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Evaluation of Risk Factors for Cardiovascular Disease in Patients of Chronic Kidney Disease

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Abstract

Introduction: *We sought to determine the influence of risk factors of chronic kidney disease (CKD) on cardiovascular disease. We studied the risk factors for cardiovascular disease (lipid profile, HbA1C, coronary artery calcium score) in predialysis patients of CKD.*

Material and methods: *100 patients of CKD and 30 healthy controls with age and sex matched were enrolled. CKD patients were further divided into stages according to eGFR. Patients with history of CVD, history of predisposing factors to dyslipidaemia were excluded from the study. The following biochemical parameters were done in all patients-haemoglobin (g/dL), blood urea (mg/dL), serum creatinine (mg/dL), serum calcium corrected for albumin (mg/dL), serum phosphorus (mg/dL), iPTH, serum uric acid (mg/dL), eGFR, urine examination, serum albumin (g/dL), ECG, ultrasonography bilateral kidneys, CRP, homocysteine, fasting blood sugar, HbA1C, lipid profile, etc. Coronary artery calcification was detected with computed tomography.*

Results: *In present study, the most common cause of CKD was hypertension (45%) followed by type-2 diabetes mellitus (30%). Systolic blood pressure (mmHg) and diastolic blood pressure (mmHg) in CKD patients were 142.3 ± 15.99 and 85.04 ± 7.04, respectively and it was significantly increased with a decline in eGFR (p value = < 0.05). The triglycerides, cholesterol and LDL lipids were significantly increased in CKD patients than control group and these were significantly increased with increasing in CKD stages (p value = < 0.0001, 0.018, < .0001 respectively) and HDL lipid was significantly low with an increasing in CKD stages. HbA1C (%) in CKD patients was 5.77 ± 1.21, and in control group was 4.99 ± 1.42, (p value = 0.009) but this was not statistically significant in different stages of CKD. CAC score was significantly present in CKD patients than in the control group (p value = < .0001). In CKD patients, out of 100 patients, calcification was present in 65 patients, while in the control group, only minimal calcification was present in 6 patients. The mean ± SD of CAC score in stage 5 was 110 ± 79.48, which was significantly high as compared to stage 4 (67.42 ± 61.41) and stage 3 (22.91 ± 39.44) (p value = < .0001), while in control group, the mean ± SD of CAC score was 1.27 ± 2.79. On performing multivariate regression analysis, With the increase in triglyceride (mg/dL), serum homocysteine (µmol/L), CRP (mg/dL), i-PTH (pg/mL), eGFR (mL/min/1.73 m^2) by 1-unit, CAC score significantly increased by 0.477, 2.802, 12.255, 0.426, 1.412 units, respectively.*

Conclusion: *Risk factors of CVD are highly prevalent in CKD patients. Cardiac calcification should be considered as a marker of CVD risk in CKD patients and it improves risk prediction for CVD. Various traditional and non-traditional risk factors such as increased CRP, iPTH, homocysteine, anaemia, hyperphosphataemia, dyslipidaemia, insulin resistance etc., accelerate the rate of cardiac calcification. Present study showed that CAC score, lipid profile and CVD are reliable markers for screening CVD in CKD patients.*

Key words: *CKD, CVD, HbA1C, hyperphosphataemia, dyslipidaemia.*

Introduction

Chronic kidney disease (CKD) is one of the leading causes of non-communicable diseases globally, with rising incidence and prevalence. Patients with CKD are a considerable social and economic burden, both directly in terms of resource use and indirectly in lost productivity and reduced quality of life. The worldwide increase of CKD is mainly driven by the rise in the prevalence of diabetes mellitus, hypertension, obesity, and aging[1]. According to the 2015 Global Burden of Disease Study, CKD was the 12th leading cause of death, accounting for 1.1 million fatalities worldwide, and the 17th leading cause of disability. Overall, CKD mortality has increased by 31.7%, making it one of the fastest rising significant causes of death[2]. The global all-age mortality rate due to CKD is increased by 41.5% between 1990 and 2017. CKD also became the 19th leading cause of years of life lost in 2013, compared with being the 36th leading cause in 1990. Subsequent GBD (Global burden of disease) reports indicate that the CKD will become the fifth highest cause of years of life lost globally by 2040[3].

CKD patients suffer from many complications, such as hypertension, cardiovascular diseases, anaemia, metabolic acidosis, altered immune response, mineral and bone disturbances, and neurological complications. Among these complications, cardiovascular complications are

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widespread and the most common cause of death. CVD is approximately three times more frequent in patients with CKD than in other known cardiovascular risk groups. Similar cardiovascular mortality is approximately 10-fold more frequent in CKD patients than in the age- and sex-matched segments of the non-renal population[4]. Cardiovascular mortality has been estimated to be around 9% per year. Patients with CKD are predisposed to CVD in various forms, including coronary artery disease, atrial or ventricular arrhythmias, myocardial infarction, stroke, congestive heart failure, or peripheral vascular disease[5].

The presence of cardiac calcification is an appropriate marker and strong predictor of cardiovascular disease and all cause mortality in CKD patients and correlates directly with the amount of coronary plaque and cardiac calcification in highly advanced CKD patients. Also, the degree of cardiac calcification can be prognosticating by coronary artery calcium score (CACS). It may affect the arterial media, atherosclerotic plaques, myocardium, and heart valve. Pathomorphologically, in the general population, calcification involves the intimal layer more than the medial layer i.e., atherosclerosis, while in CKD patients calcification involves the medial layer more than the intimal layer, i.e., arteriosclerosis.

Numerous risk factors are accountable for cardiovascular disease and CAC in CKD patients, which are predominantly classified in traditional and non-traditional groups. Traditional risk factors include age, gender, race, menopause, family history, dyslipidaemia, hypertension, DM; and non-traditional risk factors include anaemia, hypoalbuminaemia, abnormal mineral metabolism, inflammation, oxidative stress, hyperhomocysteinaemia, uric acid, etc., in the development of cardiovascular disease in CKD patients. Cardiovascular mortality and morbidity are much higher in CKD patients and cannot be fully explained on the basis of traditional risk factors only. Hence more interest has focussed on the role of other non-traditional risk factors.

This study was conducted to evaluate the ideal modality of assessment of CVD in CKD patients and importance of CAC in assessment of the CVD in CKD patients. This study also assesses the rate and potential risk factors of CAC progression in CKD patients. The available data indicates the burden of CVDs and CAC in CKD patients on dialysis, but there is scarcity of data in patients who are not yet on dialysis. Hence, the present study was planned to evaluate the risk factors of CVD in CKD patients, especially CAC.

Materials and method

The present study was a single centre, cross-sectional, observational study. 100 patients of CKD of age 18 - 60 years were enrolled, who were not yet on haemodialysis and classified into stages (stage 3 - 5) based on eGFR, calculated by MDRD equation. Chronic kidney disease was defined according to KDIGO 2012 Guidelines as eGFR < 60 mL/min/1.73 m^2 body surface area for more than three months, irrespective of the cause[6]. 30 patients were also taken, age and sex-matched, who had no evidence of structural kidney disease and no radiological and biochemical evidence of renal insufficiency as control.

Patients with aged less than 18 years and greater than 60 years of age, history of CVD, history of predisposing factors to dyslipidemia like liver disease and hypothyroidism, history of drug treatment causing altered lipid levels like statins, alpha-, and beta-blockers, history of inflammatory disease, autoimmune disorders, neoplasms, and disseminated intravascular coagulation (DIC), history of genetic lipid disorders, pregnancy or known psychiatric disorder, were excluded from the study.

After screening the patients for inclusion and exclusion criteria, a detailed history was taken, and a thorough physical examination was performed. Various anthropometric parameters like height and weight were measured in all patients. Waist hip ratio (WHR) was calculated from standard nomograms. The following biochemical parameters were done in all patients-haemoglobin (g/dL), total leukocyte count, blood urea (mg/dL), serum creatinine (mg/dL), serum electrolytes (mEq/L): Sodium and potassium, serum calcium corrected for albumin (mg/dL), serum phosphorus (mg/dL), iPTH, serum uric acid (mg/dL), eGFR, urine complete examination, serum albumin (g/dL), ECG, ultrasonography bilateral kidneys, CRP, homocysteine, fasting blood sugar, HbA1C, lipid profile. HbA1c was measured using high-performance liquid chromatography (HPLC).

Fasting lipid profiles were obtained for each patient at the time of enrollment. The patient's serum was used to estimated total cholesterol, HDL cholesterol, and triglycerides. LDL cholesterol was then calculated by using the Friedewald equation: LDL cholesterol (mg/dL) = total cholesterol – HDL cholesterol – (triglycerides/5). Patients underwent non-contrast, high resolution computed tomography scans for CAC score. HRCT cine acquisition was collected contiguous axial slices from the tracheal carina to the inferior margin of the heart. All areas of calcification with a minimum density of 130 Hounsfield Units within the borders of the coronary arteries were computed. Images were recorded during breath-holding sessions. The acquired images were reviewed on a dedicated workstation, and the Agatston method was applied for calculating a calcium score which incorporates the density of calcification, multiplying the calcification volume by a weighted density co- efficient. A CACS was calculated individually for the left main, left circumflex, left anterior descending, posterior

descending, and right coronary arteries. The score was then summed to calculate the total score.

CAC score	Atherosclerotic plaque burden	Probability of significant CAD	Implication for cardiovascular risk
0	No identifiable plaque	Very low, <5%	Very low
1 - 10	Minimal identifiable plaque burden	Very unlikely, <10%	Low
11 - 100	Definite, at least mild plaque burden	Mild or minimal coronary stenosis is likely	Moderate
101 - 400	Undeniable, at least reasonable plaque burden	Non-obstructive CAD likely	Moderately high
> 400	Extensive plaque burden	High likelihood (> 90%) of at least one significant coronary stenosis	High

Statistical analysis

The quantitative data were presented as the means ± SD. The following statistical tests were applied for the results: The quantitative variables' comparison was analysed using an Independent t-test (for two groups) and ANOVA (for more than two groups). The qualitative variables' comparison was analysed using the Chi-Square test. Pearson correlation co-efficient was used for correlation of quantitative parameters. Univariate and multivariate linear regression was used to determine the coronary calcium score factors. A p-value of less than 0.05 was considered statistically significant for statistical significance.

Results

Of the 130 patients enrolled in this study, 100 patients had CKD and 30 were age and sex matched control. Out of 100 CKD patients, 27 patients were in stage 3, 48 were in stage 4 and 25 patients were present in stage 5 CKD. The baseline clinical and biochemical data of control group and CKD patients, and in different stages of CKD are reported in Table I and II, respectively.

In present study, the mean ± SD age (years) in control was 58.4 ± 8.14 and in cases was 54.76 ± 12.06, (p value = 0.061) and the mean ± SD age (years) in CKD stage 5 was 55.6 ± 13.09, which was slightly higher than CKD stage 4 (55.77 ± 11.06) and CKD stage 3 (52.19 ± 12.85), (p value = 0.434). In this study, total 64 males and 66 females were included, from which 13 females and 17 males were taken in the control group and 53 females and 47 males were taken as cases, (p value = 0.353). The common aetiology of renal disease in study population were hypertension (45%), diabetes mellitus (30%), chronic glomerulonephritis (9%), autosomal polycystic kidney disease (10%), obstructive uropathy (3%), pyelonephritis. Diabetic patients were comparable in both group (30%) and hypertension were significantly high in CKD patients than control group (p value < 0.001).

Table I: Comparison of biochemical investigations between control and CKD patients.

Biochemical investigations	Control (n = 30)	CKD (n = 100)	P value
Systolic blood pressure (mmHg)	126.27 ± 17.22	142.3 ± 15.99	< .0001
Diastolic blood pressure (mmHg)	80.53 ± 7.52	85.04 ± 7.04	0.003*
Waist hip ratio	0.86 ± 0.05	0.86 ± 0.05	0.672
Diabetes mellitus type 2	9 (30%)	30 (30%)	1§
Blood urea (mg/dL)	22.57 ± 5.18	100.14 ± 24.92	< .0001*
Serum creatinine (mg/dL)	0.73 ± 0.09	3.04 ± 1.05	< .0001*
Sodium (mEq/L)	140 ± 0	139.15 ± 3.2	0.009*
Potassium (mEq/L)	4.11 ± 0.07	4.23 ± 0.51	0.03*
Serum uric acid (mg/dL)	4.38 ± 0.67	6.19 ± 2.12	< .0001*
eGFR (mL/min/1.73 m^2)	98.8 ± 6.96	23.1 ± 9.17	< .0001*
Serum albumin (g/dL)	4.18 ± 0.31	3.21 ± 0.46	< .0001*
Serum calcium corrected for albumin (mg/dL)	9.3 ± 0.73	8.61 ± 0.61	< .0001*
Serum phosphorus (mg/dL)	3.64 ± 0.59	5.36 ± 1.55	< .0001*
i-PTH (pg/mL)	44.53 ± 19.3	197.95 ± 38.52	< .0001*
Product of serum calcium corrected and phosphorus	33.87 ± 6.43	45.56 ± 11.42	< .0001*
Triglyceride (mg/dl)	134.47 ± 26.48	149.67 ± 31.37	0.017*
Cholesterol(mg/dl)	191.83 ± 37.1	213.27 ± 21.54	0.005*
HDL (mg/dl)	46.57 ± 5.53	39.41 ± 5.35	< .0001*
LDL(mg/dl)	134.7 ± 23.12	151.83 ± 32.13	0.008*
Fasting blood sugar (mg/dl)	105.07 ± 13.64	113.14 ± 27.55	0.032*
HbA1C (%)	4.99 ± 1.42	5.77 ± 1.21	0.009*
Serum homocysteine (µmol/L)	7.23 ± 2.36	26.56 ± 7.23	< .0001*
C-reactive protein (mg/dL)	0.39 ± 0.22	5.67 ± 2.76	< .0001*

* *Independent t-test*

Table II: Baseline characteristics of CKD patients in different stages.

Characteristics	Stage 3 (n = 27)	Stage 4 (n = 48)	Stage 5 (n = 25)	P value
Age	52.19 ± 12.85	55.77 ± 11.06	55.6 ± 13.09	0.434‡
Female	13 (48.15%)	26 (54.17%)	14 (56%)	0.83**
Male	14 (51.85%)	22 (45.83%)	11 (44%)	0.83**
Waist hip ratio	0.86 ± 0.05	0.86 ± 0.05	0.84 ± 0.05	0.441‡
Systolic blood pressure (mmHg)	128.67 ± 13.64	146.92 ± 12.11	148.16 ± 16.7	< .0001‡
Diastolic blood pressure (mmHg)	80.59 ± 7.82	86.83 ± 5.23	86.4 ± 7.33	0.0004‡
Diabetes Mellitus	10 (37.04%)	11 (22.92%)	9 (36%)	0.331§
Haemoglobin (g/dL)	11.93 ± 1.04	10.23 ± 0.82	8.14 ± 0.67	< .0001‡
Blood urea (mg/dL)	84.95 ± 20.24	98.94 ± 21.63	118.86 ± 23.97	< .0001‡
Serum creatinine (mg/dL)	2.03 ± 0.44	2.88 ± 0.6	4.43 ± 0.63	< .0001‡
Serum sodium (mEq/L)	139.33 ± 2.9	138.29 ± 3.43	140.6 ± 2.52	0.012‡

Serum potassium (mEq/L)	4.29 ± 0.55	4.14 ± 0.48	4.34 ± 0.53	0.244‡
Uric acid (mg/dL)	6.04 ± 2.66	6.16 ± 2.04	6.41 ± 1.66	0.817‡
eGFR (mL/min/1.73 m^2)	35.26 ± 5.62	21.6 ± 4.03	12.84 ± 0.9	< .0001‡
Serum albumin (g/dL)	3.57 ± 0.43	3.15 ± 0.41	2.93 ± 0.35	< .0001‡
Serum calcium corrected for albumin (mg/dL)	9.3 ± 0.41	8.58 ± 0.32	7.91 ± 0.36	< .0001‡
Serum phosphorous (mg/dL)	3.84 ± 0.93	5.31 ± 0.87	7.09 ± 1.31	< .0001‡
iPTH (pg/mL)	179.3 ± 28.39	197.12 ± 34.23	219.68 ± 45.49	0.0005‡
Product of serum calcium corrected and phosphorus	35.73 ± 8.78	45.62 ± 7.84	56.05 ± 10.53	< .0001‡
Triglyceride (mg/dL)	131.74 ± 22.47	143.6 ± 23.96	180.68 ± 30.87	< .0001‡
Total cholesterol (mg/dL)	205.07 ± 23.64	213.4 ± 17.75	221.88 ± 23.22	0.018‡
LDL (mg/dL)	136.22 ± 30	144.54 ± 22.97	182.68 ± 29.63	< .0001‡
HDL (mg/dL)	44.52 ± 4.3	39.6 ± 3.36	33.52 ± 3.19	< .0001‡
CRP (mg/dL)	3.11 ± 1.32	5.53 ± 1.86	8.69 ± 2.4	< .0001‡
Homocysteine (µmol/L)	19.7 ± 4.95	26.27 ± 4.56	34.52 ± 5.41	< .0001‡
HbA1C (%)	5.48 ± 1.17	5.78 ± 1.09	6.08 ± 1.45	0.208‡

‡ANOVA

Mean values of systolic blood pressure (mmHg) and diastolic blood pressure (mmHg) in CKD patients were 142.3 ± 15.99 and 85.04 ± 7.04, respectively and in control group were 126.27 ± 17.22 and 80.53 ± 7.52, respectively (p value = < .0001, 0.003). In present study, we observed that BP was significantly increased with a decline in eGFR (p value = < 0.05). Mean ± SD of waist-hip ratio in control was 0.86 ± 0.05 and in CKD patients was 0.86 ± 0.05, (p value = 0.672).

In the present study, haemoglobin, blood urea, serum creatinine, eGFR, serum albumin, uric acid, calcium, phosphorus and their products, iPTH, serum sodium and potassium were significantly high in cases than the control group, (p value = < .0001). It also found significant differences in haemoglobin, blood urea, serum creatinine, eGFR, serum albumin, serum calcium, serum phosphorus, serum phosphorous and corrected serum calcium product and iPTH between different stages of CKD, (p value = < 0.05). The mean ± SD of HDL (mg/dL) in control was 46.57 ± 5.53 and in CKD was 39.41 ± 5.35 (p value = < 0.0001)). Mean ± SD values of triglyceride (mg/dL), cholesterol (mg/dL), LDL (mg/dL) in CKD patients were 149.67 ± 31.37, 213.27 ± 21.54, 151.83 ± 32.13, respectively and in control group were 134.47 ± 26.48, 191.83 ± 37.1, 134.7 ± 23.12, respectively (p value = 0.01, 0.005, 0.008 respectively). The HDL and non- HDL lipids were significantly different in different stages of CKD, (p value = < 0.0001). The mean ± SD of fasting blood sugar (mg/dL) and HbA1C (%) in CKD patients were 113.14 ± 27.55 and 5.77 ± 1.21, respectively and in control group were 105.07 ± 13.64 and 4.99 ± 1.42, respectively (p value = 0.032, 0.009 respectively). But HbA1C and fasting blood sugar were statistically non significant in different stages of CKD, (p value = 0.612, 0.208). There were significant difference in serum homocysteine (µmol/L) and CRP (mg/dL) levels in cases and controls (p value = <.05). The mean ± SD of serum homocysteine (µmol/L) and CRP (mg/dL) in cases were 26.56 ± 7.23, 5.67 ± 2.76, respectively and in control group were 7.23 ± 2.36, 0.39 ± 0.22, respectively (p value = < .0001)). It also showed significant difference in homocysteine and CRP in different stages of CKD (p value < .0001).

Baseline CAC is shown in Table III (A and B). In present study, CAC score was significantly present in CKD patients than in the control group (p value = < .0001). In CKD patients, out of 100 patients calcification was present in 65 patients. Out of 100 patients, minimal calcification was present in 2 patients, mild calcification was present in 37 patients and moderate calcification was present in 26 patients. We didn't find severe calcification (> 400) in any group. In the control group, only minimal calcification was present in 6 patients. It also found that the severity of calcification was increased with increasing CKD stages (p value = 0.005). The mean ± SD of CAC score in stage 5 was 110 ± 79.48, which was significantly high as compared to stage 4 (67.42 ± 61.41) and stage 3 (22.91 ± 39.44) (p value = < .0001).

Table III (a): Comparison of coronary calcium score between control and CKD patients.

CAC score	Control (n = 30)	CKD (n = 100)	Total	P value
CAC score 0	24 (80%)	35 (35%)	59 (45.38%)	< .0001†
CAC score 1 - 10	6 (20%)	2 (2%)	8 (6.15%)	
CAC score 11 - 100	0 (0%)	37 (37%)	37 (28.46%)	
CAC score 101 - 400	0 (0%)	26 (26%)	26 (20%)	
CAC score > 400	0 (0%)	0 (0%)	0 (0%)	
Mean ± SD	1.27 ± 2.79	66.05 ± 68.75	51.1 ± 66.18	< .0001*

**Independent t test, †Fisher's exact test.*

Table III (b): Comparison of CAC score between stages of CKD.

CAC Score	Stage 3 (n = 27)	Stage 4 (n = 48)	Stage 5 (n = 25)	Total	P value
CAC score 0	15 (55.56%)	15 (31.25%)	5 (20%)	35 (35%)	0.005†
CAC Score 1 - 10	2 (7.41%)	0 (0%)	0 (0%)	2 (2%)	
CAC Score 11 - 100	7 (25.93%)	22 (45.83%)	8 (32%)	37 (37%)	
CAC Score 101 - 400	3 (11.11%)	11 (22.92%)	12 (48%)	26 (26%)	
CAC Score > 400	0 (0%)	0 (0%)	0 (0%)	0 (0%)	
Mean ± SD	22.91 ± 39.44	67.42 ± 61.41	110 ± 79.48	66.05 ± 68.75	< .0001‡

†Fisher's exact test, ‡ANOVA.

In the present study we found, severity of CAC was increased with age but it was statistically non-significant, (p value - 0.931). CAC was significantly increased with decrease in eGFR. In particular, with 0 CAC score, the mean

eGFR was 26.63 ± 9.83 and with CAC score 1 - 100, the mean eGFR was 22.38 ± 8.26 and eGFR was 19.42 ± 8.12 with > 100 CAC score, (p value - 0.007). Severity of calcification was also increased with increasing in severity of CRP, hypoalbuminaemia, hypocalcaemia, hyperphosphataemia, hyperparathyroidism, dyslipidaemia. It was also affected by hyperhomocysteinaemia and insulin resistance, (p value - < 0.0001) (Table IV).

Table IV: Association of various parameters with coronary calcium score in CKD.

Mean ± SD	0 (n = 35)	1 to 100 (n = 39)	> 100 (n = 26)	P value
Age (years)	54.46 ± 13.42	54.51 ± 12.6	55.54 ± 9.44	0.931‡
eGFR (mL/min/1.73 m^2)	26.63 ± 9.83	22.38 ± 8.26	19.42 ± 8.12	0.007‡
Serum albumin (g/dL)	3.41 ± 0.53	3.19 ± 0.39	2.95 ± 0.33	0.0004‡
Serum calcium corrected for albumin (mg/dL)	8.72 ± 0.67	8.68 ± 0.54	8.35 ± 0.6	0.039‡
Serum phosphorus(mg/dL)	3.95 ± 0.95	5.67 ± 0.86	6.79 ± 1.43	< .0001‡
i-PTH (pg/mL)	165.29 ± 10.25	195 ± 25.56	246.35 ± 28.23	< .0001‡
Product of serum calcium corrected and phosphorus	33.95 ± 6.5	48.9 ± 5.31	56.16 ± 9.66	< .0001‡
Triglyceride (mg/dL)	122.54 ± 13.54	146.26 ± 16.36	191.31 ± 19.76	< .0001‡
Cholesterol (mg/dL)	194.2 ± 11.09	216.28 ± 14.39	234.42 ± 19.16	< .0001‡
HDL (mg/dL)	43.14 ± 5.36	38.79 ± 3.85	35.31 ± 3.74	< .0001‡
LDL (mg/dL)	124.34 ± 13.01	151 ± 23.29	190.08 ± 21.28	< .0001‡
Serum homocysteine (µmol/L)	19.97 ± 4.41	26.79 ± 4.09	35.08 ± 4.34	< .0001‡
C-reactive protein (mg/dL)	3.11 ± 1.11	5.74 ± 1.43	8.98 ± 2.17	< .0001‡
HbA1C (%)	4.89 ± 1.29	6.17 ± 0.69	6.37 ± 1.07	< .0001‡

‡ANOVA

In the present study, significant positive correlation of CAC score was found with triglyceride (mg/dL), cholesterol (mg/dL), LDL (mg/dL), HbA1C (%), serum homocysteine (µmol/L), CRP (mg/dL), i-PTH (pg/mL), serum phosphorus(mg/dL), product of serum calcium corrected and phosphorus with correlation coefficient of 0.871, 0.679, 0.787, 0.48, 0.87, 0.89, 0.868, 0.77, 0.791, respectively. There was also significant negative correlation of CAC score with HDL (mg/dL), serum calcium corrected for albumin (mg/dL), eGFR (mL/min/1.73 m^2), serum albumin (g/dL) with correlation coefficient of -0.628, -0.313, -0.427, -0.406, respectively. No correlation was seen between CAC score with age (years) with correlation coefficient of 0.024.

Effect of insulin resistance on CAC is shown in Table VI. In present study, CAC was comparable between diabetics and non-diabetics (p value = 0.642).

The mean ± SD of CAC score in diabetic patients was 73.07 ± 80.31, slightly higher than non-diabetic patients (63.04 ± 63.57), but it was not statistically significant (p value = 0.507).

Table V: Correlation of coronary calcium score with various parameters in CKD.

Variables	Coronary Calcium Score	
	Correlation co-efficient	P value
Triglyceride (mg/dL)	0.871	< .0001
Cholesterol (mg/dL)	0.679	< .0001
HDL (mg/dL)	-0.628	< .0001
LDL (mg/dL)	0.787	< .0001
HbA1C (%)	0.48	< .0001
Serum homocysteine (µmol/L)	0.87	< .0001
C-reactive protein (mg/dL)	0.89	< .0001
i-PTH (pg/mL)	0.868	< .0001
Serum calcium corrected for albumin (mg/dL)	-0.313	0.002
Serum phosphorus (mg/dL)	0.77	< .0001
Product of serum calcium corrected and phosphorus	0.791	< .0001
Age (years)	0.024	0.813
eGFR (mL/min/1.73 m^2)	-0.427	< .0001
Serum albumin (g/dL)	-0.406	< .0001

Pearson correlation coefficient

Table VI: Comparison of coronary calcium score between diabetic and non-diabetic in CKD.

Coronary calcium score	Diabetic CKD (n = 30)	Non-diabetic CKD (n = 70)	P value
Evidence of CAD: 0 calcium score	11 (36.67%)	24 (34.29%)	0.642†
No Minimal CAD: 1 - 10	1 (3.33%)	1 (1.43%)	
Mild CAD: 11 - 100	9 (30%)	28 (40%)	
Moderate CAD: 101 - 400	9 (30%)	17 (24.29%)	
Mean ± SD	73.07 ± 80.31	63.04 ± 63.57	0.507*

**Independent t-test, †Fisher's exact test.*

Table VII: Multivariate linear regression to determine the factors affecting coronary calcium score.

Variables	Beta co-efficient	Standard error	P value	Lower bound (95%)	Upper bound (95%)
Triglyceride (mg/dL)	0.477	0.203	0.021	0.073	0.881
Cholesterol (mg/dL)	0.010	0.165	0.952	-0.318	0.338
HDL (mg/dL)	0.161	0.783	0.838	-1.396	1.718
LDL (mg/dL)	-0.196	0.162	0.229	-0.517	0.125
HbA1C (%)	1.362	2.282	0.552	-3.174	5.898
Serum homocysteine (µmol/L)	2.802	1.164	0.018	0.487	5.116
C-reactive protein (mg/dL)	12.255	3.264	0.0003	5.767	18.744
i-PTH (pg/mL)	0.426	0.122	0.001	0.183	0.668
Serum calcium corrected for albumin (mg/dL)	6.707	14.040	0.634	-21.203	34.617
Serum phosphorus (mg/dL)	-7.410	21.030	0.725	-49.217	34.397
Product of serum calcium corrected and phosphorus	0.767	2.452	0.755	-4.107	5.641
eGFR (mL/min/1.73 m^2)	1.412	0.479	0.004	0.459	2.364
Serum albumin (g/dL)	-4.211	6.309	0.506	-16.752	8.330

On multivariate regression analysis, triglyceride, serum homocysteine, CRP, i-PTH, eGFR were significant independent factors affecting CAC score after adjusting for confounding factors. With the increase in triglyceride (mg/dL), serum homocysteine (µmol/L), CRP (mg/dL), i-PTH (pg/mL), eGFR (mL/min/1.73 m^2) by 1-unit, CAC score significantly increased by 0.477, 2.802, 12.255, 0.426, 1.412 units, respectively (Table VII).

Discussion

CKD is a global health burden with a high economic cost to health system and is an independent risk factor for CVD. All stages of CKD are associated with increased risks of cardiovascular morbidity, mortality and decreased quality of life. So, assessing the risk of CVD in CKD patients is essential. The risk of CVD can be predicted by CAC score, carotid intima-media thickness, ankle-brachial index, brachial flow-mediated dilation and high-sensitivity CRP. Many studies showed that CAC score according to Agatston Classification, is the most appropriate method to classify persons into CVD risk categories[7,8]. So, in present research, CAC score was taken as an indicator of CVD progression. It is a noninvasive marker of subclinical atherosclerosis and has been an independent predictor of cardiovascular events and also endorsed by the American Diabetes Association[9].

In present research, CAC was significantly high in CKD patients than control group. Out of 100 patients with CKD, evidence of CAC was found in 65% and in control group calcification was present in 20 % of people i.e., 6 individuals. This is not fully explained by Framingham's traditional risk factors, which are also present in the general population. Some non-traditional risk factors specifically present in CKD patients, also contributed to this. Elraoof *et al* found 90% of CKD patients with CAC and 25 % in control group[10].

Severity of calcification was significantly associated with decline in renal function. In the study of Hyun *et al* (KNOW-CKD), low eGFR was independently associated with CAC (p = < 0.001)[11]. Roy *et al* also found that patients with mild CKD had 2.2 times and moderate CKD had 6.4 times respectively, more CAC than the group with normal eGFR[12]. CAC is also associated with age, but we didn't find such association in our study. The probable reason for this may have been a small sample size. In present study, both CRP and homocysteine were significantly associated with CAC score. These markers have both atherogenic and thrombogenic effects that cause endothelial dysfunction by increasing oxidative stress, decreasing nitric oxide release and impairing vasodilatation. They also facilitate the differentiation of VSMCs into osteoblastic cells[13-15]. Kochi *et al* observed that the adjusted hazard ratios (HR, 95% confidence interval) for CVD were 1.88 (0.25 - 9.44) for patients with CKD with low CRP and 9.71 (3.27 - 31.97) for those with CKD with high CRP[16]. Cohen *et al* also found that homocysteine concentration was increased with a decline in eGFR (p = < 0.0001) and was significantly associated with CVD[17].

Hypertension is also an independent risk factor for CVD and CAC and it contributes to CAC by vascular remodelling and arteriosclerosis. The RAAS system also contributes to the pathogenesis of calcification. Amouei *et al* conducted a study and found hypertension in 40% and 62% of patients with CAC score ≤ 100 and CAC score > 100, respectively (P value = < 0.01)[18]. Chen *et al* also found that patients with CAC had higher systolic and diastolic BP than normal coronary arteries (p value = < 0.001)[19]. Hypoalbuminaemia was also significantly associated with CAC. The serum albumin is an imperative for maintaining oncotic pressure and microvascular integrity, regulating metabolic and vascular functions, providing binding ligands for substances, antioxidant activities, and anticoagulant effects. It is also involved in the vasodilatory response to NO[20]. Verma *et al* also found that serum albumin was significantly associated with aortic calcification. In their research the mean value of serum albumin (g/dL) was 3.93 ± 0.51 with aortic calcification index (ACI) < 20% and 3.56 ± 0.59 with ACI > 20% (p value = 0.0001)[21]. Anaemia also contributes to CVD and CAC. It causes medial wall calcification and osteoblastic transformation of VSMCs. Iron also plays an essential role in the oxidative phosphorylation in myocytes. Its deficiency causes cardiac remodelling and myocyte damage[22]. Mizuiri *et al* conducted a study to establish an association between iron deficiency anaemia and CAC and found that serum iron and transferrin saturation (TSAT) levels were significantly lower in patients with CAC score ≥ 400 than in those with CAC score < 400 (odds ratio 0.46, p = < 0.05)[23].

Abnormal mineral-bone metabolism was associated with higher CAC. The mechanism involved in this; abnormal mineral metabolism induces transdifferentiation of VSMCs into osteoblastic phenotype by Osf2/Cbfa1. They also accelerate the expression of osteochondrogenic markers such as Runx2, osterix, osteopontin, and alkaline phosphatase. Many studies are consistent with this association[24-26.] Russo *et al* found that higher serum concentration of phosphorus was significantly associated with greater progression of CAC[27]. Han *et al* also concluded that serum calcium, serum phosphorus and iPTH were significantly associated with CAC (p value = < 0.0001)[28]. Verma *et al* also found significant association of serum phosphorous level with vascular calcification (p value = < 0.0001)[29]. Bore *et al* found that iPTH was inversely related to eGFR (P = < 0.0001); they also found that, after adjusting for age and diabetes, iPTH was associated with myocardial infarction (OR, 1.6; 95% CI, 1.1 to 2.3 per unit natural log PTH) and congestive heart failure (OR, 2.0; 95% CI, 1.3 to

2.9 per unit natural log PTH)[30].

Insulin resistance is common in CKD patients and HbA1C can be used as a reliable marker of insulin resistance and in present research it was significantly associated with a higher CAC score. It accelerates the process of atherosclerosis by inducing oxidative stress, low-grade inflammation and endothelial dysfunction. The main mechanism involved in CAC includes an increase expression of bone matrix proteins such as osteopontin, type I collagen and alkaline phosphatase in the medial layer of blood vessels. It also accelerates the face of the osteoblast transcription factors-like RUNX2, BMP-2 and osteocalcin. Cavero-Redondo *et al* conducted a study and found that HbA1C was a reliable risk marker for cardiovascular mortality in both diabetics and non-diabetics[31]. Chen *et al* also found that HbA1C was increased with increasing calcification (p value = < .001)[19].

In the present study, dyslipidemia was significantly associated with a high CAC score, a predictor of CVD. Non-HDL lipids are highly atherogenic and leading to thrombogenesis by fibrinolysis inhibition while HDL lipids hamper atherosclerosis by cholesterol transport from the arterial wall to the liver for further excretion, inhibition of inflammation, platelet adhesion and LDL oxidation, etc. The mechanism behind collaboration of lipids with CAC is that non-HDL lipids and oxidized lipids enhance pro-calcific activity and mineralisation of vascular cells. It has a direct effect on both bone-forming and bone-resorbing cells. Alamgir *et al* found that the normolipidemic group had the highest percentage of individuals with normal vessels (60%) than hyperlipidemia group who had the highest rate of three vessels and four vessels calcification[32]. Elraoof *et al* found that dyslipidemia was associated with high CAC score[10]. Chen *et al* found that dyslipidemia was more strongly associated with CAC score > 100 than CAC score < 100 (p value = < 0.001)[33].

In the present research, significant correlation of CAC was found with eGFR, hypoalbuminaemia, abnormal mineral metabolism (hypocalcaemia, hyperphosphataemia, iPTH and product of calcium and phosphorous), CRP, homocysteine, HbA1C, dyslipidemia (decreased HDL, increased LDL, increased total cholesterol and increased triglyceride). This association was also significant on univariate regression analysis. But on performing multivariate regression analysis, only triglyceride, serum homocysteine, CRP, i-PTH, eGFR were significant after adjusting for confounding factors.

There were some limitations of this study. First, the cross-sectional study design does not establish a causal relationship between CAC and CKD and prospective studies are required for the same. The sample size of the study was only 130, including the control group. A larger sample size would have allowed a more accurate justification of the results of our research. Our study population had 30% of diabetic patients. As diabetic patients frequently have a high prevalence of cardiovascular disease, this could have been a confounding factor in our study. Due to the high prevalence of anaemia in CKD patients, HbA1C could be falsely low. Patients taking uric acid lowering agents, phosphate binders or erythropoietin stimulating agents, were not excluded.

As per study results, CAC has marked prevalence in CKD patients and being a surrogate marker of CVD, it indicates the increase risk of cardiovascular events in CKD patients. It is affected not only by abnormal mineral metabolism but also by many traditional and non-traditional risk factors such as hypertension, insulin resistance, dyslipidemia, anaemia, hyperhomocysteinaemia, CRP, uric acid, hypoalbuminaemia etc. Although CAC is more pronounced in dialysis patients, it also has prognostic significance in predialysis patients. So, by assessing CAC score high-risk patients can be distinguished early in course of disease, facilitating timely intervention and thereby preventing complications and improving quality of life in CKD patients. Follow-up and interventional studies are required for further evaluation and establishing a strong association between CAC as a major prognostic/predicting factor for CVD in CKD patients.

Conclusion

In conclusion, cardiovascular morbidity and mortality in CKD patients has become a huge problem in our country. Present study's results suggest that risk factors of CVD are highly prevalent in CKD patients. Cardiac calcification should be considered a marker of CVD risk in CKD patients and it improves risk prediction for CVD. It is also independently and significantly related to the dangers of cardiovascular disease. Various traditional and non-traditional risk factors such as increased CRP, iPTH, homocysteine, anaemia, hyperphosphataemia, dyslipidemia, insulin resistance, etc., accelerate the rate of cardiac calcification.

Further studies are warranted to determine measures to retard the rate of progression of cardiac calcification. The severity of CAC, dyslipidemia and HbA1C is negatively associated with kidney function. Present study showed that CAC score, lipid profile and HbA1C are reliable markers for screening CVD in CKD patients. Early diagnosis of CVD can help in aggressive management and hence decrease the morbidity and mortality associated with CKD. Several novel therapies to reduce the risk of CVD in CKD are in clinical development or have been already established, raising the hope that cardiovascular risk in patients with CKD may be modifiable in the future. So, the patients with even mild CKD who are not yet on haemodialysis, should be screened and treated more aggressively for heart disease than the average population.

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