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Original research article

A Study on serum level of Osteoprotegerin (OPG) in chronic kidney disease

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Abstract

Introduction: Neutralization of a key Wnt inhibitor elevated in the circulation in CKD, Dkk1, and inhibited CKD induced vascular dedifferentiation, vascular calcification, and renal osteodystrophy. This effect was surprising since Wnt signalling in the vascular smooth muscle is implicated in stimulating osteoblastic transition and vascular calcification. However, recent studies demonstrate that Dkk1 mediated inhibition of aortic Wnt7b stimulates smad mediated aortic endothelial-mesenchymal transition (EndMT) and vascular calcificatio. EndMT is a developmental physiologic process involved in the development of the cardiac valves, the cardiac septum and the aortic root, and it may or may not contribute to cardiac fibrosis in various adult disease states.
Methodology: 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.
Results: A total 68 individuals were genotyped for this study which includes 19 control subjects, 25 Non Dialysis patients and 24 Dialysis patients. The distribution of OPG gene polymorphisms among the control, Non dialysis and dialysis group were documented. Of the 19 studied control subjects 17 (85%) TT, 2 (10% TC) and 1 (5%) TC genotypes were observed.
Conclusion: Among the 25 non dialysis patients the observed genotypes are 15 (60%) TT, 6 (24%) and 4 (16%)TC. The Dialysis group 11 TT (45.8%), 8 TC (33.3%) and 5 TC (20.8%) genotypes were observed.

Keywords: Osteoprotegerin, chronic kidney disease, CKD-MBD

Introduction

Chronic kidney disease (CKD) is associated with numerous metabolic and nutritional alterations affecting among others mineral metabolism and bone health which are interrelated and together form an entity called CKD-mineral and bone disorders (CKD-MBD). CKD-MBD is associated with disturbances of phosphate and calcium homeostasis as well as with changes in key regulators of bone status such as parathyroid hormone (PTH) and fibroblast

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growth factor 23 (FGF23) [1]. These alterations may lead to renal osteodystrophy with bone loss, osteoporosis and potentially fractures and increased risk for premature vascular calcification, adding significantly to other common causes of cardiovascular disease (CVD), the leading cause of death in CKD patients. CKD-MBD is thus thought to be a major contributor to the high mortality among patients with CKD. While many of the circulating mediators of CKD-MBD can be relatively easily measured, a detailed assessment of bone status requires more complex methods some of which such as bone biopsy are not readily available. However, the measurement of bone mineral density (BMD) usually performed by dual-energy X-ray absorptiometry (DEXA) is a more convenient method in the clinical setting and is increasingly regarded as an integral component of assessment of bone mass, presence and extent of osteoporosis, and risk of fractures. BMD is a well-established key parameter for monitoring bone disease in CKD patients, although it should be noted that, there are many common factors affecting BMD not specifically related to CKD-MBD, such as age, gender, menopause, estrogen consumption, body mass, cigarette smoking, alcohol abuse, excess glucocorticoid exposure, physical activity and genetic factors. It should also be noted that BMD cannot fully describe the status of bone fragility since bone status is due to many dynamic factors such as abnormal bone turnover and remodeling, leading to impairment of bone micro-architecture [2].

Disruption in mineral metabolism occur already at early stages of CKD, leading as the disease progresses to alterations in bone mass, bone turnover, mineralization and bone health. Disorders of bone structure and bone mass may result in severe osteoporosis and marked risk of fractures. Moreover, and even more important, because of the close links between bone status and soft tissue calcification, these alterations associate with vascular calcification, sometimes described as vascular ossification, leading to clinically manifest CVD and increased mortality [3].

The following brief review which in part is based on a recent review article from our group summarizes the current understanding of causes of CKD-MBD, and its consequences for clinical outcome in CKD patients.

Multiple investigators and we have shown that kidney diseases reactivate developmental programs involved in nephrogenesis during disease stimulated renal repair. Among the nephrogenic factors reactivated in renal repair, the Wnt (portmanteau of Wingless and Integrated) family is critical for tubular epithelial reconstitution. In the control of Wnt function, canonical signalling transcriptionally induces the expression of a family of Wnt inhibitory proteins which are secreted proteins that serve to restrict the distances of Wnt stimulation to autocrine or paracrine factors. The Wnt inhibitors are circulating factors, and the family includes the Dickkopfs (Dkk) [4].

Neutralization of a key Wnt inhibitor elevated in the circulation in CKD, Dkk1, inhibited CKD induced vascular dedifferentiation, vascular calcification, and renal osteodystrophy. This effect was surprising since Wnt signalling in the vascular smooth muscle is implicated in stimulating osteoblastic transition and vascular calcification. However, recent studies demonstrate that Dkk1 mediated inhibition of aortic Wnt7b stimulates smad mediated aortic endothelial-mesenchymal transition (EndMT) and vascular calcificatio. EndMT is a developmental physiologic process involved in the development of the cardiac valves, the cardiac septum and the aortic root, and it may or may notcontribute to cardiac fibrosis in various adult disease states. Since EndMT is a process driven by smad transcription factors activated by factors in the transforming growth factor beta (TGFβ) superfamily, of the TGFβ superfamily members, activin, a known renal developmental factor and circulating hormone, was the primary candidate. Activin is increased in the circulation by CKD associated with increased expression of activin in the kidney. Surprisingly, the activin type 2A receptor (ActRIIA) was induced by CKD in the aortic vascular smooth muscle and not the endothelium [5, 6].

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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.

Subjects

The chronic kidney disease individuals, visiting Department of Nephrology, for their treatment was the main source of the samples for this present study. From the general population unrelated healthy individuals were included as a control subjects.

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.

Exclusion criteria

- Pregnant or nursing women
- Refusal to sign the informed consent form
- Active dependency on drugs or alcohol
- HIV/AIDS/liver disease/malignancy
- Any medical, psychiatric, debilitating disease/disorder or social condition that in the judgment of the investigator would interfere with or serve as a contraindication to adherence to the study protocol or ability to give informed consent or affect the overall prognosis of the patient.

Following their clinical assessments by nephrologists, the blood samples of each study subjects were collected for DNA extraction. A procedure for protection of human subjects in this study was approved by the Institutional Ethical Review Committee. Written informed consent was collected from the study participants before collecting the blood samples. Intravenous blood samples (~5 ml each) were collected from all the participants. Every participant was given a study-specific ID number. ID numbered samples without names was handled in the laboratory. The connection between names and numbers were in a database. Access to the database is permitted only for the researchers named in the research proposal and approved by the ethical committees.

Results

Present study included 69 individuals consisting of 49 males (71%) and 20 females (29%), divided into 3 groups: Group A: Consisted of 25 individuals with pre dialysis with CKD, [22

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(80%) males and 5 (20%) females], aged from 28 years to 80 years (mean ±SD was 48.72±12.43 years). Group B: Consisted of 24 patient with end stage renal disease on regular hemodialysis, [17 (70.8%) males and 7 (21.2%) females), aged from 16 years to 84 years (mean ± SD was 51.25± 15.05). Group C: Consisted of 20 healthy volunteers, [12 (60%) males and 8 (40%) females], aged from 20 years to 65 years (mean ± SD was 38.90± 12.4 years). The other clinical variables of the studied three groups individuals such as HB, Albumin, Cholesterol, Triglycerides, calcium, phosphorus, uric acid iPTH, alkaline phosphates and OPG levels in Min-Max and mean ± SD are presented in table .

There was a significant difference between the group A and group B only for iPTH. All other variables were not shown any differences. Whereas, the mean age, mean calcium, mean iPTH, mean OPG, mean phosphorus and mean CRP was found to be statistically significant difference between the group A and group C. Similarly, group B and group C was also shown significant differences of mean age, mean calcium, mean iPTH, mean OPG, mean phosphorus and mean CRP.

Table 1: Characteristics of age, gender and Laboratory Findings

Variables	**Pre dialysis**	**Dialysis**	**Control**
	(Group A)	**(Group B)**	**(Group C) N=20**
	N=25	**N=24**	**Mean ± SD**
	Mean ± SD	**Mean ± SD**	
Age (Years)	48.72±12.43	51.25 ± 15.05	38.9 ± 12.4
(Min-Max)	(28-80)	(16-84)	(20-65)
Gender (M/F) (%)	20/5	17/7	12/8 (60% /40%)
	(80%/20%)	(70.8%/21.2%)	
Total Count	7180 ±1138.7	7620.8 ± 1345.1	7385 ± 1143
(cells/mcL)	(5400-9400)	(4700-10800)	(5300-9100)
(Min-Max)			
HB (gm/dl)	9.5 ± 0.82	8.24 ± 0.99	13.1 ± 0.68
(Min-Max)	(8.4-11.0)	(6.2-10.6)	(11.4-14.0)
Albumin (g/dL)	3.51 ± 0.36	3.37 ± 0.43	4.43 ± 0.25
(Min-Max)	(2.9-4.2)	(2.8-4.2)	(4.1-4.9)
CRP (mg/L)	1.43 ± 0.47	1.66 ± 0.76	0.6 ± 0.20
(Min-Max)	(0.7-2.9)	(0.7-3.9)	(0.3-1.1)
Cholesterol (mg/dl)	162.2 ±47.9	158.5 ± 37.4	162.9± 8.91
(Min-Max)	(83-280)	(98-240)	(140-178)
Triglycerides (mg/dl)	163 ± 53.3	170 ± 56.4	131.2 ± 9.64
(Min-Max)	(108-310)	(106-300)	(110-146)
Calcium (mg/dl)	8.7 ± 0.50	8.63 ± 0.55	9.33 ± 0.17
(Min-Max)	(7.9-9.7)	(7.8-9.8)	(9-9.6)
Phosphorus (mg/dl)	5.41 ± 1.31	5.85 ± 1.41	4.02 ± 0.24
(Min-Max)	(1.8-7.2)	(3.6-10.2)	(3.6-4.6)
Uric acid (mg/dL)	6.5 ± 1.4	7.08 ± 1.58	5.39 ± 0.62
(Min-Max)	(2.4-8.9)	(3.7-11.5)	(3.6-4.6)
iPTH (pg/ml)	200.5 ± 140.5	343.0 ± 186.0	38.33 ± 6.94
(Min-Max)	(32-541)	(82-706)	(27.4-52.4)
Alkalinephosphates	117.6 ± 45.8	163.3 ± 81.4	106.9 ± 18.8
(IU/L)	(68-225)	(65-320)	(74-130)
(Min-Max)			
OPG (pmol/l)	89.8 ± 16.47	89.1 ± 17.3	52.1 ± 5.43
(Min-Max)	(71-126)	(66.7-115.9)	(43-60.2)

A total 68 individuals were genotyped for this study which includes 19 control subjects, 25 Non Dialysis patients and 24 Dialysis patients. The distribution of OPG gene polymorphisms

among the control, Non dialysis and dialysis group were documented. Of the 19 studied control subjects 17 (85%) TT, 2 (10% TC) and 1 (5%) TC genotypes were observed. Among the 25 non dialysis patients the observed genotypes are 15 (60%) TT, 6 (24%) and 4 (16%) TC. The Dialysis group 11 TT (45.8%), 8 TC (33.3%) and 5 TC (20.8%) genotypes were observed. furthermore, the based on the observed genotype frequencies, the expected genotype frequencies were calculated and the same was documented.

Table 2: Expected and observed genotypic count for *OPG* gene rs3102735 polymorphisms in different groups

		TT	TC	CC	X2	p-Value
Control	Observed	17	2	1		
	Expected	16.2	2	1	3.9	0.046
Pre-dialysis	Observed	15	6	4		
	Expected	13	10.1	2	4.09	0.04
Dialysis	Observed	11	8	5		
	Expected	10.7	8.6	1.7	2	0.156

Discussion

The identification of the OPG–RANKL–RANK system as the dominant, final mediator of osteoclastogenes is represents a major advance in bone biology. The initial cloning and characterization of OPG as a soluble, decoy receptor belonging to the TNF receptor super family was the first step that eventually led to an unravelling of this system. Soon thereafter, the molecule blocked by OPG, called RANKL, was identified as the key mediator of osteoclastogenesis in both membrane-bound form expressed on preosteoblastic/stromal cells and a soluble form. In turn, RANKL was shown to bind its receptor, RANK, on osteoclast lineage cells. The important role played by these factors in regulating bone metabolism was demonstrated by the findings of extremes of skeletal phenotypes (osteoporosis and osteopetrosis) in mice with altered expression of these molecules [7].

The current study is the first report showing that both elevated serum OPG levels and rs3102735genotypes of the OPG gene were significantly associated with increased risk CKD. More importantly, genetic effects on serum OPG levels were more evident among subjects who had higher serum OPG concentration, and also differences in OPG concentrations between control and dialysis were observed among subjects carrying the TT, TC, and CC variant genotypes of the rs3102735gene polymorphisms of the OPG gene.

Osteoprotegerin might protect bone against intensive bone loss resulting from the imbalance of bone kinetics in CKD hemodialysis patients. Higher serum OPG and lower serum RANKL were found in CKD patients in hemodialysis. Increased serum OPG levels in hemodialysis patients are believed to partly reflect a compensatory response to increased bone loss. The determination of serum OPG levels in association with PTH levels could be useful in the diagnosis of bone turnover in renal patients. Besides, it could contribute to prevent patients from developing vascular calcification, a major risk factor for cardiovascular diseases, which in turn is an important mortality indicator in CKD patients [8].

OPG-deficient mice exhibit a decrease in total bone density and medial calcification of the aorta and renal arteries, suggesting that OPG deficiency is associated with osteoporosis and vascular calcification. In addition, transgenic over expression of OPG in OPG-deficient mice effectively rescues the osteoporotic bone phenotype typically observed in these mice. On the other hand, several experimental studies showed that a pathological increase of OPG levels may contribute to inflammation in the endothelium and ischemic brain, which is characteristic to cardiovascular disease. Recent clinical studies have shown that a high OPG level is associated with cardiovascular disease including acute myocardial infarction and

heart failure, vascular calcification and low BMD in patients with CKD, which is in agreement with our findings. Although it is possible that increased OPG production and release, leading to OPG levels above the physiologic concentration, may decrease bone density and contribute to the vascular pathologic condition, further studies are required to determine the causal relationship between increased OPG levels and lower BMD in CKD patients [9, 10].

Conclusion

Osteoprotegerin might protect bone against intensive bone loss resulting from the imbalance of bone kinetics in CKD hemodialysis patients. Higher serum OPG and lower serum RANKL were found in CKD patients in hemodialysis.

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