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Correlation of Osteocalcin and Fetuin-A levels with Renal Function and Other Biomarkers in CKD patients from Baghdad

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Abstract

Background: This investigation aims to determine if a correlation existed between the levels of Osteocalcin, Fetuin-A, and other biochemical markers in patients with CKD compared to healthy.

Methods: One hundred eighty samples divided into 120 (CKD) patients in end-stage divided into 70 males and 50 females, and 60 healthy controls 35 males and 25 females were taken between November 2022 and March 2023 from three major hospitals in Baghdad. diagnosed using symptoms, biochemical testing, history, and clinical examination by specialized nephrologists. Urea, creatinine, calcium, albumin, and phosphate were measured by spectrophotometer (Roche Cobas C111). Furthermore, a spectrophotometer (Beckman coulter au480) was used to measure sodium and potassium levels. On the other hand, vitamin D3 level and PTH were measured by spectrophotometer (Roche Cobas E411). Moreover, Osteocalcin and Fetuin-A were done by ELISA technique.

Results: The present study found that the male patients are more than females, and the highest percentage of CKD cases were found in the age group of 50-59 years. also, indicates a strong positive correlation between the levels of Fetuin-A and GFR. while a strong negative correlation between the levels of Fetuin-A with creatinine, urea, and Osteocalcin, moreover, a strong negative correlation between the level of Osteocalcin with GFR and Fetuin-A. The study also found a strong positive correlation between the levels of Osteocalcin with urea and creatinine.

Conclusion: Decreased Fetuin-A concentrations have been linked to increased calcification in the vasculature and mortality, increased Osteocalcin levels in CKD may not confer a protective effect against bone disorders.

Introduction

Chronic kidney disease (CKD) is characterized by either kidney damage or perhaps an estimated glomerular filtration rate of $\leqslant 60$ ml/min/1.73 m^2, depending on the underlying etiology. A progressive reduction in kidney function characterizes the condition, which requires replacement therapy for the kidneys at the end (transplantation or dialysis). Kidney failure is considered to be diagnosed by pathologic abnormalities detected during imaging studies, kidney biopsy, abnormalities in urine sediment, or high urinary albumin excretion rates [1]. On a global scale, CKD has been regarded as a major public health concern. According to estimates, 13.4% (11.7-15.1%) of individuals worldwide have chronic kidney disease, and between (4.902 to 7.083) million people around the world are likely to need replacement therapy for the kidneys due to end-stage renal disease (ESRD) [2]. Traditional cardiovascular disease (CVD) risk factors and chronic renal disease include aging, hyperlipidemia, diabetes mellitus, hypertension, family history, and male gender. Together with tobacco use, aggressive changes can be made to elevated cholesterol levels, blood pressure, and hyperglycemia. Non-traditional risk factors, such as conditions that alter the metabolism of chemicals including phosphate, calcium, and toxic metabolites produced by uremia, are also what cause increased CVD in CKD patients [3]. The liver produces the blood protein fetuin-A, which is then released into the bloodstream. This human protein was discovered and given the name "2-Heremans-Schmid glycoprotein" (AHSG) in 1960 [4]. This name was given to the serum protein due to the fracture of α-2 globulin in the electrophoresis of cellulose acetate. The Fetuin is a member of a large group of regulatory binding proteins that control the availability and transport of various cargo substances inside the circulation. The fetuin-A gene is located on human chromosome 3q27. A plasma protein called fetuin-A has domains that mimic cystatin. A cystatin-1 domain on fetuin-A has a strong negative charge and a strong propensity to bind to calcium ions [5].

Osteocalcin (OC), a hormone produced by osteoblasts and dependent on vitamin K for its activity, affects the activity of both osteoclasts and osteoblasts and controls the energy metabolism of lipids, glucose, and bones [6]. Studies have shown that endothelial function & vascular calcification both are related to osteocalcin & vascular health as a biomarker of bone turnover[7]. Important vascular biological influences such as uremia and inappropriate mineral metabolism affect the development of cardiovascular disease (CVD) and increase the mortality and morbidity of CKD patients [8]. Recent studies looking into the impact of osteocalcin on the vascular system, especially endothelial function, and vascular wall characteristics, have generated significantly different results. Many investigations have reported higher circulating totals Osteocalcin controls metabolic parameters, which indirectly affects the health of the vascular system [9]. This is the first study that focused on the levels of Fetuin-A and osteocalcin in CKD patients in Iraq.

Methods

The current investigation took place between November 2022 and March 2023 at three major healthcare centers in Baghdad: Baghdad Teaching Hospital/the Iraqi Center for Dialysis, the National Center for Teaching Laboratories (NCFTL), and Ghazi al-Hariri Surgical Specialties Hospital/Kidney Diseases and Transplantation Center. We selected (120) patients with stage (G5) chronic renal failure of which (70) were men and (50) were women, with ages ranging from (30 to 72) years. They were diagnosed with chronic kidney failure based on previous medical records, laboratory investigations, and clinical observations by specialist nephrologists. To compare the results of those patients with the control group of (60) healthy people aged (30-72) years, (35) men, and (25) females, Healthy adults without a history of hypertension, kidney disease, or kidney stones were chosen as the control group. Using a disposable syringe, a five ml sample of blood was drawn from each patient and the control. After separation, the serum, biochemical, and electrolyte tests are directly done. Were An automated spectrophotometer (Roche Cobas C111). ware used to evaluate the levels of calcium, phosphate, albumin, urea, and creatinine. Using an automated spectrophotometer potassium, and sodium were measured by (Beckman coulter Olympus AU480). An automated spectrophotometer was used to measure the levels of vitamin D, and PTH by (Roche Cobas E411). While using the ELISA technique Fetuin-A, and Osteocalcin was measured (All ELISA researcher kits were ordered from Mybiosource in the United States).

Statistical analysis

The data were reviewed, coded, and analyzed using the "Statistical Package of Social Science (SPSS) version 26.0". To display data, using Tabular data presentation (Complex frequency distribution table), Math presentation techniques (Mean and standard error), and the T-test for independent samples, The simple correlation coefficient (r). A p-value <0.05 was considered statistically significant.

Results

baseline features of the current study participants according to age and sex, and a comparison of their significance. Table 1 shows that the highest percentage

of chronic kidney disease (CKD) cases were found in the age group of 50-59 years, with 55 out of 120 cases (45.8%). This was followed by the age group of 60-72 which had 30 cases (25.0%). The age group of 30-39 years had 18 cases (15.0%), while the age group of 40-49 years had the lowest number of cases with 17 cases (14.2%) out of the total 120 cases. Table 1 also indicates that the percentage of females with CKD was higher in the age group of 50-59 years, with 30 out of 50 cases (60.0%) compared to males in some age groups, where only 25 out of 70 males (35.7%) had CKD. This gender difference was found to be significant (S) in the case group (p=0.029), and highly significant (HS) in the control group (P≤0.001). The study compared the Glomerular filtration rate (GFR) between the chronic kidney patient group and the control in Table 2. The results showed that the control group had a mean GFR of 92.58±1.98ml/min, while the chronic kidney disease group had a significantly lower mean GFR of 5.61±0.20ml/min. The difference in mean GFR among the two groups was highly significant with a P-value of ≤0.001. The study found that out of 120 CKD patients, 37 cases (30.8%) were overweight with a BMI of 25-29.9 (Kg/m2), 18 cases (15.0%) suffered from obesity with BMI values >30 (Kg/m2), 57 cases (47.5%) had normal weight with BMI values between 18.5 to 24.9 (Kg/m2), and 8 cases (6.7%) were underweighted. These differences in the frequency and percentage of BMI were highly significant with a Chi-squared value of 13.31 and a P-value of 0.004 as shown in Table 3. The findings of this research showed there were large variations in the levels of PTH, Fetuin-A, and Osteocalcin between CKD patients and the control. The p-values associated with these differences are all ≤0.001, indicating that the differences are highly statistically significant as shown in Table 4. According to the results of the current study, it can be deduced that there is a significant positive correlation between the levels of Fetuin-A and GFR, as indicated by Pearson's Correlation Coefficient (r=.810**) and p-value of ≤0.001. There is also a moderate positive correlation between the levels of Fetuin-A with S. Calcium, and Albumin with (r=.457**, .611**) and p-value of ≤0.001, respectively. On the other hand, there is a weak positive correlation between the levels of Fetuin-A with BMI and Vit-D with (r=0.037, .298) and p-values of 0.62 and ≤0.001, respectively. This suggests that there is a very small positive association between the level of Fetuin-A and BMI and a slightly stronger positive relationship between the levels of Fetuin-A and Vit-D. Furthermore, the levels of Fetuin-A were found to be moderately negatively correlated with the levels of PTH and S.po4 with (r=-.507, -.483**) and p-value of ≤0.001, respectively. The study also found that there is a strong negative correlation between the

levels of Fetuin-A with urea, Creatinine, and Osteocalcin with (r=-.743**, -.705**, -.677) and p-values of ≤0.001, respectively. As arranged in Table 5. According to the results of the current study, it can be indicated that there is a strong negative correlation between the levels of Osteocalcin with GFR and Fetuin-A with (r=-.794**, -.677**) and p-value of ≤0.001, respectively. There is also a moderate negative correlation between the levels of Osteocalcin with S. Ca and S. albumin with (r=-.426**, -.470**) and p-value of ≤0.001, respectively. This suggests that as the levels of Osteocalcin increase, the levels of S. Ca and S. albumin decrease. Furthermore, there is a weak negative correlation between the levels of Osteocalcin with BMI and Vit-D with (r=-.126, -.321) and p-values of 0.092 and ≤0.001, respectively. This suggests that there is a very small negative correlation between the level of Osteocalcin and BMI and a slightly stronger negative correlation between the levels of Osteocalcin and Vit-D. Moreover, the study found that there is a moderate positive correlation between the levels of Osteocalcin with S.po4 and PTH with (r=.469**, .490**) and p-value of ≤0.001, respectively. The study also found that there is a strong positive correlation between the levels of Osteocalcin with urea and creatinine, as indicated by the Pearson correlation coefficient (r=.749**, .720**) respectively. As arranged in Table 6.

Age range (Years)	Cases (n=120)		Control (n=60)	
	Males	Females	Males	Females
(30-39)	10 (14.3%)	8 (16.0%)	2 (5.7%)	9 (36.0%)
(49-49)	12 (17.1%)	5 (10.0%)	12 (34.3%)	0 (0.0%)
(50-59)	25 (35.7%)	30 (60.0%)	18 (51.4%)	9 (36.0%)
(60-72)	23 (32.9%)	7 (14.0%)	3 (8.6%)	7 (28.0%)
Total	70 (100.0%)	50 (100.0%)	35 (100.0%)	25 (100.0%)
p-value	0.029 (S)		≤0.001 (H.S)	

Table 1: Distribution of Studied groups according to Age groups (Years).

Test	Study Groups	Mean	Std. Error Mean	T-test	*p*-value
GFR	Case	5.61	0.20	43.57	≤0.001
	Control	92.58	1.98		

Table 2: Determination of GFR between the chronic kidney disease patients' group and control groups.

BMI (Kg/m²)	Cases (n=120)	Controls (n=60)	Total	
<18.5	8 (6.7%)	0 (0.0%)	8 (4.4%)	Chi-square =13.13
18.5-24.9	57 (47.5%)	17 (28.3%)	74 (41.1%)	
25-29.9	37 (30.8%)	32 (53.3%)	69 (38.3%)	
>30	18 (15.0%)	11 (18.3%)	29 (16.1%)	*p*-valu e=0.004 (H.S)
Total	120(100.0%)	60 (100%)	180 (100%)	

Table 3: Distribution of the BMI (Kg/m2) among patients and controls.

Test	Study Groups	Mean	Std. Error Mean	T-test	*p*-value
PTH	Case	387.42	23.67	14.83	≤0.001 (H.S)
	Control	35.81	1.305		
Fetuin-A	Case	2.61	0.038	15.90	≤0.001 (H.S)
	Control	5.16	0.156		
Osteocalcin	Case	32.05	0.45	19.20	≤0.001 (H.S)
	Control	17.84	0.58		

Table 4: Comparative the levels of PTH, Fetuin-A, and Osteocalcin between case and control.

Parameter	r	P-value	Correlation status
GFR	.810**	≤0.001	Strong positive correlation
S. Ca	.457**	≤0.001	Moderate positive correlation
S. albumin	.611**	≤0.001	
BMI (Kg/m2)	0.037	0.62	Weak positive correlation
Vit-D	.298**	≤0.001	
S.po4	-.483**	≤0.001	Moderate negative correlation
PTH	-.507**	≤0.001	
B. urea	-.743**	≤0.001	Strong negative correlation
S. Creatinine	-.705**	≤0.001	
Osteocalcin	-.677**	≤0.001	

Table 5: Correlation between the Fetuin-A levels with studied parameters of patients and control.

Parameter	r	P-value	Correlation Status
GFR	-.794**	≤0.001	Strong negative correlation
Fetuin-A	-.677**	≤0.001	
S. Ca	-.426**	≤0.001	Moderate negative correlation
S. albumin	-.470**	≤0.001	
BMI (Kg/m2)	-.126	.092	Weak negative correlation
Vit-D	-.321**	≤0.001	
S. po4	.469**	≤0.001	Moderate positive correlation
PTH	.490**	≤0.001	
B. urea	.749**	≤0.001	Strong positive correlation
S. creatinine	.720**	≤0.001	

Table 6: Correlation between the Osteocalcin levels with studied parameters of patients and controls.

Discussion

The result of the current study suggests that age and gender are important factors to consider in the incidence of CKD, with higher percentages of cases found in the age group 50-59 and a higher prevalence among females in certain age groups. The findings of the current study align with those of a previous study conducted by [10], which concluded that men tend to experience a more rapid decrease in kidney function compared to women. This may be due to unhealthy lifestyle choices made by men as well as the potential protective effects of estrogen or the harmful effects of testosterone. According to [11] research, individuals between the ages of 50 and 60 had the highest incidence of chronic kidney disease, and within this age group, women were more likely to be affected than men. This finding is consistent with previous research by [12, 13] which reported that individuals over the age of 60 accounted for 25.8% of all cases of CKD. A decline in the GFR is the hallmark feature of CKD. According to the results of the current study, those who had chronic renal disease had significantly lower GFR, as seen in Table 2. The study's findings appeared to be similar to previous research [14, 15] and [16] from Iraq that showed people who had CKD had significantly lower mean GFR. This study suggests that being overweight or obese is less common among CKD patients, with the majority of patients having a normal BMI as shown in Table 3. However, it is important to remember that this research only examined BMI as a measure of weight status and did not consider other factors such as body composition or distribution of body fat, which may also influence the development of CKD. The reason for the

male-specific link between a higher BMI and CKD is still unknown, even though many studies have demonstrated that BMI more accurately reflects visceral fat in men than in women [17]. Obesity, as indicated by a high BMI has been recognized as a CVD risk factor in the general population. Obesity is associated with a higher chance of CKD and ESRD incident development [18]. The current study shows elevated PTH in CKD patients with high significance as shown in Table 4. The study by [19] supports this study and demonstrates that the primary regulator of PTH secretion is plasma calcium levels. Hypocalcemia and hyperphosphatemia are brought on by vitamin D inactivation in the kidneys as a result of CKD. In consequence, the parathyroid gland becomes more cellular and produces more parathyroid hormone, which can result in secondary hyperparathyroidism (SHP). Furthermore, the research by [20] supports this study and suggests that the main cause of phosphate concentrations is the release of parathyroid hormone (PTH). A similar investigation by [21] revealed that higher phosphate levels enhance parathyroid hormone (PTH) release while reducing vitamin D activation. Plasma levels of urea and creatinine are raised in people with advanced CKD (p≤0.001). Their results resembled those of the other investigations [16, 22]–[24] are just a few examples. Blood urea and serum creatinine are two crucial solutes that the kidneys eliminate from the body, according to [25, 26] They also showed that the first organic solvent found in the blood of people with CKD was urea. The findings of the current study agree with the results of the study by [27] which suggest levels of albumin were highly and independently correlated with a decrease in renal function in patients with CKD. According to the results of the current study, the mean S. calcium level was slightly lower in CKD patients. The study by [28] confirms the findings of this study that a decrease in serum calcium was correlated to a faster reduction in renal function. Vitamin D is slightly reduced, and the GFR usually declines when the creatinine level increases, based on a study by [29]. Another study by [21] revealed that higher phosphate levels enhance parathyroid hormone (PTH) release while reducing vitamin D activation. According to a study by [30], individuals with CKD have a positive phosphorus balance, which shows that a rise in phosphate is caused by the kidneys' inability to eliminate it. This finding is consistent with the current study. Compared with the control, the current research revealed a reduction in Fetuin-A and an elevation in osteocalcin levels in CKD patients. The present research agrees with a study by [31] which showed that the fetuin-A levels were considerably lower in those suffering from CKD while these levels decreased significantly as the disease

progressed. Further research supports the findings by [32-34] suggested that the primary cause of the development of calciphylaxis could result from a fetuin-A deficiency, which involves alteration in tiny arterioles in hemodialysis patients. Fetuin-A deficiency is considered in these patients to be an independent risk factor for an elevation in cardiovascular-related mortality [35]. Increased blood levels of OC in CKD patients may be related to either rapid bone metabolism or decreased renal clearance [36]. OC, which is regarded to be the primary cause of atherosclerosis progression, had significantly different levels in chronic renal disease patients, according to [37]. Patients with stage I/II CKD had low OC levels, whereas those with stage 5/dialysis had the highest levels. Another study by [38] that found OC had a positive association with PTH but just a negative association with both BMI and eGFR concurs with the current study.

Fetuin-A and Osteocalcin are two proteins that have been studied with CKD and its associated complications. While decreased Fetuin-A concentrations have been linked to increased calcification in the vasculature and mortality, increased Osteocalcin levels in CKD may not confer a protective effect against bone disorders. Further investigation is required to fully understand the roles of these proteins in CKD and to develop strategies for their clinical Use in the managing of CKD patients.

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Author Contributions

Wissam Jabbar Hussein: the research article proposal, experiment design, explaining the findings, and article writing.
Dr. Hiba Abid Al-Hussein Hassan: Supervision, Data analysis, proof editing, Review and editing.
Abbas Muhsin Gata: Supervision, methodology, Review and editing.

Competing Interests

The authors declared that there were no conflictsof interest.

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