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Original Article

Biochemistry Section

Role of Calcium and Phosphate Ionic Product as an Early Marker of Vascular Calcification to Predict Cardiac Risk in Chronic Kidney Disease: A Case-control Study

M DEEPTHI[1], MANGALA N SIRSIKAR[2], A SHAILAJA[3], SHRABANI MOHANTHY[4]

ABSTRACT

Introduction: Cardiovascular disease is the most leading cause of death in patients with Chronic Kidney Disease (CKD) and Vascular Calcification (CV) is one of the strongest predictors of cardiovascular risk. CV is a process characterised by thickening and loss of elasticity of muscular arteries walls which occurs in two distinct sites, the intimal associated with atherosclerotic plaques and medial calcification is characterised by vascular stiffening and arteriosclerosis with adverse clinical outcomes leading to cardiovascular mortality. Disturbed mineral metabolism such as increased serum phosphorus and ionic product may be one such risk factor and is emerging as a principle modifier of CV in the CKD subjects.

Aim: To determine serum phosphorus and calcium in CKD patients and to compare with calcium phosphorus ionic product as an early independent marker of CV in CKD to predict cardiac mortality.

Materials and Methods: This was duration based case-control study conducted in Department of Nephrology, Vydehi Institute of Medical Sciences and Research Centre, Karnataka, India. Fifty cases of CKD presented in stage 3, 4 and 5 and 50 healthy individuals as controls between the age group 21-78 years were included. Serum levels of calcium, and phosphorous were analysed in autoanalyser, Ca x P ionic product was calculated, estimated Glomerular Filtration Rate (eGFR) was calculated by Modification of Diet in Renal Disease (MDRD) formula. All measured variables were compared with eGFR and compared between cases and controls. The results were presented as a mean±Standard Deviation (SD) and p-value <0.05 was considered as significant.

Results: Mean age of cases were 47.74±11.01 years and 45.66±11.46 years were of controls. Clinically, confirmed CKD was found more in male patients 31 (62%) compared to female 19 (38%). eGFR (mL/min) cases (14.12±10.72) and (102.97±27.46) in control, Serum Creatinine were 7.04±5.34 mg/dL in cases which was significantly more compared to control (0.84±0.20 mg/dL). Serum Calcium (Ca) 8.11±1.09 mg/dL in cases less compared with control 9.31±0.42 mg/dL, indicating hypocalcaemia. Serum Phosphrous (P) 5.29±1.83 mg/dL) in CKD suggesting hyperphosphatemia compared to control (3.27±0.54 mg/dL). Calcium Phosphorus Ionic Product was 46.91±14.77 in cases, elevated in CKD as compared to control (30.46±5.03). Statistically, significant result was found between serum phosphorus and calcium phosphorus ionic product ($p<0.05$), well compared with eGFR of stage 3, 4 and 5 CKD patients.

Conclusion: Hyperphosphatemia and elevated calcium-phosphate ionic product is an early independent marker of calcification to predict cardiovascular risk in late stages of CKD.

Keywords: Calcific atherosclerosis, Cardiovascular risk, Chronic kidney disease, Disturbed mineral metabolism, Intimal plaque

INTRODUCTION

The Chronic Kidney Disease (CKD) is a serious public health issue that affects more than 10 of the world's population and become much more common in recent years with increasing trend [1,2]. Renal Mineral Bone Disease (MBD) is a systemic disease characterised by an abnormal metabolism of calcium, phosphorus, Parathormone (PTH) and vitamin D, which in addition to affecting the skeletal system, is also significantly related to the appearance of cardiovascular and soft tissue calcifications, which, in turn, is associated with cardiovascular emergencies in patients with CKD and is the leading cause of death [3]. CV is defined as inadequate and pathological mineral deposition in the form of calcium phosphate salts in vascular tissues. This can happen as part of normal ageing, accelerated in some disease states, including diabetes mellitus, cardiovascular disease, and specific genetic diseases.

Calcification of arterial media (calcific arteriosclerosis or Mönckeberg's sclerosis) and increased calcification of the intimal plaque (calcific atherosclerosis) were two types of CVs which affects a majority of people with long-term CKD and especially, in dialysis patients. Calcific atherosclerosis is thought to be the last stage of classical atherosclerosis, whereas medial calcification is generally non inflammatory and connected with haemodialysis, calcium-phosphate imbalances, diabetes, and ageing [4]. This CKD-related increased cardiovascular morbidity/death has been identified in patients with no signs of ischaemic heart disease [5]. It was the cause of increased mortality rate among individuals in the early stages of CKD (20, 2, and 6 at 5 years, accordingly). Phosphorus {mainly in the form of inorganic P (Pi)} is an important component of cellular and systemic homeostasis. As glomerular filtration rate decreases in advanced CKD, inefficient urinary excretion of P combined with disordered bone remodeling and continued ingestion of P results in hyperphosphataemia. The role of hyperphosphatemia as a significant risk factor in the development of CV may be related to the role of sodium-dependent phosphate transporters [6]. Of the three types of sodium-dependent phosphate transporters, types I and II are mainly expressed in the kidney and intestinal epithelium and play an important role in phosphorus homeostasis in the body.

In contrast, the type III sodium-dependent phosphate transporters Pit1 and Pit2 are ubiquitously expressed in cells, including kidney, brain, heart, lung, and liver, as well as in osteoblasts and vascular smooth muscle cell. It is believed that they are involved in local and intracellular phosphate homeostasis and are associated with pathological down-regulation calcifications [7]. There was a loss of specific gene expression of smooth muscle and an upregulation of

bone differentiation genes resulting in differential factors (osteocalcin, osteopontin and Runx2, etc.), resulting in osteogenic differentiation. Calcium deposition also occurs in prolonged hypercalcaemic environments (>11 mg/dL). Calcium increases Pit1 mRNA levels, increases the sensitisation of smooth muscle cells to phosphorus and leads to osteoblastic differentiation. An increase in serum phosphate has also been associated with an elevated CaxP product, altered alkaline phosphatase, and decreased matrix Gla protein, which together play a role in VC [8]. CKD patients with elevated serum phosphorus and calcium × phosphate products accelerated medial and intimal calcification, and this calcification progresses rapidly in dialysis patients.

Therefore, an increase in serum levels of phosphorus and calcium × phosphate produced was directly related to an increase in mortality in a large number of haemodialysis patients in CKD patients. Limited data were available in the Indian population studying the relationships between phosphorus and the calcium-phosphorus ion product to establish their role as an independent risk factor for the development and progression of CV in patients with advanced stages of CKD.

MATERIALS AND METHODS

The present study was a case-control duration-based study conducted in the Department of Nephrology from 1st June 2014 to 1st June 2016 at Vydehi Institute of Medical Sciences and Research Centre, Bangalore, Karnataka, India. The study was approved by Vydehi Institutional Ethical Clearance (VEC) with no. VIMS&RC/VEC/103/2013-2014. After taking informed consent with structured questionnaire 50 patients of advanced CKD (stage 4 and 5) between the age group of 21-78 years and age/sex matched 50 healthy individuals as controls were enrolled in study.

Sample size calculation: Sample size was calculated using the formula:

$$n = \frac{(2)\ \sigma^2(Z_{1-\alpha/z}+Z_{1-\beta})^2}{d^2}$$

σ=standard deviation of calcium-phosphate product (7.21) $Z_{1-\alpha/z}$=1.96 at 95 confidence level, $Z_{1-\beta}$ = 0.84 at 80 power d = clinically significant difference (4)

$n = (2)\ (7.21)^2\ (1.96 + 0.84)^2/(4)^2$

n = 50 per group.

Inclusion criteria: Patients with stage 4 and 5 of CKD, eGFR were 15-29 ml/min/1.73 m^2. 15 ml/min/1.73 m^2, respectively in men and women, age group of 21-78 years. Subjects with no intake of Ca/vitamin D supplements and phosphate binders were included in the study.

Exclusion criteria: Chronic smokers and alcoholic patients, with history suggestive of and clinically confirmed angina, myocardial infarction, stroke, and peripheral vascular disease, liver disease bone disorders and bone fractures excluded were excluded. Also patients on glucocorticoid, bisphosphonate, non steroidal anti-inflammatory drugs, phenytoin, or warfarin and on immunotherapy or immunosuppressive treatment were also excluded from the study.

Procedure

Within 24 hours of admission to the hospital before initiating treatment, 5 mL of blood samples were taken.

Serum levels of magnesium measured by Calmagite method, serum calcium (8.5-10.2 mg/dL by Ion Selective Electrode (ISE) method, serum phosphorous (2.5-4.5 mg/dL) levels by Ammonium Molybdate method. Magnesium (1.8-2.9 mg/dL) by calmagite chromogen method, serum creatinine (0.60-1.24 mg/dL (M), 0.40-1.00 mg/dL (F) by modified jaffes method. CKD was defined and classified as per Kidney Disease Outcomes Quality Initiative (KDOQI) criteria [9]. The MDRD equations were being used to determine the eGFR from serum creatinine levels [10]. Accordingly eGFR was categorised as

1) G1- ≥90 - Normal or high
2) G2- 60-89-Mildly decreased
3) G3a- 45-59-Mildly to moderately decreased
4) G3b- 30-44 Moderately to severely decreased
5) G4 15-29 Severely decreased
6) G5 <15 Kidney failure. The diagnosis of underlying basic kidney disease was made on clinical evidence. The definitions for hypocalcaemia (cCa1 8.5 mg/dL), hyperphosphatemia (phosphorus >4.5 mg/dL) [11].

STATISTICAL ANALYSIS

Data was entered into Microsoft excel data sheet and was analysed using Statistical Package for the Social Sciences (SPSS) 22.0 version software. Categorical data was represented in the form of frequencies and proportions. Chi-square test was used as test of significance for qualitative data. Continuous data was represented as mean±SD. Independent t-test was used as test of significance to identify the mean difference between two quantitative variables. Analysis of Variance (ANOVA) was the test of significance to identify the mean difference between more than two groups for quantitative data. The p-value <0.05 was considered as statistically significant after assuming all the rules of statistical tests.

RESULTS

Mean age of CKD subjects were 47.74±11.01 years as compared with control 45.66±11.46 years. Samples were age matched with p-value of 0.357. More number of cases were observed between 41-60 years [Table/Fig-1].

	Cases		Controls	
Age (years)	n	%	n	%
21-30	4	8.0	6	12.0
31-40	9	18.0	10	20.0
41-50	17	34.0	16	32.0
51-60	14	28.0	13	26.0
61-70	6	12.0	5	10.0
Total	50	100.0	50	100.0
Mean±SD	47.74±11.01		45.66±11.46	

[Table/Fig-1]: Age distribution of patients studied.
Student's t-test; Samples are age matched with p-value=0.357

More number of clinically confirmed CKD subjects belonged to male patients 31 (62%) as compared to female 19 (38%). Samples were gender matched with p-value of 0.529 [Table/Fig-2].

	Cases		Controls	
Gender	n	%	n	%
Female	19	38.0	16	32.0
Male	31	62.0	34	68.0
Total	50	100.0	50	100.0

[Table/Fig-2]: Gender distribution of patients studied.
Chi-square test; Samples are gender matched with p-value=0.529

Late stages of CKD were divided into three categories and found more patients in stage 4 i.e. 14 (28%) and 32 (64%) in stage 5 [Table/Fig-3].

CKD Stage	No. of patients	%
3	4	8.0
4	14	28.0
5	32	64.0
Total	50	100.0

[Table/Fig-3]: CKD Stage in two groups studied.

Serum calcium levels found <8.5 mg/dL in 35 CKD (70%) cases as compared to control suggestive of hypocalcaemia [Table/Fig-4].

Serum phosphorus value >4.5 to <6 mg/dL in 20 (40%) of CKD patients. In 18 (36%) of patients showed values >6.5 mg/dL as compared to control subjects. The results suggested that there was significant hyperphosphatemia in CKD patients as compared with normal control subjects [Table/Fig-5].

Serum calcium (Ca) (mg/dL)	Cases		Controls	
	n	%	n	%
<8.5	35	70.0	0	0.0
8.5-10.2	13	26.0	50	100.0
>10.2	2	4.0	0	0.0
Total	50	100.0	50	100.0

[Table/Fig-4]: Serum Calcium (Ca) (mg/dL) in two groups studied.

Serum phosphrous (P) (mg/dL)	Cases		Controls	
	n	%	n	%
<2.5	4	8	2	4
2.5-4.5	8	16	45	90
>4.5 to <6.5	20	40	1	2
≥6.5	18	36	3	6
Total	50	100	50	100

[Table/Fig-5]: Serum Phosphrous (P) in two groups studied.

Chi-square test of association showed results of Calcium Phosphorus Ionic Product was more in CKD patients as compared to control suggestive of increased calcium phosphorus ionic product in cases [Table/Fig-6].

eGFR (mL/min) in CKD cases was 14.12±10.72 found very less compared to control subjects 102.97±27.46 suggestive of end stage CKD [Table/Fig-7].

Ca x P Ionic product	Cases		Controls	
	n	%	n	%
<55	6	12.0	50	100.0
>55	44	88.0	0	0.0
Total	50	100.0	50	100.0

[Table/Fig-6]: Ca x P Ionic Product in two groups studied.

Variables	Cases	Controls	p-value
eGFR (mL/min)	14.12±10.72	102.97±27.46	<0.001**
Serum Urea (mg/dL)	119.88±71.29	23.67±5.76	<0.001**
Serum Creatinine (mg/dL)	7.04±5.34	0.84±0.20	<0.001**
Serum Calcium (Ca) (mg/dL)	8.11±1.09	9.31±0.42	<0.001**
Serum Phosphrous (P) (mg/dL)	5.29±1.83	3.27±0.54	<0.001**
Ca x P Ionic Product	46.91±14.77	30.46±5.03	<0.001**

[Table/Fig-7]: Comparison of study variables in two groups studied.
** A p-value <0.01 was considered to be highly statistically significant

There was a statistically significant results found between serum phosphorus and Ca x P ionic product (p<0.001) which was very well compared with eGFR of stage 3/4/5 CKD patients.

[Table/Fig-8] shows comparison of co-morbidities with respect to CKD stage showed 14 (43.8%) on dialysis in stage 5, in which 12 (37.5%) with type II DM and Hypertension, 23 (71.9%) found to have abnormal lipid profile.

Co-morbidities		CKD stage						p-value
		Stage 3		Stage 4		Stage 5		
		Count	%	Count	%	Count	%	
On dialysis	No	3	75.0	7	50.0	18	56.2	0.673
	Yes	1	25.0	7	50.0	14	43.8	
DM	No	2	50.0	8	57.1	20	62.5	0.862
	Yes	2	50.0	6	42.9	12	37.5	
HTN	No	1	25.0	8	57.1	20	62.5	0.357
	Yes	3	75.0	6	42.9	12	37.5	
Lipid profile	No	0	0	5	35.7	9	28.1	0.374
	Yes	4	100.0	9	64.3	23	71.9	

[Table/Fig-8]: Comparison of co-morbidities with respect to CKD stage.

DISCUSSION

The primary goal in patients with CKD is to manage Ca and P levels under control range to avoid vascular damage associated with poor mineral metabolism. Abnormal Ca and P homeostasis has been found to play an important role in the impulse of lenited Vascular Smooth Muscle Calcification (VSMC) in CKD. Hyperphosphatemia is prominent in patients with CKD and End Stage Renal Disease (ESRD) due to impaired renal phosphate clearance. The phosphate as well as calcium is not only a constituent of hydroxyapatite, but also contributes CV through signaling pathways. Serum phosphate concentration is usually maintained within 2.5-4.5 mg/dL by a variety of mechanisms until renal disease has progressed to approximately CKD stage 5 or ESRD. Hyperphosphatemia result in development of secondary hyperparathyroidism, calcium and vitamin D dysregulation CV.

As consequences hyperparathyroid state and altered vitamin D status in ESRD patients results in extraosseous calcifications Thus increasing Ca × P ionic product levels can act directly or indirectly on VSMCs to leading to calcification. Thus, it was found that hyperphosphatemia leads to development and progression of secondary hyperparathyroidism and a predisposition to metastatic calcification as the product of serum calcium and phosphorus (Ca x PO4) is elevated. Both of these conditions reinforce one another for significant morbidity and mortality seen in patients with ESRD. The proposed mechanism could be generation of free radicals, advanced glycation end products, proinflammatory markers which were enhanced by associated co-morbidities and treatment before and after dialysis.

Reactive Oxygen Species (ROS) produced in response to high P loads have also been implicated as downstream mediators of the osteogenic conversion of VSMCs during CV. The degradation of the vascular matrix [12], evident from the predominant medial arterial calcification induced by CKD and hyperphosphatemia, is due to the accumulation of linear mineral deposits along the arterial elastic lamina (elastocalcinosis) [13]. It also promotes, together with calcium and vitamin D, the formation and deposition of calcium phosphate crystals in soft tissues, in particular in the vessel wall, heart valves and periarticular regions. As a result of the increased oxidative stress, oxidised Low-Density Lipoproteins (LDLs) formed, which were found to trigger VSMC proliferation and differentiation towards a bone phenotype. Oxidative stress, on the other hand, inhibits the development of osteoblasts and bone marrow stromal cells [12,13].

Ganesh SK et al., demonstrated an association between calcium x phosphate product and causes sudden death from coronary artery disease [14]. They found that higher mortality risk was seen for patients in the high PO(4) group (>6.5 mg/dL) compared with the lower PO(4) group (< or =6.5 mg/dL). The Relative Risk (RR) of sudden death was also strongly associated with elevated Ca x PO(4) product (>72 mg^2/dL^2) had a relative mortality risk of 1.34 relative to those with products of 42 to 52 mg^2/dL^2. Similar results were obtained in present study. Block GA et al., found an association between calcium x phosphate product and the risk of hospitalisation for cardiovascular disease and demonstrated that an increase in calcium x phosphate product of 4.0-4.4 $mmol^2/L^2$ compared with the reference range of 3.2-3.6 $mmol^2/L^2$ is associated with an increased risk of all-cause of mortality and hospitalisation. Mortality in ESRD patients is strongly correlated with serum P levels >5.5 mg/dL [15].

Cardiovascular mortality was significantly higher for phosphorus concentrations above the K/DOQI-threshold in PD (2.4; 95 CI: 1.3-4.2) and HD patients (1.5; 95 CI: 1.1-2.1), and for elevated Ca×P in PD (2.2; 95 CI: 1.3-3.8) which was very well established in our study 38 patient reported phosphorus level more than 5.5 mg/dL. Young EW et al., demonstrated the association between the calcium x phosphate product and cardiovascular mortality. Hyperphosphatemia was present in 73.8% of the study population, while 31.3% presented with high calcium-phosphate product [16]. Kestenbaum B et al., found in their study that relatively small elevations in serum P in the high normal range (3.5-4.5 mg/dL) have been correlated with increased risk of cardiovascular and all-cause mortality in CKD patients [17]. Tonelli M et al., found that even that the general population with normal renal function also risk for development of cardiovascular complications [18]. The present study also found similar results.

Thus, to summarise male patients with advanced age with hyperphosphatemia, elevated calcium and phosphorus product in end stages of renal failure could be used as early marker of calcification which predict future cardiac mortality.

Limitation(s)

Seum intact Parathormone (PTH) was not analysed to rule out hyperparathyroidism. Sample size less and duration of study restricted to two years.

CONCLUSION(S)

Biochemical parameters such as calcium, phosphate, Ca × P, PTH, and vitamin D must be kept within target ranges to prevent bone disease and extraskeletal calcification, reduce the risk of heart disease, and maintain homeostasis of the body systems. Hyperphosphatemia is one of the main factors in the development of calcification that increases the ionic product calcium-phosphorus, very few studies are available in the Asian population. In addition, increasing evidence implies that elevated plasma phosphorus and Ca×P concentrations could be important stimuli for excess CV in end stage renal failure patient. More research is needed to establish a partnership.

Although much has been learned recently about the mechanisms of ectopic calcification, more research and a better understanding of this complicated process is needed. Effective therapeutic options for the prevention and treatment of this complex disease are only possible with a better understanding of the pathophysiology of CV.

Authors' contributions: DM was principle investigator planned for study, sample collection, statistical analysis. MS designed, drafted and written the manuscript and being a corresponding author. AS and SM participated in its design and coordination, and reviewed the manuscript. All of the authors contributed to the discussion and approved the final manuscript.

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PARTICULARS OF CONTRIBUTORS:
1. Assistant Professor, Department of Biochemistry, Vydehi Institute of Medical Sciences and Research Centre, Bangalore, Karnataka, India.
2. Associate Professor, Department of Biochemistry, Vydehi Institute of Medical Sciences and Research Centre, Bangalore, Karnataka, India.
3. Professor, Department of Biochemistry, Vydehi Institute of Medical Sciences and Research Centre, Bangalore, Karnataka, India.
4. Professor and Head, Department of Biochemistry, Vydehi Institute of Medical Sciences and Research Centre, Bangalore, Karnataka, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Mangala N Sirsikar,
Vydehi Institute of Medical Sciences and Research Centre, #82, Nallurahalli,
Whitefield, Bangalore-560066, Karnataka, India.
E-mail: mangalans81@gmail.com

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