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Original research article

Relationship between the gene polymorphism of osteoprotegerin, serum osteoprotegerin level and chronic kidney disease in south Indian populations

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Abstract

Introduction: CKD-MBD is thus thought to be a major contributor to the high mortality among patients with CKD. The negative regulation of osteoclastic bone resorption exerted by OPG could increase BMD and bone volume by decreasing the active osteoclasts as demonstrated by *in vitro* studies
Methodology: This is an observational study with no interventions carried out on any subject. Furthermore, all the CKD individuals were made two groups based on the dialysis. Finally, the analyses was done between the predialysis, dialysis and control population to find the possible or potential diagnostic marker for CKD-MBD.
Results: Distribution of rs3102735genotypes between the non-dialysis and dialysis groups were not found significant differences for allelic (OR: 1.54, 95% CI (0.65-3.60)), genetic TT vs TC (OR: 1. 81, 95% CI (0.48-6.76) p=0.37) TT vs CC (OR: 1. 70, 95% CI (0.36-7.85) p=0.49) and dominant (OR: 1.77, 95% CI (0.57-5.50) p=0.32) models. Similarly, we have not found significant difference for rs3102735 in recessive model (OR: 0.72, 95% CI (0.16-3.09) p=0.662).
Conclusion: The serum OPG may be a useful biomarker for early diagnosis of CKD-MBD.

Keywords: Gene polymorphism, osteoprotegerin, serum osteoprotegerin level

Introduction

Patients with mild to moderate chronic kidney disease (CKD), or end-stage renal disease have an increased risk for fracture because reduced kidney function is associated with bone loss. First note on bone abnormalities associated with renal disease date back to 19th century and the first epidemiological reports were published during the 1970s and 1980s when fractures in significant number were associated to the use of aluminium-containing dialysis fluids. The identification of aluminium-related osteomalacia led to changes in composition of dialysis fluids and thereafter the clinical entity of aluminium-related osteodystrophy has practically vanished [1].
Prevalence of impaired renal function in general adult population is estimated to be approximately 11%. Rix *et al.* have analyzed biochemical markers of bone turnover

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together with bone mineral density data in mild-to-moderate CRI patients and concluded that skeletal changes seem to initiate early in the course of CKD. In unselected population of predialysis CRI patients, abnormal bone histology was found in 68% of the patients with severe impairment of renal function. Among dialysis patients, 46% have been reported to display gross histological bone abnormalities [2].

Epidemiological studies on U.S dialysis population report approximately four-fold increases in the incidence of hip fractures in comparison to age matched population. In other populations on dialysis, the prevalence of vertebral fracture has been reported to be as high as 21% (ref) and even higher incidence of hip fractures has been reported. However, in this context, one should recall that CKD-BMD is usually asymptomatic and the clinical complications appear late in the course of CKD [3].

The osteoprotegerin (OPG) and the receptor activator of nuclear factor-kappa B ligand (RANK/RANKL) systems play important roles in the regulation of osteoclast formation, activity and survival in normal and pathological states of bone remodeling. Biochemical markers, such as C-telopeptide cross laps (CTX) and bone-specific alkaline phosphatase (B-ALP) are markers of bone resorption and bone formation that have been used for prediction of fracture risk, independent of other methods for monitoring osteoporosis, such as BMD. The negative regulation of osteoclastic bone resorption exerted by OPG could increase BMD and bone volume by decreasing the active osteoclasts as demonstrated by *in vitro* studies. OPG levels increase with increasing age; this age-dependent increase in OPG might be a counter-regulatory mechanism preventing further bone loss in elderly subjects. OPG, and also FGF23, associate with myocardial damage and aortic pulse wave velocity in CKD patients, thereby linking CKD-BMD with CVD. OPG is linked to osteoporosis, and loss of muscle mass as well as of fat mass. There is a positive relationship between OPG and femoral neck BMD in HD patients indicating that OPG perhaps could be used as an initial screening tool of bone loss and presence of CKD-MBD in ESRD patients. Apart from being a bone biomarker, OPG associates with severity of coronary calcification in non-dialysis CKD patients. Unlike OPG, the free and total RANKL levels decrease with age, possibly due to a general age-related reduction of cell activity. The circulating concentration of OPG increases independently of the changes of serum PTH in uremic patients. In pre-dialysis CKD stage 1-5 patients, serum RANKL negatively, and OPG positively, were found to be associated with femoral neck BMD. OPG and adipose tissue derived leptin associated with osteoporosis in patients with chronic obstructive pulmonary disease suggesting that links between these markers and loss of bone mass could be influenced by fat mass [4].

Methodology

This Retrospective Cohort study included 50 chronic kidney disease individuals at different stages (predialysis and dialysis) and twenty unrelated healthy individuals. The clinical variables of study participants such as hemoglobin (HB), albumin, CRP, total cholesterol, triglycerides, calcium, phosphorus, uric acid, iPTH, alkaline phosphate and OPG level were collected from the hospital data base. Further, the age, gender, family history of diabetics, hypertension and chronic kidney disease were collected using through the standard questionnaire. This is an observational study with no interventions carried out on any subject. Furthermore, all the CKD individuals were divided in to two groups based on the dialysis. Finally, the statistical analyses were performed between the predialysis, dialysis and control population to find the possible or potential diagnostic marker for CKD-MBD.

Inclusion criteria

Those patients within age 18-75 years who satisfied the following criteria were offered enrollment in the study:

- CKD stage 3, 4 and 5 (pre HD).
- CKD stage 5 on regular dialysis for at least one year.

Reagents used for DNA extraction

All the plastic ware and glassware used throughout this study were sterilized by autoclaving and Millipore water was used for the preparation of solutions.

Results

Discordance in Hardy Weinberg equilibrium (HWE) with respect to genotype frequency was observed in non-dialysis patients (0.042) and no deviation from Hardy-Weinberg equilibrium was observed with controls (0.05). The of miner allele frequencies was higher in Non-dialysis patients (28.0) than the controls (10.0). There was no significant difference in the genotype distributions between the control and Non dialysis patients in genetic model (TT vs TC p=0.154; TT vs CC p=0.168), dominant model (TT vs TC+CC p=0.065) and recessive model (CC vs TT+TC p=0.243). But, inallelic model (T vs A p=0.033) between the control and Non dialysis patients were found to be significant association.

Table 1: Genetic association analysis between control and Non-dialysis

Genotype	Control N=19 (%)	Non-dialysis N=25 (%)	OR (95% CI)	p-Value
TT	17 (85)	15 (60)	Reference	
TC	2 (10)	6 (24)	3.4 (0.59-19.4)	0.154
CC	1 (5)	4 (16)	4.5 (0.45-45.1)	0.168
TC+CC	3 (15)	10 (40)	3.7 (0.87-16.3)	0.065
CC	1 (5)	4 (6)	Reference	
TT+TC	19 (95)	21 (84)	0.27 (0.02-2.69)	0.243
T	36 (90)	36 (72)	Reference	
C	4 (10)	14 (28)	3.5(1.05-11.6)	0.033
MAF	10	28		
HWE	0.05	0.042		

Accordance in Hardy Weinberg equilibrium (HWE) with respect to genotype frequency was observed in both control (0.05) and Dialysis group (0.05). The of miner allele frequencies was higher in Dialysis patients (37.5) than the controls (10.0). Distribution of rs3102735genotypes between the control and dialysis groups was found statistically significant differences for allelic (OR: 5.4, 95% CI (1.64– 16.6)), genetic TT vs TC (OR: 6. 18, 95% CI (1.10–34.7) p=0.027) TT vs (OR: 7. 72, 95% CI (0.79–75.3) p=0.049) and dominant (OR: 6.69, 95% CI (1.54–29.0) p=0.007) models. But we have not found significant difference for rs3102735 in recessive model (OR: 0.2, 95% CI (0.02–1.8) p=0.127).

Table 2: Genetic association analysis between control and Dialysis group

Genotype	Control	Dialysis	OR (95% CI)	p-Value
TT	17 (85)	11 (45.8)	Reference	
TC	2 (10)	8 (33.3)	6.18 (1.10-34.7)	0.027
CC	1 (5)	5 (20.8)	7.72 (0.79-75.3)	0.049
TC+CC	3 (15)	13 (54.1)	6.69 (1.54-29.0)	0.007
CC	1 (5)	5 (20.8)	Reference	
TT+TC	19 (95)	19 (79.2)	0.2 (0.02-1.8)	0.127
T	36 (90)	30 (62.5)	Reference	
C	4 (10)	18 (37.5)	5.4 (1.64-17.6)	0.003
MAF	10	37.5		

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HWE	0.05	0.156		

Distribution of rs3102735genotypes between the non-dialysis and dialysis groups was not found significant differences for allelic (OR: 1.54, 95% CI (0.65–3.60)), genetic TT vs TC (OR: 1. 81, 95% CI (0.48–6.76) p=0.37) TT vs CC (OR: 1. 70, 95% CI (0.36–7.85) p=0.49) and dominant (OR: 1.77, 95% CI (0.57–5.50) p=0.32) models. Similarly, we have not found significant difference for rs3102735 in recessive model (OR: 0.72, 95% CI (0.16–3.09) p=0.662).

Table 3: Genetic association analysis between non-dialysis and Dialysis patients

Genotype	Non-dialysis	Dialysis	OR (95% CI)	p-Value
TT	15 (60)	11 (45.8)	Reference	
TC	6 (24)	8 (33.3)	1.81 (0.48-6.76)	0.37
CC	4 (16)	5 (20.8)	1.70 (0.36-7.85)	0.491
TC+CC	10 (40)	13 (54.1)	1.77 (0.57-5.50)	0.32
CC	4 (6)	5 (20.8)	Reference	
TT+TC	21 (84)	19 (79.2)	0.72 (0.16-3.09)	0.662
T	36 (72)	30 (62.5)	Reference	
C	14 (28)	18 (37.5)	1.54 (0.65-3.60)	0.316
MAF	28	37.5		
HWE	0.042	0.156		

Table 4: Studied characteristics between the OPG genotypes

Characteristics	Groups	TT	TC	CC	p-Value [a]	p-Value [b]	p-Value [c]
		Mean±SD	**Mean**±SD	**Mean**±SD			
OPG	Non Dialysis	85.2±12.3	99.2±17.9	89±24.7	0.507	0.573	0.905
	Dialysis	82.7 ±13.3	93±20	96.9±19			
iPTH	Non Dialysis	177.6 ± 156.1	193±84	296±129.7	0.009	0.043	0.016
	Dialysis	353±176	93±20	96.9±19.9			
CRP	Non Dialysis	1.51±0.34	1.11±0.37	1.62±0.88	0.281	0.414	0.73
	Dialysis	1.91±0.86	1.52±0.72	1.3±0.40			
Calcium	Non Dialysis	8.8±0.45	8.6±0.61	8.67±0.58	0.087	0.662	0.905
	Dialysis	8.6±0.46	8.53±0.68	8.8±0.55			
Phosphorous	Non Dialysis	5.58±0.97	5.06±1.61	5.3±2.15	0.61	0.414	0.905
	Dialysis	6.03±1.65	5.8±1.33	5.5±1.1			
Uric acid	Non Dialysis	6.4±1.55	6.5±1.15	6.8±1.68	0.61	0.181	0.73
	Dialysis	7.03±1.98	7.4±0.66	6.6±1.7			
Alkali Phosphate	Non Dialysis	107.5±40.7	123±55.1	148±47	0.061	0.95	0.73
	Dialysis	164±78.5	150±93	180±82.8			

Among the studied characteristics the iPTH was found to be significant differences between the groups. But the other studied characteristics were not found significant differences. This indicates that the genotypes of OPG gene not regulating the circulating OPG levels.

Discussion

The elevated level of OPG theoretically should be a protective factor for bone metabolism; however, conflicting results were produced. Interestingly, just like some previous reports, the serum OPG level had a negative correlation with the BMD. So JIAN-QING *et al*. [5]

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speculated that the increasing of OPG should be a compensatory response to counteract the bone loss. Also, Rasmussen *et al*., reported, the route of elimination for the molecule is unknown, but a decline in kidney function has been found associated with increasing OPG levels [6].

Elevated OPG levels have been interpreted as a failed compensatory mechanism trying to counteract the ongoing calcification process; however, because OPG production and expression are highly regulated by several inflammatory cytokines, it could also be speculated that increased OPG levels in CKD may in part be a consequence of systemic inflammation [7].

Hofbauer and Schoppet, reported, the initial step of the mineral bone disorder in CKD was traditionally thought to be the disturbed calcium and phosphorus metabolism induced by reduction of renal function. After this, the increased iPTH would activate the RANKL/OPG system and induced bone disorder. However, JIAN-QING *et al*. study showed, the OPG increased earlier than the serum Ca, P and iPTH levels, so there should be some other factors regulating the production of OPG in CKD patients [8].

Martin *et al*., conclude that OPG in combination with iPTH can be used as a marker for non-invasive diagnosis of renal osteodystrophy in hemodialysis patients. OPG correlated positively with circulating surrogate markers of inflammatory, endothelial dysfunction and oxidative stress. Increased OPG levels per se were related to higher cardiovascular and all-cause mortality even after adjustment for age, sex, C-reactive protein, diabetes mellitus and baseline CVD.

In CKD, the extra-skeletal calcification has even been associated with increased cardiovascular mortality in CKD. So the early finding of extra skeletal calcification may be more important than ROD. As we all know, the occurrence of calcifications in the soft tissues and the arterial vessels cannot be found with bone biopsy, or be found by computer tomography in the early stage. So the purpose of the measurement of serum OPG in CKD patients should not just be confined to the bone protection.

A number of polymorphisms in the OPG gene have been described in previous investigations and associated with bone mineral density, vertebral fractures, coronary artery disease, Pagets disease, osteoarthritis and osteoporosis in different populations. To our knowledge, this is the first study investigating the association between polymorphisms in the OPG gene and CKD. We found no association between the study OPG polymorphism and CKD. We have recently identified an association of a polymorphism in the vitamin D receptor (VDR) gene with CKD. However, other polymorphismsin the OPG gene and in other genes of the host bone metabolism response may also be involved in the determination of susceptibility to and/or progression of CKD [9].

OPG gene polymorphisms have been associated with osteoporosis and vascular impairment. Furthermore, subjects with a C allele in the promoter region at position 950 (TC and CC) have significantly higher circulating OPG serum levels, and genetic variations in the OPG gene confer an increased risk of CVD and carotid plaque vulnerability in Caucasians. However, Rhee *et al*. Observed that polymorphisms in the promoter region of the OPG gene were not associated with aortic calcification or coronary artery disease in Koreans [10]. Previous studies have shown that the C allele of the T950C (rs2073617) polymorphism in the OPG gene is significantly and independently associated with increased serum osteoprotegerin levels. Interestingly, Biscetti *et al*. also showed that polymorphisms in the OPG gene were associated with increased risk of ischemic stroke in diabetic patients. A study conducted in 2008 by Baioni *et al*. was found that the no association between the studied OPG polymorphism and susceptibility to CKD or periodontal disease. However, our study observed that the significant differences between control and dialysis group for genetic, dominant and allelic models [11]. Furthermore, the increase levels of serum OPG was found in the dialysis group when compared to non-dialysis and control. These findings further support

that conclusion that osteoprotegerin is a risk factor for progressive CKD and BMD. These data further suggest a role for OPG as a reliable biomarker in CKD dialysis. Although the mechanisms linking OPG and CKD dialysis require further study, the association between OPG and CKD in dialysis patients shown here requires further investigation to clarify the possible role of OPG as a biomarker for identifying patients with, or at risk of, CKD progressions [12].

Conclusion

We found that the serum OPG concentration was significantly increased in dialysis group compared to non-dialysis and control group. Further, the OPG gene polymorphisms were found significant differences between the control and dialysis group in all models except recessive model. Furthermore, there are no independent associations of serum OPG concentration with dialysis and pre dialysis group. Moreover, serum iPTH, CRP and other clinical variables was also found significant differences between the control, dialysis and pre-dialysis.

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