Original Article

Osteopontin, Cardiovascular Risk Factors and Carotid Intima-Media Thickness in Chronic Kidney Disease

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Abstract

The pleiotropic cytokine osteopontin (OPN) is found to be involved in the pathogenesis of both kidney and cardiovascular disease (CVD). We evaluated the relationship between OPN, other cardiovascular risk factors and carotid intima-media thickness (CIMT) in chronic kidney disease (CKD) (predialysis) patients. This is a 2-year cross-sectional prospective study involving 75 patients with CKD from stage 1 to stage 5 attending the nephrology outpatient department and 25 healthy controls. Routine biochemical parameters were analyzed on clinical chemistry Autoanalyzer Beckman Coulter DXC 600 Synchron, USA. OPN was estimated by ELISA method. Carotid intima-media wall thickness was estimated by Doppler of carotid vessels. Serum OPN and other nontraditional cardiovascular risk factors such as CIMT, lipoprotein (a) Lp(a), fibrinogen, and homocysteine were significantly increased in patients of CKD compared to controls. OPN, Lp(a), fibrinogen, CIMT, parathyroid hormone, and homocysteine progressively increased from early stages of CKD and increased further with progression of the disease, but nitric oxide (NO) level progressively decreased with progression of CKD. OPN showed a positive correlation with CIMT, Lp(a), fibrinogen, and homocysteine and negative correlation with estimated glomerular filtration rate and NO. There was a close direct association between circulating levels of OPN and the presence of atherosclerotic plaques in carotid arteries of patients with CKD. Osteopontin and nontraditional CVD risk factors are altered in early stages of CKD and might predict adverse outcomes in these patients.

Keywords: *Cardiovascular disease risk factors, carotid intima-media wall thickness, chronic kidney disease, osteopontin*

Introduction

Impaired renal function is an independent risk state for cardiovascular disease (CVD) and associated mortality.[1] The global burden of both CVD and chronic kidney disease (CKD) is increasing, which is partially attributable to the growing prevalence of shared traditional risk factors such as advanced age, diabetes mellitus, and hypertension. The Framingham Heart Study has adjusted for traditional CVD risk factors such as age, sex, diabetes mellitus, hypertension, smoking, and lipids and reported that nontraditional risk factors increase as kidney function declines and are hypothesized to be stronger CVD risk factors in individuals with CKD.[2] The risk of cardiovascular events is increased markedly in patients with severe CKD, with a cardiovascular mortality being 10–30-fold higher than that of age-matched controls. Traditional and nontraditional or novel risk factors contribute to elevated risk of cardiovascular events in patients with reduced estimated glomerular filtration rate (eGFR).[3] Osteopontin (OPN) has been implicated as the key factor in a variety of biological processes such as immune cell activation, bone resorption, inhibition of vascular calcification, extracellular matrix remodeling, and atherosclerosis.[4] OPN as T-helper 1 cytokine is believed to exacerbate inflammation in several chronic inflammatory diseases, including atherosclerosis. OPN besides proinflammatory functions, physiologically, is a potent inhibitor of mineralization, prevents ectopic calcium deposits, and is a potent inducible inhibitor of vascular calcification.[5] OPN expression on atherosclerotic plaques is shown to be closely associated with the severity of atherosclerosis and calcification. The diverse roles of OPN related to cardiovascular diseases such as atherosclerosis, valvular stenosis, hypertrophy, myocardial infarction and heart failure were discussed by

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Singh *et al*.[6] Abnormalities in lipid metabolism occur in patients with all stages of CKD. The most common dyslipidemia in CKD is hypertriglyceridemia, whereas the total cholesterol levels can be high, normal, or low, mostly due to malnutrition.[7] Elevated plasma low-density lipoprotein cholesterol (LDL-C) concentration is not a typical feature of advanced CKD, especially those who are on hemodialysis (HD). There could be more qualitative changes in LDL in patients with CKD.

Elevation of several nontraditional risk factors was associated with an increased risk of CVD in CKD and HD patients.[8] Elevated plasma levels of lipoprotein (a) (Lp(a)) were present in patients with CKD.[9] In CKD patients, plasma fibrinogen concentration was also reported to be increased and correlated with systemic markers of inflammation such as C-reactive protein (CRP) and interleukin-6.[10] The association between total homocysteine and risk for atherothrombotic disease is not a consistent finding in end-stage renal disease (ESRD). Some cross-sectional studies report higher levels whereas others report no difference or even paradoxically lower total homocysteine levels in ESRD patients with CVD.[11] Hyperparathyroidism, hypovitaminosis D, and hyperphosphatemia are found in ESRD and develop progressively as renal function deteriorates.[12] Endothelial dysfunction has a complex pathophysiology involving multiple mechanisms. Either reduced synthesis or decreased bioavailability of nitric oxide (NO) is one of the fundamental mechanisms in the development of endothelial dysfunction.

Increased intima-media thickness (IMT) of the common carotid artery represents a feature of atherosclerosis that is manifested as diffuse arterial wall thickening, whereas increased IMT of the proximal internal carotid artery is a surrogate for focal atherosclerotic plaque.[13] The mean IMT of the common carotid artery is a more reproducible measure than the IMT of the internal carotid artery and is believed to be better suited for cardiovascular risk assessment.[14]

Kennedy *et al*. found an increased carotid IMT (CIMT) in predialysis patients in their study. Preston *et al*. studied the association between CIMT and cardiovascular risk factors in CKD. They found that the arterial changes occur early in the course of renal disease progression and might be related to dyslipidemia in the early stages.[15] The aim of present study was to assess the cardiovascular risk factor profiles by both traditional (dyslipidemia) and nontraditional risk factors (serum Lp (a), fibrinogen, alkaline phosphatase, homocysteine, parathyroid hormone (PTH), Vitamin D, NO, and OPN) in CKD patients with varying degrees of renal dysfunction. We also aimed to investigate the potential relationship between plasma OPN levels with other cardiovascular risk markers and carotid IMT.

Materials and Methods

A 2-year, cross-sectionalstudy was undertaken to study cardiovascular risk factors in CKD. A total number of 75 patients with CKD from stage 1–5 attending the Nephrology Outpatient Department at Sri Venkateswara Institute of Medical Sciences, were divided into three groups based on the stage of the disease (classified as per NKF K/DOQI) with 25 patients in each group being included into the study after obtaining written informed consent. Twenty-five healthy controls were included as Group 1 in the study. Inclusion criteria included Group 1: controls, Group 2: CKD stage 1 and 2, Group 3: CKD stage 3 and 4, and Group 4: CKD stage 5. Exclusion criteria included acute kidney injury, acute on CKD, smoking, pediatric age group (<18 years), pregnant women, unwilling patients, diabetes mellitus, vasculitis, liver disease, malignancy, and history of alcohol abuse. Controls included healthy individuals, who were the patients' relatives and hospital staff, nonsmokers, nondiabetics as per ADA criteria,[16] nonhypertensive as per the Joint National Committee 8 guidelines[17] after obtaining informed consent.

Sample collection

Six milliliters of fasting venous blood was collected and 2 ml of blood was transferred into EDTA bottle for the estimation of plasma fibrinogen, and the remaining blood was collected in additive-free tubes for other investigations. The blood samples were allowed to stand for 30 min and centrifuged at 3000 rpm for 15 min and the separated serum was stored at −80°C until further analysis. Serum urea, creatinine, total cholesterol, triglycerides, high-density lipoprotein (HDL), calcium, phosphorus, albumin, alkaline phosphatase, and homocysteine were estimated using commercial kits. All the above parameters were analyzed on clinical chemistry Autoanalyzer Beckman Coulter DXC 600 Synchron, USA. LDL and very LDL (VLDL) were calculated by Friedewald's equation.[18] Fibrinogen and Lp(a) were estimated by immunoturbidimetry method by commercial kits. PTH was estimated by chemiluminescent assay and Vitamin D was estimated by immunoradiometric assay on Beckman analyzer. Serum NO was estimated by spectrophotometric method using PerkinElmer spectrophotometer, Lambda 25 UV/VIS Spectrophotometer, USA.[19] OPN was estimated by ELISA method using the commercially available human OPN ELISA kit (Cloud clone, USA). Carotid intima-media wall thickness was estimated by Doppler of carotid vessels on Voluson 730 GE Medical Systems.

Statistical analysis

All continuous variables were tested for normal distribution with the Kolmogorov–Smirnov test. Normally distributed values were presented as mean ± standard deviation (SD) and for data in nonnormal distribution as median and IQR. Categorical values were presented

as numbers and percentage. Unpaired student's *t*-test was used for comparison of means between controls and cases. Comparison of means across the groups was done by ANOVA followed by *post hoc* analysis. Pearson's correlation coefficient was used for testing the association between the parameters studied. Binary logistic regression analysis was performed to show the association between categorical variable and numerical variable. Statistical analysis was performed using Microsoft Excel spreadsheets and SPSS Version 22.0 (IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp). $P < 0.05$ was considered statistically significant.

Results

All patients of the study were from the Department of Nephrology, Sri Venkateswara Institute of Medical Sciences. Seventy-Five CKD patients and 25 controls were included in the study. The mean ± SD values of the traditional and nontraditional biochemical parameters of the 25 controls and 75 CKD patients using unpaired *t*-test are shown in Table 1. Statistical analysis using one-way ANOVA of comparison of various parameters among different groups of CKD compared to controls is shown in Table 2. Significance of difference within the groups is shown in Table 3 using ANOVA followed by Bonferroni *post hoc* analysis. Correlation of OPN with other cardiovascular risk factors is shown in Table 4. There was no significant correlation of OPN with serum alkaline phosphatase, Vitamin D, and PTH in CKD patients in our study. Serum OPN levels showed significant association with the presence of atherosclerotic plaques in carotid arteries with odds ratio of 1.110, 95% confidence intervals (1.040–1.184) ($P = 0.002$*).

Table 1: The mean±standard deviation values of biochemical parameters in patients of chronic kidney disease and controls

Parameter	Controls (*n*=25)	CKD patients (*n*=75)	*p*
Serum urea (mg/dl)	24.04±9.63	59.01±39.27	0.001*
Serum creatinine (mg/dl)	0.87±0.22	3.53±2.45	0.000*
Serum cholesterol (mg/dl)	181.56±31.07	187.23±55.73	0.528†
Serum TGL (mg/dl)	129.28±33.51	169.57±60.59	0.000*
Serum HDL-C (mg/dl)	36.32±7.55	43.30±11.62	0.006*
Serum VLDL-C (mg/dl)	25.88±6.89	33.89±12.14	0.000*
Serum LDL-C (mg/dl)	119.36±26.46	112.22±42.06	0.428†
Serum albumin (g/dl)	3.92±0.64	4.23±0.26	0.024*
Serum calcium (mg/dl)	9.10±0.45	9.13±0.60	0.786†
Corrected calcium	9.16±0.76	8.94±0.64	0.160†
Serum phosphorus (mg/dl)	4.29±0.621	4.64±0.85	0.063†
Calcium–phosphorus product	39.36±6.73	41.47±7.96	0.236†
Serum ALP (U/L)	86.72±23.30	90.48±32.74	0.597†
Serum HCY (µmol/L)	12.92±1.98	22.24±7.55	0.000*
Serum OPN (ng/ml)	10.20±2.38	33.89±9.57	0.000*
Fibrinogen (mg/dl)	200.88±21.69	419.36±61.42	0.000*
Serum lipoprotein (a) (mg/dl)	18.12±4.90	44.08±14.40	0.000*
Serum NO (µmol/L)	40.80±3.28	17.21±6.08	0.000*
Serum Vitamin D (ng/ml)	23.16±15.17	22.46±10.94	0.805†
Serum PTH (pg/ml)	21.20±15.66	55.53±99.65	0.005*
CIMT (mm)	0.47±0.06	0.55±0.14	0.000*

*Significant at the 0.05 probability level, †NS: Not significant at the 0.05 probability level. Group 1: Controls, Group 2: CKD Stage 1-5 CKD, CKD: Chronic kidney disease, TGL: Triglycerides, HDL-C: High-density lipoprotein cholesterol, LDL-C: Low-density lipoprotein cholesterol, VLDL-C: Very low-density lipoprotein cholesterol, ALP: Alkaline phosphatase, HCY: Homocysteine, OPN: Osteopontin, NO: Nitric oxide, PTH: Parathyroid hormone, CIMT: Carotid intima-media thickness

Discussion

CKD is associated with substantially increased risk of cardiovascular morbidity and mortality, independent of traditional cardiovascular risk factors such as diabetes, hypertension, and tobacco use.[20] In addition, several nontraditional cardiovascular risk factors are more prevalent in persons with CKD and have been discussed as potential mechanisms for increased cardiovascular risk in CKD.[21]

OPN is a new cardiovascular risk factor whose association with renal function was not extensively studied. OPN is implicated as a key factor in development of atherosclerosis and it is an important inhibitor of vascular calcification. Plasma levels of OPN are elevated in patients with CKD.[22] The serum OPN levels in the present study were increased in all the stages of CKD compared to controls. There was a significant increase in the serum OPN level with progression of CKD similar to the study done by Lorenzen *et al*. They found a direct association between OPN and markers of renal function (serum creatinine, homocysteine, and symmetric dimethylarginine) as well as with cardiovascular risk factors. In the present study, serum OPN levels were found to have a significant positive correlation with CIMT, which is a marker of cardiovascular risk and negative correlation with eGFR in CKD patients, emphasizing the influence of renal function on serum OPN levels. This was in concordance to the findings of Lorenzen *et al*.[23]

In the present study, among the lipid parameters, triglycerides, VLDL, and HDL were increased and total cholesterol and LDL were decreased in CKD patients compared to control group. In a study, Pennell *et al*., documented elevated triglycerides and VLDL and decreased HDL with less frequent elevations of LDL and total cholesterol in dialysis patients.[24] Rao *et al*. studied the altered lipid, lipoprotein, and apoprotein abnormalities along with Lp(a) in CKD patients with stage 1–5 and reported increased triglyceride levels in stage 1 and 2 CKD patients compared to controls and significantly high VLDL-C, Lp(a), and apoB levels in all the stages of CKD patients when compared to controls. Similar to the

Table 2: Comparison of various biochemical parameters among different groups of the study using one-way ANOVA

Parameter	Group 1	Group 2	Group 3	Group 4	*p*
Serum urea (mg/dl)	24.04±9.63	25.4±7.51	51.2±15.80	100.44±37.70	0.001*
Serum creatinine (mg/dl)	0.87±0.21	1.45±0.31	2.84±0.72	6.30±2.24	0.001*
Serum cholesterol (mg/dl)	181.56±31.07	218.72±56.44	178.68±55.91	164.28±39.93	0.001*
Serum TGL (mg/dl)	121.28±33.51	191.4±63.77	189.6±50.35	127.72±45.41	0.001*
Serum HDL-C (mg/dl)	36.32±7.54	51.76±10.02	42.16±11.80	36±6.70	0.743†
Serum VLDL-C (mg/dl)	25.88±6.89	38.32±12.57	37.84±10.05	25.52±9.24	0.001*
Serum LDL-C (mg/dl)	119.36±26.46	129.24±48.31	104.68±39.18	102.76±33.60	0.046*
Serum albumin (g/dl)	3.92±0.64	4.24±0.23	4.14±0.28	4.33±0.28	0.002*
Serum calcium (mg/dl)	9.10±0.45	9.06±0.80	9.30±0.43	9.04±0.50	0.344†
Corrected calcium	9.16±0.75	8.78±0.57	8.86±0.78	9.19±0.47	0.059†
Serum phosphorus (mg/dL)	4.29±0.62	4.22±0.56	4.54±0.75	5.15±0.94	0.000*
Calcium–phosphorus product	39.36±6.72	37.53±6.86	41.73±6.97	45.15±8.33	0.002*
Serum ALP (U/L)	45.15±8.33	39.36±6.72	37.53±6.86	41.73±6.96	0.002*
Serum HCY (µmol/L)	12.92±1.97	18.28±6.65	23.48±7.14	24.96±7.40	0.002*
Serum OPN (ng/ml)	10.20±2.38	23.40±4.12	33.39±4.62	42.47±7.51	0.001*
Fibrinogen (mg/dl)	200.88±21.69	360.65±34.41	426.04±40.11	471.28±49.06	0.001*
Serum lipoprotein (a) (mg/dl)	18.12±4.90	35.68±10.01	44.4±13.87	52.16±14.33	0.001*
Serum NO (µmol/L)	40.8±3.27	23.68±2.99	17.48±3.51	10.48±1.36	0.001*
Serum Vitamin D (ng/ml)	23.16±15.17	18.28±7.17	28.04±13.66	21.08±8.87	0.030*
Serum PTH (pg/ml)	17.00 (11-27.5)	17.00 (11-20.5)	18.00 (18-22)	61.00 (39.5-166)	0.000*
CIMT (mm)	0.47±0.06	0.48±0.14	0.57±0.14	0.59±0.01	0.001*
eGFR (ml/min/m^2)	86.76±23.95	49.52±11.71	26.16±9.76	9.29±3.33	0.001*

*Significant at the 0.05 probability level. †NS: Not significant at the 0.05 probability level. Group 1: Controls, Group 2: CKD Stage 1 and 2, Group 3: CKD Stage 3 and 4, Group 4: CKD Stage 5, CKD: Chronic kidney disease, TGL: Triglycerides, HDL-C: High-density lipoprotein cholesterol, LDL-C: Low-density lipoprotein cholesterol, VLDL-C: Very low-density lipoprotein cholesterol, ALP: Alkaline phosphatase, HCY: Homocysteine, OPN: Osteopontin, NO: Nitric oxide, PTH: Parathyroid hormone, CIMT: Carotid intima-media thickness, eGFR: estimated Glomerular filtration rate

above-mentioned studies, the present study also found an increase in triglycerides and VLDL-C in CKD patients compared to healthy controls. On contrary to other studies, a significant increase in serum HDL-cholesterol (HDL-C) was seen in patients of CKD compared to that of controls. However, serum total cholesterol, LDL-C, HDL-C, VLDL-C, and triglycerides progressively decreased in different stages of CKD with progression of disease, which is in contrast to dyslipidemia expected with CKD. This finding might be due to administration of statins as a part of management protocol.

In the present study, there was a progressive increase in the levels of serum Lp(a) with progression of CKD, but the elevation was not statistically significant between Groups 2 and 3. Mannangi and Jayasree studied 30 CKD patients and 30 healthy controls and found significant increase in serum Lp(a) levels in cases compared to controls.[25] Muntner *et al*. and Ponnudhali and Nagarajan compared the abnormal lipid profile pattern with the prevalence of decreased apolipoprotein A1 levels and increased apolipoprotein B, plasma fibrinogen, Lp(a), homocysteine, and CRP levels by eGFR. Bostom *et al*. in their study concluded that combined hyperhomocysteinemia, hyperfibrinogenemia, and elevated Lp(a) contributed to the higher incidence of vascular disease in dialysis patients, which was inadequately explained by traditional CVD risk factors.[26] From the above studies, it is evident that Lp(a) levels increased in early stage of CKD and adds to atherogenic lipid profiles of CKD. This correlated with the results of the present study as observed by a serial increase in serum Lp (a) with progressive increase in severity of CKD.[27] The fibrinogen levels were increased significantly in CKD patients and increases with progression of CKD. Goicoechea *et al*. included 128 outpatients with eGFR of <60 ml/min/1.73 m^2 in their study. The results in their study showed both high CRP and fibrinogen levels in CKD stages 3 and 4 and shown to be the independent risk factors for all-cause mortality supporting the findings of the present study.[28] In the present study, serum homocysteine levels were significantly higher in patients of CKD compared to controls and elevation was observed from early stages of CKD and there was a progressive increase in serum homocysteine level with progression of CKD. It was concluded by the findings of Mallamaci *et al*. and Moustapha *et al*. that hyperhomocysteinemia is an independent risk factor for cardiovascular mortality and morbidity in ESRD, with an increased relative risk of 1% per µmol/L increase in total homocysteine concentration.[29] From the above studies, it is evident that homocysteine levels were increased in CKD which was similar to the results in present study. Hyperparathyroidism, hypovitaminosis D, and hyperphosphatemia are nearly

Table 3: Comparison of various biochemical parameters between the groups of the study by *post hoc* Bonferroni analysis

Parameter	Gr1 vs. Gr2	Gr1 vs. Gr 3	Gr1 vs Gr4	Gr2 vs Gr3	Gr2 vs Gr 4	Gr3 vs Gr4
Serum urea (mg/dl)	1.000†	0.001*	0.001*	0.000*	0.001*	0.001*
Serum creatinine (mg/dl)	0.512†	0.000*	0.001*	0.001*	0.001*	0.001*
Serum cholesterol (mg/dl)	0.038*	1.000†	1.000†	0.028*	0.001*	1.000†
Serum TGL (mg/dl)	0.000*	0.001*	1.000†	1.000†	0.000*	0.001*
Serum HDL-C (mg/dl)	0.001*	0.167†	1.000†	0.002*	0.001*	0.123†
Serum VLDL-C (mg/dl)	0.001*	0.001*	1.000†	1.000†	0.001*	0.001*
Serum LDL-C (mg/dl)	1.000†	1.000†	0.740†	0.142†	0.089†	1.000†
Serum albumin (g/dl)	0.022*	0.318†	0.002*	1.000†	1.000†	0.472†
Serum calcium (mg/dl)	1.000†	1.000†	1.000†	1.000†	1.000†	1.000†
Corrected calcium	1.000†	1.000†	1.000†	0.763†	1.000†	0.654†
Serum phosphorus (mg/dl)	0.646†	1.000†	0.246†	0.458†	1.000†	0.165†
Calcium–phosphorus product	1.000†	1.000†	0.001*	0.763†	0.001*	0.026*
Serum ALP (U/L)	1.000†	1.000†	0.035*	0.259	0.002*	0.598†
Serum HCY (µmol/L)	0.018*	0.001*	0.001*	0.023*	0.002*	1.000†
Serum OPN (ng/ml)	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*
Fibrinogen (mg/dl)	0.001*	0.001*	0.000*	0.001*	0.001*	0.001*
Serum lipoprotein (a) (mg/dl)	0.001*	0.001*	0.000*	0.049*	0.001*	0.110
Serum NO (µmol/L)	0.000*	0.001*	0.001*	0.001*	0.001*	0.001*
Serum Vitamin D (ng/ml)	0.861†	0.861†	1.000†	0.024*	1.000†	0.228†
Serum PTH (pg/ml)	1.000†	1.000†	0.000*	1.000†	0.000*	0.000*
CIMT (mm)	1.000†	0.017*	0.006*	0.053†	0.019*	1.000†
eGFR (ml/min/m^2)	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*

*Significant at the 0.05 probability level, †NS: Not significant at the 0.05 probability level. Group 1: Controls, Group 2: CKD Stage 1 and 2, Group 3: CKD Stage 3 and 4, Group 4: CKD Stage 5, TGL: Triglycerides, HDL-C: High-density lipoprotein cholesterol, LDL-C: Low-density lipoprotein cholesterol, VLDL-C: Very low-density lipoprotein cholesterol, ALP: Alkaline phosphatase, HCY: Homocysteine, OPN: Osteopontin, NO: Nitric oxide, PTH: Parathyroid hormone, CIMT: Carotid intima-media thickness, eGFR: Estimated glomerular filtration rate, CKD: Chronic kidney disease, Gr-group

universally found in ESRD and develop progressively as renal function deteriorates. There is a growing evidence to support the view that elevated PTH, reduced production of active Vitamin D metabolites, altered tissue responsiveness to these hormones, sustained hyperphosphatemia, and aggressive use of calcium supplements and Vitamin D analogs are important in pathogenesis of CVD in CKD. In this study, statistically significant increase in PTH levels was found in patients of CKD compared to control group but significant increase in PTH levels was seen only in later stages of CKD (Stage 4-5) compared to controls. A large cohort study by Levin *et al*. of more than 4000 nondialyzed patients with stage 4–5 CKD (mean GFR 33 ml/min) and iPTH (median: 105 pg/ml, IQR: 57–188 pg/ml) and elevated phosphorus were associated with an increased risk of death and the progression of renal failure, whereas Vitamin D therapy was associated with better survival.[30] Vitamin D deficiency is common among patients with CKD. A higher level of serum Vitamin D is expected in residents of tropics in relation to inhabitants of nontropical regions, due to greater sun exposure and increased production of Vitamin D. The serum Vitamin D, i.e., 25(OH)D levels are lower in patients of CKD compared to controls in the present study. However, there were no significant changes in levels of serum 25(OH)D among different stages of CKD in this study. In the Brazilian study of predialytic CKD patients by Diniz *et al*. suggested that Vitamin D deficiency is associated with CVD, the most common cause of mortality.[31] Similar observations were drawn by Wolf *et al*. and Ravani *et al*. who studied the association between Vitamin D deficiency and CVD and early mortality in earlier stages of CKD patients.[32] However, in the present study, there was a decrease in serum 25(OH) D level in patients of CKD stage 5 compared to that of patients of CKD stage 3 and 4, but the difference was not significant (P = 0.228). This could be due to inadequate supplementation of active Vitamin D to the study population. Hypertension, hypercholesterolemia, and CKD are associated with diminished release of NO into arterial wall either because of impaired synthesis or excessive oxidative degradation. Diminished NO bioactivity may facilitate vascular inflammation that could lead to oxidation of lipoproteins and foam cell formation, the precursor of atherosclerotic plaque. The serum NO of patients with CKD was significantly lower than that of control group in the present study. In this study, there was a progressive decrease in the levels of serum NO with the progression of CKD and the difference of serum NO among different stages of CKD was also statistically significant except Group 3 versus Group 4 CKD.

Table 4: Pearson correlation analysis of osteopontin versus cardiovascular risk factors in patients of chronic kidney disease

Parameter	*r*	*p*
Blood urea	0.599	0.001*
Serum creatinine	0.591	0.001*
Serum total cholesterol	−0.350	0.002*
Serum TGL	−0.373	0.001*
Serum HDL-C	−0.488	0.001*
Serum VLDL-C	−0.376	0.001*
Serum LDL-C	−0.199	0.001*
Serum HCY	0.244	0.035*
Serum fibrinogen	0.710	0.001*
Serum ALP	−0.023	0.846†
Serum lipoprotein (a)	0.309	0.007*
Serum NO	−0.739	0.001*
Serum Vitamin D	0.181	0.121†
Serum PTH	0.162	0.166†
CIMT	0.273	0.018*
eGFR	−0.732	0.001*

*Significant at the 0.05 probability level, †NS: Not significant at the 0.05 probability level. *r*: Correlation coefficient, HDL-C: High-density lipoprotein cholesterol, LDL-C: Low-density lipoprotein cholesterol, VLDL-C: Very low-density lipoprotein cholesterol, PTH: Parathyroid hormone, ALP: Alkaline phosphatase, CIMT: Carotid intima-media thickness, eGFR: Estimated glomerular filtration rate, TGL: Triglycerides, HCY: Homocysteine, NO: Nitric oxide

Endothelial dysfunction provides an important link between renal disease and the increased risk of CVD in CKD patients. Either reduced synthesis or decreased bioavailability of NO is one of the fundamental mechanisms in the development of endothelial dysfunction. In a study done by Reddy *et al*., plasma NO levels were found to be significantly lower in all stages of CKD patients when compared to controls.[33] In the present study, carotid wall IMT was measured in patients with varying degrees of renal dysfunction. CIMT is a surrogate measure of atherosclerosis and an independent predictor of cardiovascular events. In this study, statistically significant increase in CIMT levels were observed in patients of CKD compared to healthy controls. Modi *et al*. evaluated the efficacy of using CIMT to predict the presence of CAD in patients with ESRD and observed significantly higher in those with CAD as compared to those without. The sensitivity and specificity of using CIMT > 0.75 mm as a predictor of CAD was 90.47% and 73% and its positive and negative predictive values were 0.73 and 0.92, respectively.[34] Preston *et al*. and Zhang *et al*. showed that there was an association between CIMT and cardiovascular risk factors in CKD. The above study suggested that arterial changes occur early in the course of renal disease progression and are related to dyslipidemia in early stages of CKD.[35] In concordance to the findings of the previous studies, the present study also observed increased CIMT in all stages of CKD than in control population and the increase of CIMT progressively increased with stages of CKD. Along with CIMT, plaque occurrence in carotid arteries is also a strong predictor of cardiovascular events in general population. Szeto *et al*. reported that in their study, 121 (59.6%) patients had visualized atherosclerotic plaques, but in our study, 22 (29.3%) patients had visualized atherosclerotic plaques.[36] The difference could be due to different study population and sample size in both studies. Both diabetics and nondiabetics with CKD were studied by Szeto *et al*., but in our study, only nondiabetic CKD group was studied. Moreover, in the present study, early stages of CKD were also included but Szeto *et al*. studied only CKD stage 3 and 4 patients. In our study, the presence of atherosclerotic plaques in patients with CKD was significantly higher than the control group and none of the patients of stages 1 and 2 CKD had atherosclerotic plaques in carotid arteries, but 9 patients of Group 3 and 13 in Group 4 had atherosclerotic plaques. Hence, it is evident that there was a progressive increase in the number of patients having atherosclerotic plaques in carotid arteries with progression of CKD. There was no evidence of atherosclerotic plaques in early stages of CKD, but they were found in later stages of CKD (from CKD stage 3 onward) and the difference was statistically significant between early and late stages of CKD ($P = 0.026$).

Conclusions

The observations of the present study revealed a progressive correlation between nontraditional risk factors with stages of CKD. The present study also reported OPN to be an early marker of cardiovascular risk, which progressively increased with the severity of the CKD when compared to controls. CIMT and atherogenic plaques also showed a correlation with the severity of the CKD, and there is a close direct association between circulating levels of OPN and the presence of atherosclerotic plaques in carotid arteries of patients with CKD.

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Conflicts of interest

There are no conflicts of interest.

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