, and A. Mourad, "Evaluation of peripheral vascular calcification and serum magnesium level in Egyptian hemodialysis patients," Arab J. Nephrol. Transplant., vol. 3, no. 1, pp. 11–16, 2010.

[15] F. Turgut, M. Kanbay, M. R. Metin, E. Uz, A. Akcay, and A. Covic, "Magnesium supplementation helps to improve carotid intima-media thickness in patients on hemodialysis," Int. Urol. Nephrol., vol. 40, no. 4, pp. 1075–1082, 2008.

[16] R. C. Shroff, R. McNair, N. Figg, J. N. Skepper, L. Schurgers, A. Gupta, et al., "Chronic mineral dysregulation promotes vascular smooth muscle cell adaptation and extracellular matrix calcification," J. Am. Soc. Nephrol., vol. 21, no. 1, pp. 103–112, 2010.

[17] H. Uedono, M. Fujii, H. Takata, S. Yamada, K. Tsuruya, T. Kitazono, et al., "Effects of fetuin-A-containing calciprotein particles on posttranslational modifications of fetuin-A in HepG2 cells," Sci. Rep., vol. 11, Art. no. 11865, 2021.

[18] N. X. Chen and S. M. Moe, "Pathophysiology of vascular calcification," Curr. Osteoporos. Rep., vol. 13, no. 6, pp. 372–380, 2015.

[19] W. V. de Oliveira Junior, A. P. Sabino, R. C. Figueiredo, D. R. A. Rios, and M. G. Carvalho, "Association between dyslipidemia and CCL2 in hemodialysis patients," Cytokine, vol. 125, Art. no. 154858, 2020.

[20] M. Ketteler, P. Bongartz, R. Westenfeld, J. E. Wildberger, A. H. Mahnken, R. Böhm, et al., "Low fetuin-A levels and cardiovascular mortality in dialysis patients," Lancet, vol. 361, no. 9360, pp. 827–833, 2003.

[21] Z. Zhou, B. Sun, X. Li, and C. Zhu, "Circulating fetuin-A and all-cause mortality in CKD: A meta-analysis," Front. Physiol., vol. 10, Art. no. 966, 2019.

[22] G. Favretto, A. Stasi, C. Divella, R. Franzin, P. Pontrelli, L. Gesualdo, et al., "Uremic endothelial-derived extracellular vesicles: Mechanisms of formation and role in inflammation and oxidative stress," Toxicol. Lett., vol. 347, pp. 12–22, 2021.

[23] A. Galassi, M. Cozzolino, P. Ciceri, and F. Conte, "Current treatment options for secondary hyperparathyroidism in stage 3–4 CKD," Expert Opin. Drug Saf., 2021.

[24] C. L. Lu, J. F. Shyu, T. H. Yen, C. F. Hung, and C. C. Wu, "Emerging role of nutritional vitamin D in secondary hyperparathyroidism in CKD," Nutrients, vol. 10, no. 12, Art. no. 1890, 2018.

[25] G. Toft, U. Heide-Jørgensen, H. van Haalen, G. James, K. Hedman, H. Birn, et al., "Anemia and clinical outcomes in patients with severe chronic kidney disease," J. Nephrol., vol. 33, no. 1, pp. 147–156, 2020.

[26] L. F. Fried, R. A. Kronmal, A. B. Newman, D. E. Bild, M. B. Mittelmark, J. F. Polak, et al., "Renal insufficiency as a predictor of cardiovascular outcomes in elderly individuals," J. Am. Coll. Cardiol., vol. 41, no. 8, pp. 1364–1372, 2003.

[27] A. P. Guérin, G. M. London, S. J. Marchais, and F. Metivier, "Arterial stiffening and vascular calcifications in end-stage renal disease," Nephrol. Dial. Transplant., vol. 15, no. 7, pp. 1014–1021, 2000.

[28] J. H. Ix, M. G. Shlipak, V. M. Brandenburg, S. Ali, M. Ketteler, and M. A. Whooley, "Association between fetuin-A and the metabolic syndrome," Circulation, vol. 113, no. 14, pp. 1760–1767, 2006.

[29] Z. Sun, "Aging, arterial stiffness, and hypertension," Hypertension, vol. 65, no. 2, pp. 252–256, 2015.

[30] M. Temmar, S. Liabeuf, C. Renard, S. Czernichow, N. E. Esper, I. Shahapuni, et al., "Pulse wave velocity and vascular calcification at different stages of chronic kidney disease," J. Hypertens., vol. 28, no. 1, pp. 163–169, 2010.

[31] K.-U. Eckardt, R. Agarwal, A. Aswad, A. Awad, G. A. Block, M. R. Bacci, et al., "Safety and efficacy of vadadustat for anemia in patients undergoing dialysis," N. Engl. J. Med., vol. 384, no. 17, pp. 1601–1612, 2021.

[32] P. E. Pergola, R. N. Woodward, A. Florez, S. Rai, P. Delafontaine, C. Bowers, et al., "Ziltivekimab for treatment of anemia of inflammation in patients on hemodialysis," J. Am. Soc. Nephrol., vol. 32, no. 1, pp. 211–222, 2021.