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Expression of sclerostin and bone morphogenetic protein-7 (BMP-7) in serum of patients with chronic kidney disease-mineral and bone disorder (CKD-MBD) and their correlation with calcium and phosphorus metabolism

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Abstract

Calcium and phosphorus metabolism involves in patients with chronic kidney disease-mineral and bone disorder (CKD-MBD). Sclerostin and bone morphogenetic protein-7 (BMP-7) are closely related to bone formation. This study aims to assess sclerostin and BMP-7's role in renal function, blood calcium and phosphorus in CKD-MBD. 86 CKD-MBD patients were collected as the case group and 86 healthy subjects were selected as control group. ELISA was used to detect Sclerostin and BMP-7 serum level. The automatic biochemical analyzer was used to detect liver and kidney function. Blood calcium and phosphorus level, and iPHT level was detected by immunoradiation. No differences were found regarding age, gender, and BMI between case group and control group ($P > 0.05$). Compared to controls, CKD-MBD patients had significantly reduced blood calcium and eGFR level, and increased Scr, BUN, blood phosphorus and iPTH level ($P < 0.05$) with increased Sclerostin ($t = 33.86$, $P < 0.001$), and reduced BMP-7 level ($t = 329.38$, $P < 0.001$). Scr, BUN, blood phosphorus levels and iPTH were correlated with Sclerostin positively ($P < 0.05$) and BMP-7 negatively ($P < 0.05$). eGFR and blood calcium were correlated with Sclerostin negatively ($P < 0.05$) and BMP-7 positively ($P < 0.05$). Scr, eGFR, blood calcium levels, blood phosphorus levels, and iPTH are independent risk factors for serum Sclerostin and BMP-7 level. Sclerostin and BMP-7 changes may be important factors affecting the occurrence of CKD-MBD. Renal function, blood calcium and blood phosphorus are independent risk factors for serum Sclerostin and BMP-7 level.

Keywords: CKD-MBD; bone sclerostin; bone morphogenetic protein-7.

Practical Application: Our study showed that Renal function, blood calcium and blood phosphorus are independent risk factors for serum Sclerostin and BMP-7 level. However, large cohort clinical studies are required to confirm this.

1 Introduction

According to a 2012 survey, the prevalence of chronic kidney disease (CKD) in China was as high as 10.8% (Zhang et al., 2012). Meta-analysis shows that among 6,908,440 researchers, the global prevalence of CKD is about 13.4% (Hill et al., 2016). At present, the survival time of CKD patients is gradually extended, but the quality of life of patients is seriously affected by various complications of CKD. Several studies have demonstrated the potential benefits of nutrition supplement or diets in delaying CKD progression (Molina et al., 2021; Naber & Purohit, 2021). Chronic kidney disease-mineral and bone disorder (CKD-MBD) is a CKD complication with manifestations being mineral and bone metabolism abnormalities, which stimulates hyperparathyroidism, metastatic calcification of blood vessels and soft tissues. CKD-MBD occurs in the early stages of CKD and in the stage of CKD4-5, obvious hypocalcemia, hyperphosphatemia, and hyperthemia PTH appear. Patients with CKD-MBD in early period have no obvious clinical symptoms. At present, common biochemical indicators such as bone mineral density, blood calcium, blood phosphorus, parathyroid hormone (iPTH), and bone alkaline phosphatase are useful for preventing and treating CKD-MBD with great diagnostic significance. However, clinically, CKD-MBD patients have already been treated with calcium supplementation and phosphorus reduction due to their long-term chronic course. The serum calcium and phosphorus detected by them may not reflect the actual changes in the disease. Sclerostin is a product of the SOST gene discovered in 2001. It is a soluble blocker of the classic Wnt-β signaling pathway and regulates bone mass via Wnt-β-chain signaling (Delgado-Calle et al., 2017). Studies have found that antibody treatment with Sclerostin can increase bone mass in mice and normalize the level of phosphorylation in the blood (Carpenter & Ross, 2020), so some researchers have suggested that Sclerostin may be a promising molecule target for treating osteoporosis. However, other studies reported that Sclerostin level is lower in osteoporosis patients and Sclerostin may not be a contributing factor to osteoporosis (Basir et al., 2019). Therefore, it is uncertain whether Sclerostin can be applied to the treatment of bone diseases. Recent research shows that Sclerostin is not only closely related to bone metabolism, but also has important links with calcium and phosphorus metabolism, renal function, PTH, vitamin D, alkaline phosphatase, etc. (Elsalam et al., 2019; Ji et al., 2018). However, there is no research on the correlation between Sclerostin and calcium and phosphorus

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metabolism in CKD-MBD. Therefore, this study determined the relationship between Sclerostin and calcium and phosphorus metabolism in CKD-MBD. BMP-7 is mainly expressed in kidney and bone tissues. In CKD patients, the function of the nephron is destroyed and the survival of BMP-7 is reduced accordingly. Recent studies have shown that BMP-7 is a protective factor of the kidney (Ćelić et al., 2018), which participates in maintaining the structure and function of the kidney tissue (Manson et al., 2015), and can also inhibit renal fibrosis of diabetic nephropathy (Liu et al., 2019; Xiao et al., 2019). In addition, BMP-7 is also closely related to bone formation (Lee et al., 2019). For example, BMP-7 can affect the osteogenic differentiation of gingival stem cells (Levey et al., 2011). Since BMP-7 is closely related to bone formation, whether its expression level is related to calcium and phosphorus metabolism and renal function in patients with CKD-MBD is unknown. Our study also tested the level of BMP-7 in patients with CKD-MBD, clarified its correlation with calcium and phosphorus metabolism, and provided new clues for CKD-MBD treatment.

2 Materials and methods

2.1 Main reagents and instruments

Human Sclerostin ElISA Kit (Shanghai Lanji Biotechnology Co., Ltd.), BMP-7 ElISA Kit (Shanghai Senxiong Technology Industrial Co., Ltd.), Microplate Reader (American Bio Tek Company), Fully Automatic Chemical Immunoassay (US BACKMAN), low-speed desktop centrifuge (Shanghai Leibo Analytical Instrument Co., Ltd.), electric thermostatic water bath (Shanghai-Hengheng Equipment Factory), -80 °C low temperature refrigerator (SANYO)

2.2 CKD diagnostic criteria

The diagnostic criteria was referred to the 2012 KDIGO revised criteria (Musgrove & Wolf, 2020): renal structural or functional abnormalities ≥ 3 months, with or without a decrease in GFR. One of the following manifestations: abnormal renal pathological examination; positive indicators of renal damage: abnormal blood or urine components or abnormal imaging; GFR < 60mL/(min/1.73 m^2) ≥ 3 months, with or without renal damage.

2.3 Diagnostic criteria of CKD-MBD

This was referred to the "Guidelines for the Diagnosis and Treatment of Chronic Kidney Disease Minerals and Bone Abnormalities" issued by the Nephrology Branch of the Chinese Medical Association in 2013: 1) abnormal metabolism of serum calcium, phosphorus, iPTH or active vitamin D (VitD); 2) Bone transformation, mineralization, bone mass, linear growth or abnormal strength; 3) Calcification of blood vessels or other soft tissues. One or more of the three items above can be diagnosed.

2.4 Collection of study subjects and specimens

All patients with CKD-MBD were collected from January 1, 2018 to December 31, 2018 in Guangzhou, and were set as case groups. Inclusion criteria: 1) Those who met the CKD-MBD diagnostic criteria and CKD diagnostic criteria; 2) Those whose clinical data were completed and approved by the hospital ethics committee to participate in this trial; 3) All patients and family members were aware of the entire research process and willing to cooperate and sign Informed Consent; 4) Age over 18 years. Exclusion criteria: 1) those with autoimmune diseases and malignant tumors; 2) those who underwent total or subtotal parathyroidectomy; 3) postmenopausal osteoporosis, primary hyperparathyroidism, etc. patients with metabolic bone disease; 4) Those with severe heart, liver, and lung dysfunction; 5) Those who had used immunosuppression for the past six months; 6) Those who regularly took VitD for calcium and phosphorus treatment in the past month; 7) Parathyroid disease Such as parathyroid adenoma, parathyroid cancer caused by pseudoparathyroidism; 8) Pregnancy, lactation, mental illness or those who did not agree to participate in the study. Finally, 86 patients met the criteria, consisting of 47 males and 39 females. In addition, 86 healthy people with comparable age receiving physical examination during the same period were selected from the physical examination center of our hospital as the control group, including 45 males and 41 females. After informed consent obtaining from the two groups, 5 mL fasting blood was drawn and centrifuged at 3000 r/min for 5 min to collected serum which was stored at -80 ° C for subsequent testing.

2.5 Detection of sclerostin and BMP-7 in serum by ELISA

The ELISA kit was used to detect the contents of Sclerostin and BMP-7 in the serum of the two groups of people. Following the instructions of the kit, the experimental steps were as follows: Set blank holes, standard holes and sample holes. Add 50 μL of standard to the standard well, and add 40 μL of the sample dilution to the sample well for 30 min incubation at 37 °C. Peel off the sealing plate membrane, discard the liquid in the enzyme-labeled wells and dry them. Each well is filled with washing solution and left for 30 s, then discarded. Repeat this 5 times and pat dry. Add 50 μL of enzyme-labeled reagent to each well, except for the blank wells. Repeat the warm-bath washing step. Add 50 μL of developer A and B respectively for 15 min incubation followed by addition of 50 μL stop solution. Zero the blank hole, and measure the absorbance (OD) at 450 nm within 15 min. Draw a standard curve according to the absorbance values obtained from different concentrations of standards, and find the corresponding concentration on the standard curve.

2.6 Detection of liver and kidney function, blood calcium, blood phosphorus, and iPHT

These were measured using an automatic biochemical analyzer and iPHT level was detected by immunoradiation.

2.7 Statistical methods

SPSS 19.0 software was adopted for processing data which were displayed as mean ± standard deviation (SD) and compared by t test. Pearson correlation or multiple linear regression analysis was used to analyze the correlation between each index and serum Sclerostin and BMP-7 levels. $P < 0.05$ indicates a difference.

3 Results and discussion

3.1 Comparison of basic clinical data and laboratory indicators

The basic clinical data and related laboratory indicators of the two groups were compared and shown in Table 1 and found no significant differences in age, gender, and BMI between the two groups (P > 0.05). Compared with the healthy people, the eGFR of CKD-MBD patients was significantly reduced and Scr and BUN were significantly elevated (P < 0.05). CKD-MBD patients also showed a significant decrease in blood calcium, increase in blood phosphorus and iPTH (P < 0.05).

3.2 Comparison of sclerostin and BMP-7 serum contents

The ELISA method was used to detect the contents of Sclerostin and BMP-7 in the serum of the two groups. As shown in Figure 1, the content of Sclerostin was (88.91 ± 10.94) pmol/L in the case group, and (38.32 ± 8.50) pmol/L in the control group. The content of Sclerostin in case group was significantly elevated compared to control group (P < 0.001). The content of BMP-7 was (30.78 ± 10.66) pg/L in the case group and (81.83 ± 12.08) pg/L in the control group. The content of BMP-7 in case group was significantly decreased compared to control (P < 0.001).

3.3 Correlation analysis of sclerostin, BMP-7 and various indicators

The correlation between the contents of Sclerostin and BMP-7 in serum and various indicators was analyzed. As shown in Table 2, Sclerostin and BMP-7 were not correlated with age and BMI (P > 0.05); Sclerostin was positively correlated with Scr, BUN, blood phosphorus levels and iPTH (P < 0.05) and negatively with eGFR, Serum calcium levels (P < 0.05); BMP-7 was negatively correlated with Scr, BUN, blood phosphorus levels, and iPTH (P < 0.05) and positively correlated with eGFR and blood calcium levels (P < 0.05). P < 0.05). Through comparing the contents of Sclerostin and BMP-7 in different genders, we found that the contents of Sclerostin and BMP-7 were (63.56 ± 27.08) pmol/L and (56.40 ± 27.98) pg/L in males, and (63.68 ± 27.46) pmol/L, (56.20 ± 28.21) pg/L in females. No difference of Sclerostin and BMP-7 level was found in different genders (P > 0.05).

3.4 Multiple linear regression analysis of sclerostin, BMP-7 and various indicators

In order to exclude the influence of confounding factors, we used Sclerostin and BMP-7 as the dependent variables, age,

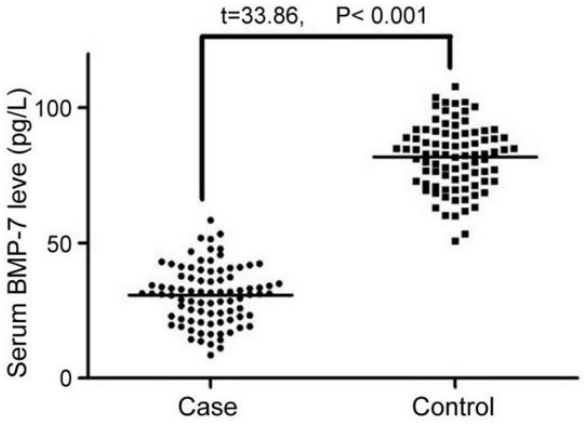

Figure 1. Comparison of serum levels of sclerostin and BMP-7 in control and case.

Table 1. Comparison of basic clinical data and laboratory indicators between control group and case group.

	Control	Case	T value	P value
Gender				
Male	45	47	0.94	0.76
Female	41	39		
Age	49.33 ± 7.88	49.79 ± 8.52	0.37	0.71
BMI (kg/m^2)	24.22 ± 2.34	24.51 ± 2.04	0.86	0.39
eGFR (mL/min)	106.50 ± 15.50	19.96 ± 7.91	47.72	< 0.001
Scr (μmol/L)	60.35 ± 6.49	384.22 ± 89.12	33.61	< 0.001
BUN (mmol/L)	4.20 ± 0.52	19.50 ± 7.72	18.32	< 0.001
Ca (mmol/L)	2.35 ± 0.14	1.94 ± 0.17	17.61	< 0.001
P (mmol/L)	1.15 ± 0.11	1.91 ± 0.20	30.13	< 0.001
iPTH (pg/mL)	44.98 ± 5.01	275.37 ± 62.42	34.12	< 0.001

BMI: Body mass index; eGFR: epidermal growth factor receptor; Scr: serum creatinine; BUN: Blood urea nitrogen; Ca: Calcium; P: phosphorus; iPTH: parathyroid hormone.

Table 2. Correlation analysis of sclerostin, BMP-7 and various indicators.

		aGE	BMI	eGFR (mL/min)	Scr (μmol/L)	BUN (mmol/L)	Ca (mmol/L)	P (mmol/L)	iPTH (pg/mL)
Sclerostin	r	0.09	0.05	-0.90	0.87	0.75	-0.78	0.84	0.82
	p	0.26	0.51	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
BMP-7	r	0.10	-0.04	0.89	-0.85	-0.74	0.94	-0.89	-0.95
	p	0.19	0.58	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

gender, BMI, BUN, Scr, eGFR, blood calcium, blood phosphorus, and iPTH as independent variables for multiple linear regression analysis. As shown in the Table 3 and Table 4, serum Sclerostin and BMP-7 levels were closely related to Scr, eGFR, blood calcium levels, blood phosphorus levels, and iPTH, suggesting that they might be independent risk factors for serum Sclerostin and BMP-7 levels.

3.5 Discussion

Disorders of calcium and phosphorus metabolism are one of the common clinical complications in patients with CKD. When GFR decreases, urinary phosphorus elimination gradually decreases, blood phosphorus concentration gradually increases, and the increase in blood phosphorus concentration leads to an increase in binding to blood calcium and a decrease in blood calcium, which further inhibits 1,25-dihydroxyvitamin D3 and stimulates the increase of PTH. Disturbance of calcium and phosphorus metabolism is a direct factor that causes CKD-MBD. Therefore, calcium and phosphorus regulators are currently the focus of research on the pathogenesis of CKD-MBD, such as FGF23 (Ribeiro et al., 2020), Klotho protein (Kuo et al., 2019), and so on. Previous studies have found that Sclerostin is also closely related to calcium and phosphorus metabolism (Elsalam et al., 2019), but the correlation between Sclerostin and calcium and phosphorus metabolism has not been verified in the CKD-MBD population. In addition, as a protective factor of the kidney, BMP-7 also affects bone formation, but there is little research on its correlation with calcium and phosphorus metabolism. Therefore, this study tested the serum levels of Sclerostin and BMP-7 in CKD-MBD patients to clarify their expression in CKD-MBD patients and correlation with calcium and phosphorus metabolism. Our study found that Sclerostin level in the serum of patients with CKD-MBD is significantly increased. Sclerostin is positively correlated with Scr, BUN, blood phosphorus levels, and iPTH, and negatively correlated with eGFR and blood calcium levels. Multiple linear regression results suggest that Scr, EGFR, blood calcium level, blood phosphorus level, and iPTH are independent risk factors for serum Sclerostin levels in patients with CKD-BMD. Ji et al. (2018) showed that the expression of Sclerostin in serum of patients with stage 3-5 of CKD was significantly increased, which was negatively correlated with eGFR and blood calcium levels, and significantly positively correlated with SCr, blood phosphorus, PTH and calcium-phosphorus products. According to the diagnostic criteria of CKD-MBD, patients with stage 3-5 of CKD could be diagnosed as CKD-MBD. Kuo TH (Pietrzyk et al., 2019) research found that serum Sclerostin serum levels were closely related to bone density and PTH in renal dialysis patients; Kanbay et al. (2014) also found that plasma Sclerostin levels and iPTH, blood phosphorus, and alkaline phosphate in renal dialysis patients showed a significant positive correlation. These results are mutually corroborative to the conclusions of our study. Serum Sclerostin levels are expected to be a sensitive indicator of the occurrence of CKD-MBD. In addition, some scholars have pointed out that Sclerostin is closely related to the prognosis of CKD (Oštrić et al., 2019), which further shows

Table 3. Multiple linear regression analysis of sclerostin and related indicators in CKD-MBD patients.

Parameters	B value	SE	β	T value	P value
Constant	137.576	7.329		18.773	0.000
Age	-0.009	0.027	-0.003	-0.332	0.740
Gender	-0.276	0.430	-0.005	-0.643	0.521
BMI	0.011	0.100	0.001	0.112	0.911
BUN	0.007	0.038	0.002	0.173	0.863
Scr	0.010	0.003	0.062	3.118	0.002
eGFR	-0.071	0.014	-0.122	-5.268	0.000
Ca	-44.143	2.212	-0.415	-19.953	0.000
P	7.870	1.323	0.119	5.950	0.000
iPTH	0.072	0.007	0.326	10.905	0.000

Table 4. Multiple linear regression analysis of BMP-7 and related indicators in CKD-MBD patients.

Parameters	B value	SE	β	T value	P value
Constant	-66.965	9.926		-6.746	0.000
Age	0.030	0.036	0.009	0.833	0.406
Gender	0.351	0.582	0.006	0.603	0.547
BMI	-0.037	0.135	-0.003	-0.273	0.785
BUN	0.037	0.052	0.013	0.720	0.472
Scr	-0.012	0.004	-0.077	-2.960	0.004
eGFR	0.090	0.018	0.150	4.918	0.000
Ca	64.667	2.996	0.591	21.581	0.000
P	-11.050	1.791	-0.163	-6.168	0.000
iPTH	-0.018	0.009	-0.080	-2.031	0.044

that Sclerostin might involve in CKD pathogenesis. Studies have found that BMP-7 is a protective factor of the kidney (Ćelić et al., 2018) and its expression level is closely related to the coronary artery calcification score in patients with CKD (Lv et al., 2018). At the same time, the results of this study also found that serum BMP-7 levels in patients with CKD-MBD were significantly reduced. It is suggested that the level of BMP-7 also affects the development of CKD. We also found that BMP-7 had a negative correlation with Scr, BUN, blood phosphorus level, and iPTH, and a positive correlation with eGFR and blood calcium. In addition, Scr, eGFR, blood calcium level, blood phosphorus level, iPTH is an independent risk factor for serum BMP-7 levels, suggesting that observation of changes in serum BMP-7 levels can be used as one of the indicators for the diagnosis and treatment efficacy of CKD-MBD patients. Some researchers have suggested that BMP-7 may be a therapeutic target for nephritis and renal fibrosis (Denizli et al., 2013), which confirms that BMP-7 is related to renal function. In addition, the correlation between BMP-7 and blood calcium and blood phosphorus may be related to the role of BMP-7 in bone formation. This study for first time demonstrates a correlation of Sclerostin and BMP-7 level with calcium and phosphorus metabolism, and renal function in CKD-MBD patients, providing another new clue for monitoring indicators during the diagnosis and treatment of CKD-MBD. However, since this study is only a cross-sectional study, it is impossible to deduce the influence of serum levels of Sclerostin and BMP-7 on the prognosis of patients with CKD-MBD. Also due to the limitation of the sample size, the future research needs to expand the sample size for further verification.

4 Conclusion

Changes in the expression levels of serum Sclerostin and BMP-7 may be important factors affecting the occurrence of CKD-MBD. Renal function, blood calcium and blood phosphorus are independent risk factors for serum Sclerostin and BMP-7 level.

Ethical approval

Research experiments conducted in this article with animals or humans were approved by the Ethical Committee and responsible authorities of our hospital following all guidelines, regulations, legal, and ethical standards as required for humans or animals.

Conflict of interest

The authors declare no conflicts of interest.

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