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ORIGINAL ARTICLE

Serum Dickkopf-3 Level in Chronic Kidney Disease Patients and Its Association with Cardiovascular Disease

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ABSTRACT

Background: Dickkopf-3 (DKK-3) has garnered interest as a potential biomarker for diagnosing and monitoring kidney and cardiovascular diseases (CVDs). Herein, we aimed to evaluate the level of DKK-3 in individuals with chronic kidney disease (CKD) and detect its correlation with CVD.

Patients and methods: The study involved 68 cases categorized into four groups. The study protocol involved conducting a comprehensive medical history assessment, a standard physical examination, laboratory investigations, and an enzyme-linked immunosorbent assay (ELISA) to quantify the serum DKK3 levels.

Results: Our findings indicated a significant elevation in serum DKK-3 levels among CVDs patients who suffer from renal impairme nt and those with end-stage renal disease who were on dialysis. Additionally, our outcomes revealed a significant positive correlation between DKK-3 and various biomarkers levels, including SGPT ($r = 0.336$, $p = 0.005$), parathyroid hormone ($r = 0.425$, $p < 0.001$), creatinine ($r = 0.62$, $p < 0.001$), total cholesterol ($r = 0.312$, $p = 0.01$), low-density lipoprotein cholesterol (LDL-C) ($r = 0.268$, $p = 0.027$), and triglycerides ($r = 0.256$, $p = 0.035$). Our results indicate a significant negative correlation between DKK-3 levels and both eGFR ($r = -0.507$, $p < 0.001$) and hemoglobin levels ($r = -0.33$, $p = 0.006$). Additionally, an independent association was observed between the serum levels of DKK-3 and creatinine, LDL-C, and LVEDD.

Conclusion: DKK-3 may serve as a novel biomarker for the progression of CKD. Moreover, DKK3 can potentially function as a biomarker for CVD among individuals with renal disease.

Keywords: CVD; DKK3; CKD.

INTRODUCTION

Chronic kidney disease (CKD) is a medical condition characterized by structural or functional abnormalities of the kidneys that persist for three months or more and can have detrimental impacts on an individual's overall health. The diagnosis of CKD is associated with intensified risks of cardiovascular disease, all-cause mortality, acute kidney injury, and progression of CKD [1].

Dickkopf-3 (DKK3) is a type of circulatory protein secreted and belongs to the Dickkopf-3 family. The Wnt signaling pathway is implicated in diverse physiological phenomena, including embryonic morphogenesis, cardiovascular pathogenesis, and oncogenesis. The regulation of the pathway is primarily governed by DKKs, with a particular emphasis on DKKs 1, 2, and 4 [2]. The complete elucidation of the precise role of DKK3 in the signaling cascade of the Wnt pathway remains to be accomplished. It has been suggested that DKK3 may be implicated in CVD. Research utilizing animal models that are transgenic or knockout for DKK3 has indicated that DKK3 may have a cardioprotective effect while also potentially causing harm to the kidneys [3]. The DKK3 protein has recently attracted attention as a biomarker for kidney and CVD development. In a cohort from the general population, plasma levels of DKK3 were found to be adversely linked to the beginning of atherosclerosis [4].

The enhancement of current risk prediction models for renal function loss was observed in individuals

with pre-existing CKD who exhibited elevated urine DKK3 levels at baseline, in accordance with reference [5]. In addition, it was found that preoperative levels of DKK3 in urine were indicative of the occurrence of acute kidney injury (AKI) after surgery and a higher probability of experiencing a decline in renal function in the long term among patients who underwent cardiac surgery [6]. This investigation aimed to assess the correlation between DKK3 level and cardiovascular disease in individuals diagnosed with CKDs.

PATIENTS AND METHODS

All the participants gave their written consent after receiving comprehensive details. The Institutional Review Board of Zagazig University approved the study protocol, with the reference number IRB # 9669-14-7-2022. The study was conducted in adherence to the guidelines outlined in the Declaration of Helsinki. The research, a comparative cross-sectional study, involved 68 individuals ages over 18 and of both sexes, separated into four groups with 17 cases in each group, and was conducted in the nephrology and dialysis units of Zagazig University Hospitals between August 2022 and March 2023. The study encompassed four distinct groups: Group I, which comprised 17 patients diagnosed with predialysis CKD and CVD, and Group II, which consisted of 17 patients diagnosed with predialysis CKD without CVD. Group III consisted of 17 individuals with CKD on hemodialysis with CVD, while Group IV consisted of 17 individuals who underwent hemodialysis for CKD but did not exhibit any signs of CVD. The presence of CKD was determined in each case examined using the 2012 Kidney Disease Improving Global Outcomes (KDIGO) criteria. The CKD EPI method was utilized to calculate the estimated glomerular filtration rate (eGFR) based on the input of blood creatinine, sex, and age.

Inclusion criteria: The inclusion criteria for this study comprise individuals of both genders who are above 18 years of age. The study involved the formation of four distinct cohorts of patients suffering from CKD, with and without comorbid CVDs. **Exclusion criteria:** The study's exclusion criteria comprise a medical history of prior usage of immunosuppressive medications, presence of an active form of inflammation, acute diseases, diagnosis with liver diseases, encompassing both acute and chronic conditions, as well as any previous exposure to radiation or chemotherapy for malignancy.

Methods: The study involved conducting a comprehensive medical history assessment, a routine physical examination, and laboratory analysis of triglycerides, total serum cholesterol, high-density lipoprotein cholesterol (HDL-C), and LDL-C, and complete blood count (CBC) for all participants. This study aims to assess the lipid profile of serum samples through the utilization of an enzyme colorimetric test, specifically the cobas6000. Renal function assessment was achieved by measuring blood urea and serum creatinine levels. The eGFR was using the Modification of Diet in Renal Disease (MDRD). The MDRD formula 186 × (plasma Cr in umol/L × 0.011312) 1.154 × age-0.203 × 1.210 if black × 0.742 if the female (Uche and Osegbe, 2017).

The study included assessments of liver function tests, as well as measurements of serum calcium, phosphorus, and parathyroid hormone. The present study employed the Human DKK-3 ELISA Kit to measure serum DKK3 levels using the ELISA technique.

Test principle: The methodology utilized for the quantification of DKK-3 levels involved a double-antibody sandwich ELISA, employing a kit with a detection range of 0.2 to 60 ng/mL. Following the coating of a monoclonal antibody enzyme well with a human DKK3 monoclonal antibody, the addition of DKK3 was carried out, followed by an incubation period. Subsequently, we incorporated biotin-labeled DKK3 antibodies with a streptavidin-HRP mixture to generate an immunological complex. To remove the uncombined enzyme, we repeated the washing and incubation procedures. Upon addition of Chromogen Solution A or B, the liquid underwent a color change to blue, followed by a subsequent transition to yellow upon exposure to acid. The content of the human substance DKK3 in the sample and the color chroma were positively associated.

All patients had standard 12-lead electrocardiogram recordings during rest and stress to capture sinus rhythm. The 12-channel device was used to record electrocardiograms with a specification of 25 mm/s for a paper speed of 10 mm/mv. When modifying the speed or amplitude of the lead-12 ECG for the diagnosis of arrhythmias, we focused on the quality of the ECG rather than just the speed or amplitude. We also looked at the device's automatic heart rate detection. To prevent cardiac arrest and changes in volume status caused by hemodialysis, echocardiography was performed on dialyzed patients at least 24 hours after dialysis.

STATISTICAL ANALYSIS

Data were collected using SPSS (Statistical Program for the Social Sciences) version 26. analysis. When necessary, the chi-square, fisher exact, and Monte Carlo tests were used to compare

categorical variables. Categorical variables were presented using their absolute frequencies. We used the Shapiro-Wilk test to validate the assumptions employed in parametric tests. The means, standard deviations, medians, and interquartile ranges of the quantitative variables were used to describe them depending on the data type. To compare quantitative data between two groups, the Mann-Whitney test (for data that are not normally distributed) was created. The strength and direction of the link between two continuous variables were assessed using Spearman rank correlation coefficients for data that were not normally distributed. Quantitative data from two groups were compared using the Kruskal-Wallis test for irregularly distributed data and the one-way analysis of variance (ANOVA) test for normally distributed data. Pairwise comparison and LSD comparison were used to find differences between the two groups when the difference was significant. The best cut-off value for a particular quantitative measure was determined using the ROC curve to diagnose a particular medical condition. There was a linear regression analysis to quantify the associated independent components for the dependent factor. The level of statistical significance was established at P 0.05. There was a noticeable difference if $p \leq 0.001$.

RESULTS

Table 1 illustrates that no statistically significant differences were observed in gender, age, and body mass index among the groups under investigation. No statistically significant difference was observed between the compared groups regarding concurrent diabetes or hypertension. The study found significant statistical differences among the research groups with respect to the incidence of CVDs**. Table 2** demonstrates that ECG incidence did not differ statistically significantly from one group to the next. Abnormalities suggest bundle branch block, left ventricular hypertrophy, atrial enlargement, or ischemic heart disease in the study groups. Regarding the existence of evidence of echo findings, there was a statistically significant difference between the groups under study for ischemic heart disease, left ventricular hypertrophy, and systolic dysfunction. There was no difference between the study groups regarding the presence of additional echo findings suggestive of AR, TR, MR, AS, pulmonary hypertension, or pericardial effusion. There was a statistically significant difference in the ejection fraction between the groups under investigation. (on LSD comparison, the difference between groups is significant except when comparing groups I and III). There were statistically significant differences between the study groups regarding IVSD (on LSD comparison, the difference is significant between every two groups except when comparing groups I, II, and III). Between the studied groups, there was a statistically significant difference in LVEDD. (on LSD comparison, the difference is significant between every two groups except when comparing groups I and II). Between the studied groups, there was a statistically significant difference in LVESD. (on LSD comparison, the difference is significant between group III and both II and IV). Regarding PWD, there was a statistically significant difference between the studied groups. (on LSD comparison, the difference is significant between group IV and each other group).

Table 3 reveals statistically significant differences between the study groups in SGOT and serum creatinine. On the LSD comparison, there is a significant difference between groups, except for groups I and II. Between the studied groups, there is a statistically significant difference in SGPT. On the LSD comparison, there is a significant difference between each pair of groups, except for groups I and III. The study revealed a statistically significant variance in eGFR among the groups being examined. A significant distinction has been observed between Groups II, III, and IV subsequent to undergoing the LSD test. Furthermore, significant differentiation is present between Group I and both Groups III and IV. The results of the study suggest that no statistically significant disparity was observed among the groups examined in relation to the serum urea levels. A statistically significant difference was observed in the total cholesterol levels among the groups that were examined. The findings from the pairwise comparisons demonstrate significant dissimilarities among the groups, except for the comparisons encompassing groups II, I, and IV. The results indicate that there is a significant difference in LDL-C levels among the study groups. A significant distinction is observed in the pairwise comparison between groupings I and II. Moreover, significant variations are present between the second and third, as well as the fourth. The results of the study suggest that there were no statistically significant variations detected among the analyzed cohorts in terms of HDL cholesterol or triglycerides. A statistically significant difference in serum calcium was observed among the study groups. A significant contrast is observed between groups I and III, as well as among group IV and every other group, in relation to LSD. The study results indicate a statistically significant difference in parathyroid hormone levels between

the tested groups. A significant disparity is evident in the pairwise comparison of groups II and IV. Moreover, there is a significant difference between groups I and IV. With respect to serum phosphorus, the study groups exhibit a statistically insignificant disparity. A statistically significant variation in hemoglobin levels was observed among the study groups. There is a significant difference between Group III and other groups in comparison to LSD. Additionally, statistically significant variations existed in the serum DKK-3 levels among the groups under investigation. The pairwise comparison results indicate a statistically significant difference between all groups except for groups I and IV.

Statistically speaking, there was a strong association between DKK-SGPT, parathyroid hormone, serum creatinine, level 3, total cholesterol, LDL-C, and triglycerides are all present. The relationship between DKK-3, hemoglobin, and e-GFR is negatively correlated and statistically significant. A non-significant link exists between DKK-3 and other parameters studied **(Table 4)**.

Table 5 shows the correlation between serum DKK3 levels and various echocardiographic parameters. Data suggest that there is a significant positive correlation between serum DKK3 levels and IVSD (r=0.419, p<0.001), LVEDD (r=0.311, p=0.013), and PWD (r=0.317, p=0.011); additionally, there is a significant negative correlation between serum DKK3 levels and ejection fraction (r=-0.481, p<0.001). However, there was no statistically significant correlation between serum DKK3 levels and LVESD (r=-0.223, p=0.079).

Among factors significantly correlated with serum DKK-3, serum creatinine (unstandardized β=1.534, p<0.001), LDL-C (unstandardized β=0.079, p<0.001), and LVEDD (unstandardized β=-0.19, p=0.016) **(Supplementary Table 1)**.

Figure 1. A statistically significant relationship (p < 0.001) between cardiovascular disease and serum DKK-3 among renal patients (Significantly higher in patients with cardiovascular disease).

Figure 2. A statistically significant relationship (p < 0.001) between the presence of end-stage renal disease and serum DKK-3 among renal patients (Significantly higher in patients on dialysis).

Supplementary Table 2 shows that the best cut-off point for serum DKK-3 in the diagnosis of end-stage renal disease is ≥15.2615 with the area under curve 0.835, sensitivity 76.5%, specificity 73.5%, positive predictive value 74.3%, negative predictive value 75.8% and overall precision 75% (p<0.001). The best cut-off of serum DKK-3 in the diagnosis of cardiovascular disease is ≥15.67 with the area under curve 0.835, a sensitivity of 70.6%, specificity of 73.5%, positive predictive value of 72.7%, negative predictive value of 71.4% and overall accuracy 72.1% (p<0.001). The best cut-off of serum DKK-3 in the diagnosis of cardiovascular disease in end-stage renal disease is ≥22.05 with the area under curve 0.912, sensitivity 94.1%, specificity 82.4%, positive predictive value 84.2%, negative predictive value 93.3% and overall precision 88.2% (p<0.001) **Supplementary figure 1**.

Table (1): Comparison between the studied groups regarding demographic data and comorbidities:

	Group I	Group II	Group III	Group IV	χ2	P
	N=17(%)	N=17(%)	N=17(%)	N=17(%)		
Gender:					4.99	0.173
Female	7 (41.2%)	12 (70.6%)	12 (70.6%)	8 (47.1%)		
Male	10 (58.8%)	5 (29.4%)	5 (29.4%)	9 (52.9%)		
	Mean ± SD	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	**F**	**p**
Age	47.0 ± 17.69	50.29 ± 13.89	54.41 ± 15.15	53.65 ± 10.14	0.938	0.427
BMI	23.94 ± 2.93	23.24 ± 3.01	21.65 ± 2.29	23.41 ± 3.87	1.752	0.165
Hypertension	9 (52.9%)	15 (88.2%)	10 (58.8%)	12 (70.6%)	5.644	0.13
Diabetes	8 (41.7%)	7 (41.2%)	2 (11.8%)	8 (47.1%)	MC	0.118
CVD	0 (0%)	17 (100%)	0 (0%)	17 (100%)	MC	<0.001**

F One Way ANOVA test χ2 Chi-square test
BMI: Body Mass Index
CVD: Cardiovascular Disease
MC: Monte Carlo test
SD: Standard Deviation
**p≤0.001 is statistically highly significant

Table (2): Comparison between the studied groups regarding ECG and Echocardiographic findings:

	Group I		Group III		χ^2	p
	N=17(%)		N=17(%)			
AF	3 (17.6%)		0 (0%)		Fisher	0.227
Atrial enlargement	2 (11.8%)		5 (29.4%)		Fisher	0.398
IHD	7 (41.2%)		13 (76.5%)		3.441	0.064
LVH	4 (23.5%)		9 (52.9%)		Fisher	0.157
BBB	4 (23.5%)		9 (52.9%)		Fisher	0.157
	Group I	**Group II**	**Group III**	**Group IV**	χ^2	**p**
	N=17(%)	**N=17(%)**	**N=17(%)**	**N=17(%)**		
IHD	10 (58.8%)	-	0 (0%)	-	Fisher	<0.001**
Systolic dysfunction	0 (0%)	-	5 (29.4%)	-	Fisher	0.044*
LVH	2 (11.8%)	-	10 (58.8%)	-	Fisher	0.01*
MR	2 (11.8%)	-	8 (47.1%)	-	Fisher	0.057
TR	3 (17.6%)	-	3 (17.6%)	-	0	>0.999
AS	1 (5.9%)	-	2 (11.8%)	-	Fisher	>0.999
Portal hypertension	5 (29.4%)	-	2 (11.8%)	-	Fisher	0.398
AR	2 (11.8%)	-	1 (5.9%)	-	Fisher	>0.999
Pericardial effusion	0 (0%)	-	2 (11.8%)	-	Fisher	0.485
LVDD	9 (52.9%)	-	9 (52.9%)	-	0	>0.999
	Mean ± SD	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	**F**	**p**
EF	43.94 ± 14.82	68.53 ± 6.71	46.71 ± 8.04	56.76 ± 11.14	18.65	<0.001**
LSD	P1 <0.001**	P2 <0.001**	P3 0.008*	P4 0.452	P50.001**	P6 0.002*
IVSD	9.19 ± 1.71	8.59±1.5	10.36±1.55	15.2±4.0	24.058	<0.001**
LSD	P1 0.477	P2 0.046*	P3 0.001**	P4 0.189	P50.001**	P60.001**
LVEDD	52.77±12.66	56.47±10.39	45.21 ± 5.19	65.47±10.76	9.752	<0.001**
LSD	P1 0.299	P2 0.004*	P3 0.001**	P4 0.047*	P50.001**	P60.017*
LVESD	33.85±16.96	42.65±12.56	26.57±10.14	40.93±19.79	3.433	0.023*
LSD	P1 0.1	P2 0.005*	P3 0.015*	P4 0.195	P50.198	P60.754
PDW	9.18 ± 1.73	8.41 ± 2.53	9.42 ± 2.17	15.33 ± 3.58	25.723	<0.001**
LSD	P1 0.774	P2 0.572	P3 0.001**	P4 0.77	P50.001**	P60.001**

χ^2 Chi-square test *p<0.05 is statistically significant **p≤0.001 is statistically highly significant
p1 difference between group I and II p2 difference between group II and III
p3 difference between group III and IV p4 difference between group I and III
p5 difference between group I and IV p6 difference between groups II and IV
F One Way ANOVA test LSD least significant difference
AF: Atrial Fibrillation
HD: Ischemic Heart Disease LVH: Left Ventricular Hypertrophy
BBB: Bundle Branch Block MR: Mitral Regurgitation
TR: Tricuspid Regurgitation
AS: Aortic Stenosis
AR: Aortic Regurgitation
LVDD: Left Ventricular Diastolic Dysfunction
Fisher: Fisher's Exact Test
EF: Ejection Fraction
IVSD: Interventricular Septal Thickness
LVEDD: Left Ventricular End-Diastolic Diameter
LVESD: Left Ventricular End-Systolic Diameter
PDW: Platelet Distribution Width
SD: Standard Deviation

Table (3): Comparison between the studied groups regarding Biochemical parameter

	Group I	Group II	Group III	Group IV	F	p
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD		
liver and kidney function test						
SGOT	14.19 ± 5.19	15.59 ± 6.88	19.34 ± 6.04	26.24 ± 2.79	16.685	<0.001**
LSD	P1 0.458	P2 0.049*	P3 <0.001**	P4 0.008*	P50.001**	P60.001**
SGPT	15.04 ± 4.47	10.09 ± 3.3	15.2 ± 4.27	20.03 ± 6.47	12.331	<0.001**
LSD	P1 0.004*	P2 <0.001**	P3 0.004*	P4 0.923	P50.003*	P60.001**
Creatinine	5.09 ± 2.1	3.53 ± 1.9	12.06 ± 3.05	9.52 ± 2.85	41.352	<0.001**
LSD	P1 0.076	P2 <0.001**	P3 0.005*	P4 0.001**	P50.001**	P60.001**
Urea	126.62±50.29	109.74±43.66	120.56±24.93	119.97±29.2	0.565	0.64
	Median(IQR)	**Median(IQR)**	**Median(IQR)**	**Median(IQR)**	**KW**	**p**
e-GFR	37(32.5–54.5)	47(39.5-57.5)	8(5.7 – 10.5)	8(4.85 – 11)	45.884	<0.001**
Pairwise	P1 0.409	P2 <0.001**	P3 0.889	P4 <0.001**	P50.001**	P60.001**
lipid profile						
HDL	31.82 ± 11.4	30.94 ± 7.78	35.58 ± 9.76	30.88 ± 7.92	0.963	0.416
	Median(IQR)	Median(IQR)	Median(IQR)	Median(IQR)	KW	p
Cholesterol	190 (132-214)	119 (91.5-148)	133 (123.5-162)	160.9 (142.5-207)	13.5	0.004*
Pairwise	P1 0.003*	P2 0.087	P3 0.622	P4 0.219	P50.69	P60.001**
Triglycerides	131 (99.5–192)	131 (76 – 179)	187 (119–223.5)	130 (95 – 172.5)	4.863	0.182
LDL	94.6 (56-141.1)	56.2 (47.95-92.1)	68.6 (50.3–95.6)	115 (72.6-130.2)	10.682	0.014*
Pairwise	P1 0.022*	P2 0.482	P3 0.031*	P4 0.112	P50.573	P60.004*
Parathyroid Hormone and Minerals						
Calcium	9.58 ± 1.1	8.63±0.66	8.21 ± 0.64	6.58 ± 3.52	7.364	<0.001**
	P1 0.151	P2 0.518	P3 0.015*	P4 0.039*	P5 <0.001**	P6 0.003*
Phosphorus	3.91±0.76	4.25±1.37	4.54 ± 1.47	4.82 ± 1.66	1.409	0.248
	Median (IQR)	Median (IQR)	Median (IQR)	Median (IQR)	KW	p
PTH	61 (23 – 78.75)	62.5 (58 –84.5)	276 (183.7-728)	307.9 (175.5-555.95)	36.351	<0.001**
	P1 0.466	P2 <0.001**	P3 0.683	P4 0.155	P5 0.001**	P60.001**
Hemoglobin	11.08 ± 1.61	10.37 ± 1.94	8.89 ± 1.39	11.42 ± 2.47	5.988	0.001**
Serum DKK-3						
DKK-3	15.28 (11.62-18.38)	7.72 (4.71 – 8.98)	29.08 (26.11–39.37)	15.24 (12.8 - 1828)	40.225	<0.001**
Pairwise	P1 0.002*	P2 <0.001**	P3 0.004*	P4 <0.001**	P50.699	P60.003*

p1 difference between group I and II p2 difference between group II and III
p3 difference between group III and IV p4 difference between group I and III
p5 difference between group I and IV p6 difference between groups II and IV
F One Way ANOVA test KW Kruskal Wallis test LSD least significant difference
*p<0.05 is statistically significant **p≤0.001 is statistically highly significant
SGOT: Serum Glutamic Oxaloacetic Transaminase
SGPT: Serum Glutamic Pyruvic Transaminase
SD: Standard Deviation IQR: Interquartile Range
e-GFR: estimated Glomerular Filtration Rate HDL: High Density Lipoprotein
LDL: Low Density Lipoprotein PTH: Parathyroid Hormone

Table (4): Correlation between serum DKK3 and the studied parameters:

	r	p
Age (year)	-0.03	0.81
BMI	-0.166	0.177
WBCs	0.035	0.774
Hemoglobin	-0.33	0.006*
Platelet count	-0.102	0.406
SGOT	0.134	0.275
SGPT	0.336	0.005*
Creatinine	0.62	<0.001**
Urea	0.046	0.709
eGFR	-0.507	<0.001**
Calcium	-0.115	0.349
Phosphorus	0.079	0.522
PTH	0.425	<0.001**
Total cholesterol	0.312	0.01*
Triglycerides	0.256	0.035*
LDL	0.268	0.027*
HDL	0.201	0.101

r Spearman rank correlation coefficient *p<0.05 is statistically significant
BMI: Body Mass Index
WBCs: White Blood Cells
SGOT: Serum Glutamic Oxaloacetic Transaminase
SGPT: Serum Glutamic Pyruvic Transaminase
LDL: Low-Density Lipoprotein
HDL: High-Density Lipoprotein
eGFR: estimated Glomerular Filtration Rate
PTH: Parathyroid Hormone
**p≤0.001 is statistically highly significant

Table (5): Correlation between serum DKK3 and Echo parameters:

	r	p
IVSD	0.419	0.001**
LVEDD	0.311	0.013*
LVESD	-0.223	0.079
PWD	0.317	0.011*
Ejection fraction (%)	-0.481	<0.001**

r Spearman rank correlation coefficient *p<0.05 is statistically significant
IVSD: Interventricular Septal Thickness
LVEDD: Left Ventricular End-Diastolic Diameter
LVESD: Left Ventricular End-Systolic Diameter
PWD: Posterior Wall Thickness
**p≤0.001 is statistically highly significant

Supplementary Table (1): Linear stepwise regression analysis of factors significantly correlated to serum DKK-3 among renal patients:

	Unstandardized Coefficients		Standardized Coefficients	t	p	95.0% Confidence Interval	
	β	Std. Error	Beta			Lower	Upper
(Constant)	9.809	5.061		1.938	0.057	-.322	19.941
Creatinine	1.534	0.219	0.608	7.020	<0.001**	1.097	1.971
LDL cholesterol	0.079	0.018	0.374	4.342	<0.001**	.042	.115
LVEDD	-0.190	0.077	-0.214	-2.475	0.016*	-.343	-.036

*p<0.05 is statistically significant **p≤0.001 is statistically highly significant
β: Unstandardized regression coefficient
Std. Error: Standard error of the estimate
Beta: Standardized regression coefficient

Supplementary Table (2): Performance of DKK-3 in diagnosis of end stage chronic kidney, cardiovascular disease and cardiovascular disease in end-stage in renal patients:

Cutoff	AUC	Sensitivity	Specificity	PPV	NPV	Accuracy	p
End Stage Chronic Kidney							
≥15.2615	0.835	76.5%	73.5%	74.3%	75.8%	75%	<0.001**
Cardiovascular Disease in renal patients							
≥15.67	0.796	70.6%	73.5%	72.7%	71.4%	72.1%	<0.001**
Diagnosis of cardiovascular disease in end-stage renal patients							
≥22.05	0.912	94.1%	82.4%	84.2%	93.3%	88.2%	<0.001**

AUC area under curve PPV positive predictive value NPV negative predictive value **p≤0.001 is statistically highly significant

cardiovascular disease and serum DKK-3 level

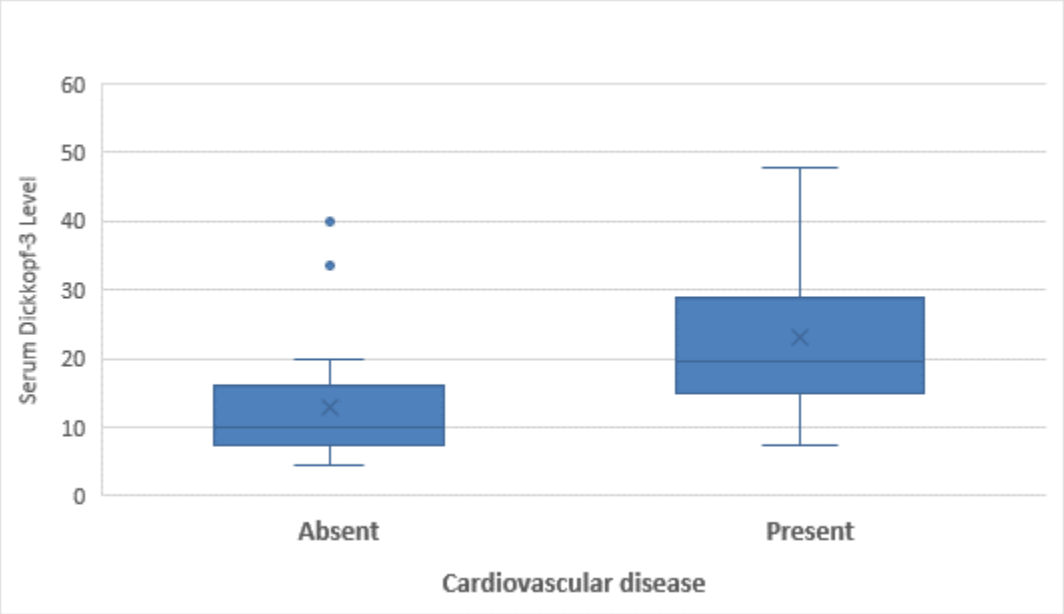


Figure (1): Boxplot showing relation between

Figure 1. A statistically significant relationship ($p < 0.001$) between cardiovascular disease and serum DKK-3 among renal patients (Significantly higher in patients with cardiovascular disease).

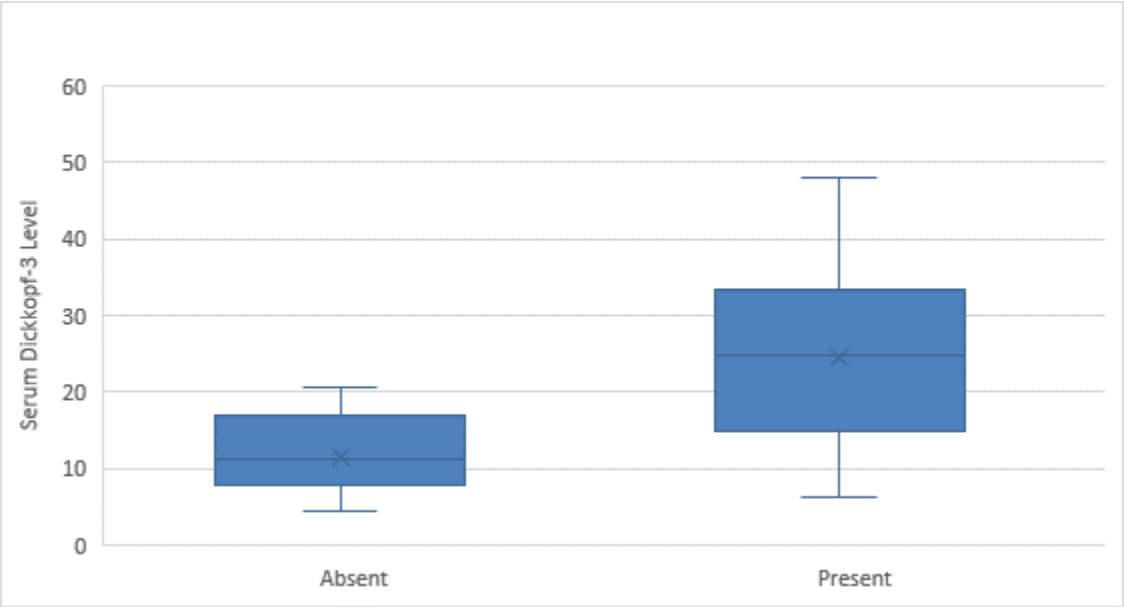
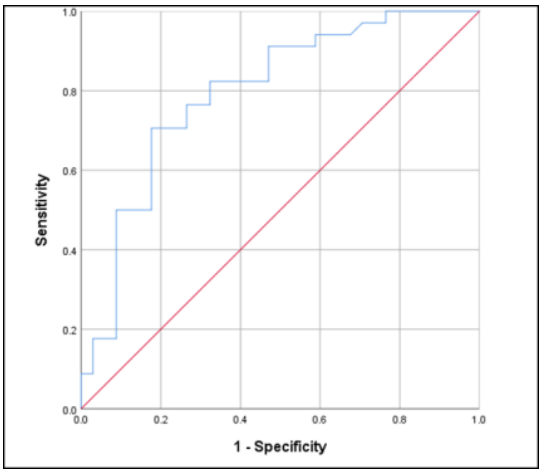
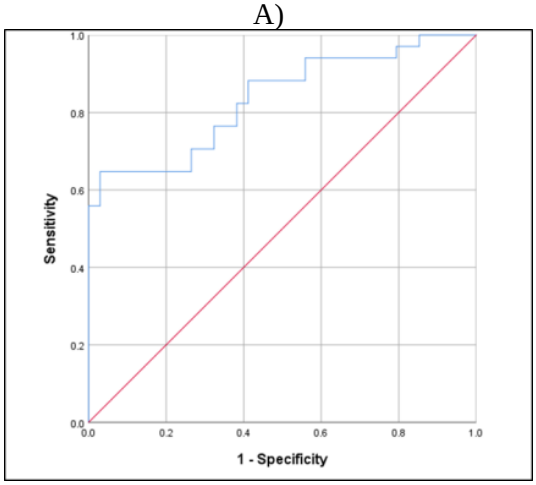


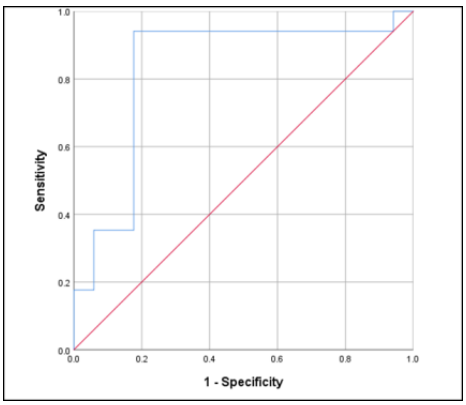
Figure (2): Boxplot showing relation between end-stage renal disease and serum DKK-3 level.
Figure 2. A statistically significant relationship ($p < 0.001$) between the presence of end-stage renal disease and serum DKK-3 among renal patients (Significantly higher in patients on dialysis).



A)



B)



c)

Supplementary Figure 1. ROC curve showing performance of DKK-3 in diagnosis of A) end stage chronic kidney disease, B) cardiovascular disease in renal patients, and C) cardiovascular disease in end-stage renal patients.

DISCUSSION

The study's results revealed a significant statistical variance in the levels of blood DKK-3 among the groups that were analyzed. The study found that there were both negative and positive associations between DKK-3 and hemoglobin, as well as eGFR. Specifically, there was a statistically significant negative association between these variables. However, there were also statistically significant positive associations between DKK-3 level and SGPT, parathyroid hormone, serum creatinine, total cholesterol, LDL-C, and triglycerides. There was no statistically significant correlation observed between DKK-3 and the other parameters that were examined. The levels of DKK-3 in blood were found to have independent correlations with serum creatinine, LDL-C, and left ventricular end-diastolic diameter (LVEDD).

A prior investigation by Piek et al. [7] centered on a cohort of 8420 participants from the PREVEND Cohort. The aim of the research was to mitigate the incidence of end-stage vascular and renal diseases. The research findings indicate a significant association between the concentrations of plasma DKK3 and the risk factors linked to cardiovascular diseases. The aforementioned risk factors encompassed age, male sex, body mass index (BMI), and blood glucose levels. **Pratama's** [8] study suggests that T cells play an essential role in developing kidney fibrosis, which can occur due to prolonged inflammation. Interestingly, maintaining kidney function and structure in the absence of DKK3 (a protein linked to fibrosis) was found to be associated with increased T cell proliferation and the production of Th1 cells, Tregs, and interferon-gamma in the interstitium of the kidney. This suggests that Th1 cells, in particular, may be beneficial in preventing kidney fibrosis**.**

Analysis of the echocardiographic data in our investigation found that DKK-3 levels and all IVSD, LVEDD, and PWD cases had a statistically significant positive connection. DKK-3 and ejection fraction had a statistically significant inverse relationship. There was no significant correlation observed between DKK-3 and LVESD.

In the same line as our findings, Bao, Piek, et al. [9,10]. also analyzed the correlation between DKK-3 and various echocardiographic parameters IVSD, LVESD, LVEDD, and PWD, and their results were in agreement with our findings**.**

Our findings demonstrated a statistically significant relationship between cardiovascular disease and serum DKK-3 among renal patients (Significantly higher in patients with cardiovascular disease). The ideal serum cut-off value for DKK-3 is 15.67, with an area under the curve of 0.835, a sensitivity of 70.6%, a specificity of 73.5%, a positive predictive value of 72.7%, a negative predictive value of 71.4%, and overall precision of 72.1% for the detection of cardiovascular disease in patients with renal disease.

Piek et al. explored the link relationship between DKK3, CVD, and existing and developing CVD. After correcting for common risk factors and clinical characteristics against us, they did not find an independent relationship between DKK3 and CVD in this general population cohort, which was more prominent and, therefore, more powerful than the study by Yu et al. This study cohort is generally younger than the cohort used in the study of **Yu et al.,** and DKK3 was strongly correlated with age; this could partly explain these differences [7,4]**.**

Subsequent studies need to consider investigating the plausible function of trimeric serine hydrolase at elevated temperatures 1 (HtrA1) in modulating the concentrations of plasma DKK3 protein. This study further supports the correlation between DKK3 and cardiovascular risk factors, as previously reported by **Toprak et al.** [11]**.**

Our results revealed that end-stage renal disease and serum DKK-3 had a statistically significant inverse relationship among renal patients. (Importantly higher in dialysis patients). The optimal serum DKK-3 cut-off for the diagnosis of end-stage renal disease is ≥15.2615 With an overall accuracy of 75%, an area under the curve of 0.835, a sensitivity of 76.5%, specificity of 73.5%, a positive predictive value of 74.3%, and a negative predictive value of 75.8%. In line with these findings, **Schunk et al. [3]** revealed urine DKK3 as a biomarker for the rapid progression of CKD, which may be essential for nephrologists, in particular, to monitor medications that can stop or at least postpone progression**.**

Toprak et al. [11] suggested that the assessment of short-term GFR loss differs from the determination of long-term prognosis of CKD based on specific renal endpoints such as ESRD or a 40% decline in GFR over several years. The biomarker DKK3 indicates a patient's acute decline in renal function, while the latter metric offers a prognostic evaluation of the percentage of individuals who may encounter a renal endpoint. Further investigation is necessary to assess the correlation between DKK3 and the renal outcomes mentioned above**.**

To visualize the individual course of DKK3 in patients with primary kidney diseases, **Schunk et al. [3]** examined urine DKK3 concentrations in these patients following the start of a particular medication. Our findings indicate a reduction in DKK3 levels within a 30-day period following the initiation of treatment, which aligns with the outcomes of our study. DKK3 returned to normalcy before kidney function did. Recently, more extensive research has been started to see if DKK3 could indicate treatment efficacy for various CKD entities even before kidney function (i.e., GFR) improves or stabilizes**.**

On the other hand, we discovered that the optimal serum DKK-3 threshold for detecting cardiovascular disease in end-stage renal illness is 22.05, with an AUC of 0.912 and a sensitivity of 80%.94.1%, specificity 82.4%, and Overall accuracy was 88.2%, with a 93.3% negative predictive value and an 84.2% positive predictive value. The optimal cut-off value for serum DKK-3 in the diagnosis of cardiovascular disease in predialysis disease is 9.0195, with an area under the curve of 0.926, a sensitivity of 88.2%, a specificity of 76.5%, a positive predictive value of 78.9%, negative predictive value of 86.7%, and overall accuracy of 82.4%.

Our conclusions are supported by ROC studies by **Alaaraji et al. [12]** DKK3, as shown by its values of AUCs 0.9896 (95% CI 0.9737 to 1.006) in male Iraqi patients with CKD; it can be a very precise biomarker to predict and diagnose CKD. This study found a relationship between higher levels of DKK3, KIM-1, NT-pro-BNP, GDF-15, and CPP with a higher risk of poor renal outcomes in patients with CKD. However, more research is necessary to pinpoint the precise role of DKK3 in the pathophysiology of CKD**.**

Sanchez et al. [13] have presented findings on the potential utility of uDKK3 as a novel biomarker for monitoring the advancement of CKD, which aligns with the outcomes of our investigation. The utilization of uDKK3 as a noninvasive diagnostic biomarker may be considered due to the ongoing development of CKD. A prospective study was conducted on two cohorts comprising 351 individuals diagnosed with stages 2 to 3 of chronic kidney disease. **Fliser et al. [14]** reported that the randomization of the therapy arms had no bearing on the prediction of CKD progression. A separate analysis of the CARE FOR HOME trial reported that uDKK3 was significantly and independently related to cardiovascular events during the follow-up period. This demonstrated the tight relationship between cardiovascular problems and the development of CKD**.**

In the same study comprising 458 individuals, a baseline DKK3 ≥322 pg/mg was the most accurate indicator of persistent renal disease, with a consistent decline in eGFR of >25% at one month after exposure to contrast media. Adding baseline DKK3 to different clinical prediction scores derived from the patient's clinical and laboratory data significantly increased integrated discrimination improvement (IDI) [15]**.**

In addition, a prospective study of 490 patients who underwent coronary angiography revealed that uDKK3, as demonstrated by **Seibert et al. [16],** was a reliable marker of contrast-induced AKI, even in the absence of apparent CKD. According to **Fliser et al. [14],** patients with a high risk of a significant reduction in e-GFR throughout follow-up were identified by baseline uDKK3 but not by e-GFR or proteinuria. A promising tool for the early detection of clinically "silent" progressive diseases, the superiority of uDKK3 was evident in individuals with an e-GFR >90 mL/min/1.73 m2 and no proteinuria CKD**.**

In agreement with our study, the relationship between DKK3 levels and renal disorders and function was examined previously. First, elevated levels of DKK3 at baseline were independently associated with an increased risk of future kidney function loss in patients with CKD [5,17]**.**

In the study by **Schunk et al. [3],** Although DKK3 was strongly correlated with kidney

function in blood plasma, it was not independently associated with both prevalent and newly onset CKD. Although DKK3 is produced in a variety of organs and tissues, circulating levels of the protein are probably a reflection of its production in all these tissues, as opposed to urinary DKK3, which will inevitably originate primarily from the tubular epithelium and is therefore much more specific to the kidney**.**

The study's robustness is attributed to its large sample size and the application of multiple regression analyses to determine autonomous predictors of serum DKK-3 levels. The results align with prior research and offer significant perspectives on the possible function of DKK-3 as a biomarker for renal and cardiovascular ailments. The study's limitations are attributed to its cross-sectional design, which restricts the ability to establish a causal relationship between serum DKK-3 levels and renal or cardiovascular disease. Furthermore, it is noteworthy that the study solely comprised individuals with a confirmed renal ailment; consequently, the findings may not be universally applicable to the broader populace. Subsequent investigations ought to utilize longitudinal methodologies to establish causal relationships and evaluate the efficacy of DKK-3 as a biomarker in more extensive demographic groups.

CONCLUSIONS

We concluded that DKK3 could represent a new biomarker of CKD progression. Furthermore, DKK3 may serve as a biomarker of CVD in patients with renal disease.

We recommend considering DKK3 assessment as a tool to achieve better outcomes in CKD patients and paying attention to this fact while presenting recent guidelines for the treatment of CKD. In addition, further studies must be done to analyze all aspects of this issue.

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