IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 17, Issue 2 Ver. 4 February. (2018), PP 85-90
www.iosrjournals.org

Comparison of Specific Bone Biomarkers in Cronic Kidney Disease Bulgarian Patients with secondary hyperparathyroidsm

Sv. Staykova [1], Y. Bocheva [1], K. Prodanova [2]
[1] *Clinic of Dialysis at University Hospital"St. Marina "-Varna, Bulgaria*
[2]*Department of Applied Mathematics&Informatics, Technical University of Sofia, Bulgaria*

Abstract: *Secondary hyperparathyroidism (sHPTH) is one of the most common complications of CKD (chronic kidney disease), which alters the mineral balance with the development of bone mineral disorders and soft tissue calcifications. The treatment of bone-mineral disorders requires continuous monitoring of Ca-P exchange, PTH (parathyroid hormone), serum Vitamin D levels and the protein bone markers - osteocalcin, bone alkaline phosphatase - BAP. In the Clinic of Dialysis at University Hospital "St. Marina " are followed and compared serum biomarker levels - BAP, Osteocalcin, Vit D in two groups of patients-dialysis and CKD patients in 2 and 3 stage with sHPTH. The results show statistically significant differences between the mean values of the two groups and between female and male in the tested serum levels of the indicators.*

Keywords: *biomarkers, hemodialysis treatment , predialysis stage, secondary hyperparathyroidism*

Date of Submission: 26-01-2018 Date of acceptance: 13-02-2018

I. Introduction

Secondary hyperparathyroidism (sHPTH) is one of the most common complications of CKD (chronic kidney disease), which alters the mineral balance with the development of bone mineral disorders and soft tissue calcifications. When glomerular filtration (GFR) is reduced (GFR<60ml/min), phosphorus clearance is significantly decreased, and this results in phosphorus retention and stimulation of PTH synthesis and secretion [8]. Kidney Disease Outcomes Quality Initiative (KDOQI) and Kidney Disease Improving Global Outcomes (KDIGO) experts have recognized that vitamin D insufficiency and deficiency should be avoided in CKD and dialysis patients (HD) by using supplementation to prevent SHPT. Despite the widespread use of the metabolites of vitamin D, phosphate binders and calcimimetics [1], in many patients with CKD and dialysis treatment, inadequate biochemical control has been observed.

The treatment of bone-mineral disorders requires continuous monitoring of Ca-P exchange, PTH (parathyroid hormone), serum Vitamin D levels and the protein bone markers - osteocalcin, bone alkaline phosphatase - BAP. Applied by us monitoring and comparison of the serum biomarker levels in two groups of patients-on HD and CKD – 2 and 3 stage with sHPTH identify statistically significant differences between the mean values of the two groups and between the male and female in the tested serum levels.

Vitamin D deficiency is an important component in the pathogenesis of sHPTH and it is defined as a reduced serum level of 25(OH)D (25 - hydroxyvitamin D). This deficit is rarely found in the general population but it is more commonly distributed among patients with CKD [20,17]. Vitamin D toxicity is increased by higher calcium intake, calcitriol analogs, and adynamic bone disease in dialysis patients. The frequency of this toxicity is not known but appears very rare. Diagnosis mainly includes hypercalcemia with the risk for extra osseous calcification.

Impaired renal function is associated with a 32% higher risk of vitamin D deficiency compared to the general population. It develops early in the course of chronic renal failure, and its proliferation increases with the progressive loss of kidney tissue [2]. Vitamin D belongs to the group of fat-soluble sex-sterols with several existing forms. The major compounds in humans are - vitamin D3 (cholecalciferol) and vitamin D2 (ergocalciferol). Synthesis of vitamin D3 is induced by sunlight in the skin and is a major source of vitamin D in the general population but can also be delivered from dietary sources [3, 4]. The usage of vitamin D receptor activator in the pre-dialysis stage has an inhibitory effect on elevating of the serum levels of PTH at the time of initiation of dialysis.

In chronic kidney disease (CKD), the hyperphosphaturic osteocyte-derived hormone FGF-23 increases to compensate for phosphate retention and further inhibits renal 1-hydroxylase expression, and induces the expression of 24-hydroxylase responsible for the degradation of 1,25-(OH2)D. However, 24,25(OH)D levels are lower in dialysis patients than in the normal population. Thus, the impaired uptake of 25(OH)D by altered kidneys remains the main cause of 1,25(OH2)D deficiency since the metabolic clearance rate of calcitriol seems not altered .In addition to the direct effect of high 25(OH)D levels, local osteoblastic conversion of 25(OH)D to

1,25(OH2)D appears to be an important positive regulator of FGF-23 production, particularly in uremia. Together with decreased kidney function, a decrease in 1,25(OH)2D leads to hypocalcemia and SHPT, which are the main causes of secondary osteoporosis.

The Bone alkaline phosphatase (BAP) is the bone-specific alkaline phosphatase isoform. A glycoprotein that is found on the surface of osteoblasts, BAP represents the biosynthetic activity of these bone-forming cells. It was shown to be a sensitive and reliable indicator of bone metabolism [5]. The BAP serum testing is of great importance for the monitoring of the treatment in the clinical practice in the patients on dialysis.

II. Material and Methods

A study of the biomarkers Vitamin D, Osteocalcin and BAP was performed in two groups of patients. The first group of 45 patients (23 man and 22 women) is in HD treatment at the Dialysis Clinic of University Hospital "St. Marina ", scheduled on dialysis sessions for 4 hours with a duration of the dialysis treatment of 2-9 years. Mean value $\pm$ Std. Dev. of the age of the patients is 51,53 $\pm$ 11,85 years.

The second group is with the same sample size of 45 patients (19 man and 26 women) in pre-dialysis stage with CKD. Mean value of the age is 68,88 $\pm$ 14,67 years.

The following methods were used to study 25 OH Vitamin D, Osteocalcin and BAP OSTASE®:

1. The LIAISON® 25 OH Vitamin D TOTAL test applies a chemiluminescent immune test (CLIA) technology for the quantification of 25-hydroxyvitamin D and other hydroxylated metabolites of vitamin D in human serum.
2. The LIAISON® Osteocalcin Test of DiaSorin is a single-step chemiluminescent analysis (IHLA) designed to quantify the osteocalcin polypeptide in human serum.
3. DiaSorin LIAISON® BAP OSTASE® is a one-step supplemental chemiluminescent assay (IHLA) based on the "sandwich" method designed for quantification of bone alkaline phosphatase (BAP) in human serum.

The precision of the LIAISON® analysis was evaluated according to CLSI EP5-A2.

All blood samples for the tested biomarkers were taken at the beginning of the dialysis session in a "yellow" vacutainer - 5 ml of the blood hemlines before the anticoagulant was placed in the extracorporeal system. The blood sample was centrifuged for 10 minutes at 3600 revolutions and, after coagulation, the serum was separated from the clot.

The statistical analysis of the data is made by a specialized software package STATISTICA [6].

The descriptive statistics as the frequencies of distribution of the examined variables, mean values $\pm$ standard deviations and 95% confidence of the intervals of the means are presented. Student's test (t-test) for two independent samples for identification of statistically significant differences in the mean is used. Analysis of variance (ANOVA) test are used to detect significance of the factor "sex" for the serum Vitamin D levels, levels of protein bone markers osteocalcin and BAP. Values $p<0,05$ were adopted for statistically significant.

III. Results and Discussion

Osteocalcin, as a major non-collagen protein in human bones, is released from the bone matrix and enters the blood vessels. It is synthesized by osteoblasts and odontoblasts of the bone tissue. The main part of the synthesized protein is a part of the extracellular bone matrix, which then mineralizes with the formation of new bone and the rest goes into the bloodstream. After the bone resorption under the actions of the osteoclasts, osteocalcin is released from the bone matrix and falls into the blood. Blood levels of osteocalcin are an important indicator of the metabolic changes in bone tissue [7, 8].

Comparison of the serum biomarker levels of osteocalcin in the two groups of patients - on HD and predialysis using t-test for two independent samples identify statistically significant difference (p=0,027) between the mean values of the groups (Table1).

Table 1. Comparison of the mean values of the osteocalcin in patients on HD (h_PTH) and predialysis (n_PTH).

T-test for Independent Samples. Note: Variables were treated as independent samples									
osteocalcin	Mean Group 1	Mean Group 2	t-value	df	p	Valid N Group 1	Valid N Group 2	Std.Dev. Group 1	Std.Dev. Group 2
h_PTH vs. _n_PTH	1257.06	678.26	2.243	88	0.027	45	45	1517.29	800.36

The frequencies distribution of observed osteocalcin levels in patients of the researched two groups are presented on Fig.1.

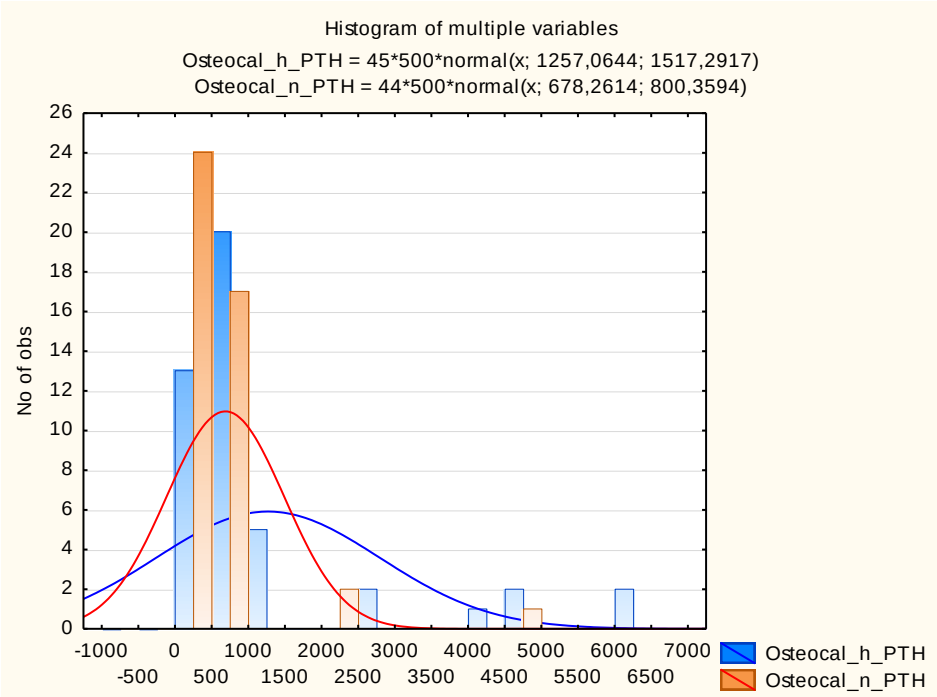

Fig.1: Histogram of osteocalcin levels in patients on HD (h_PTH) and predialysis (n_PTH).

The statistically significant difference in mean values of osteocalcin among patients with CKD with bone and mineral metabolism disorders before and after hemodialysis is associated with changes in serum PTH levels in these patient groups.

Vitamin D deficiency is associated with metabolic syndrome and obesity [9] in HD patients. In patients on peritoneal dialysis, low levels of vitamin D are associated with cognitive changes [10]. In transplanted patients, the low serum level of 25 (OH) D is associated with a rapid reduction of the renal function. Vitamin D deficiency is associated with insulin resistance, ventricular hypertrophy, atherosclerosis and vascular calcifications [3]. Vitamin D deficiency, which includes both serum levels of 25 (OH) D and levels of 1,25 (OH) D, is usually seen in patients with CKD and on chronic dialysis. The main consequence is sHPTH and the active metabolites of vitamin D remain the first line of prevention and treatment. Lower 25 (OH) D levels are associated with high bone metabolism, secondary hyperparathyroidism and reduced bone mineral density (BMD) in patients with CKD and on dialysis.

From our study, no significant difference in the mean values of Vitamin D values (p=0,102) was found in the two groups of patients on dialysis (18,74 ± 8,83) and in predialysis stage (15,95 ± 6,85

ANOVA test remained significantly differences in the measured values of Vitamin D for female and male in the both groups - with high PTH (p=0,02) and with normal PTH (p=0,047) (Table2).

Table 2. Mean values and St Dev. of Vitamin D measured in female and male patients in the two groups.

Mean ± Std. Dev. of Vit.D for female and male			
	N	Vit.D Mean	Vit.D Std.Dev.
Female- high_PTH	22	14,72	6,13
Male- high_PTH	23	22,58	9,48
Female_normal_PTH	20	13,71	7,38
Male_normal_PTH	25	17,65	6,01

On the Fig.2 the distribution of values of Vitamin D in the two groups is presented.

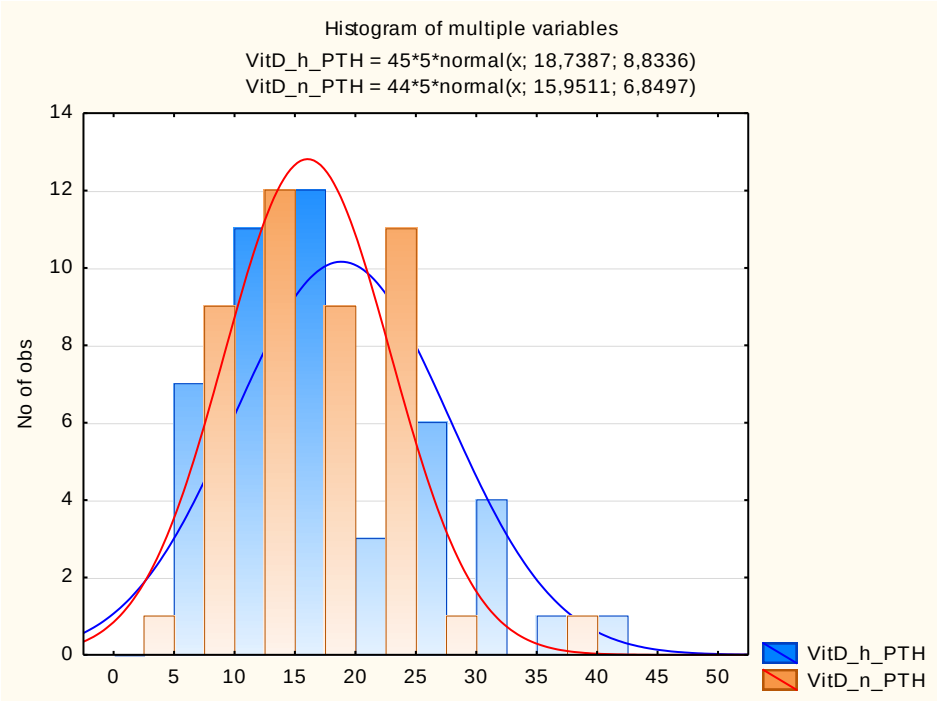

Fig.2: Histogram of the distributions of values of Vitamin D in the two groups.

In [12] the reorganization of the bone with the bone-specific alkaline phosphatase and bone biopsy in hemodialysis patients with CKD and diagnosed a dynamic disease and different bone changes in hyperparathyroidism is examined. At a reference value of 22 ng/mL this enzyme is an optimal prognostic factor for bone biopsy. At levels above 22 ng/mL and those of intact parathyroid hormone below 726 pg / mL, mild to moderate reorganization of bone tissue is considered. High BAP levels are associated with disability and high mortality in dialysis patients and its dominant effect on bone metabolism is emphasized.

Our results show a significant difference in the serum BAP in the studied groups of patients - with high PTH and normal PTH in the HD patients, which is consistent with studies in the foreign literature. The measured values of BAP in the patients with high PTH remained significantly higher as compared to the values in the group of patients with normal PTH (54,30 ± 75,20 vs 22,60 ± 29,10, p=0,011). On the Fig.3 the mean values ± St. Error of BAP and its 95% confidence intervals in the two researched groups are presented.

No significant difference was established between the values of the BAP for female and male in the two researched groups. In the Table 3 the mean values ± Std. Dev. of BAP measured in female and male patients are presented.

Table 3. Mean values, std. dev. of BAP measured in female and male patients in the two groups.

Mean ± Std. Dev. of BAP for female and male			
	N	BAP Mean	BAP Std.Dev.
BAP_high_PTH: Total	45	54,30	75,20
Female_high_PTH	22	63,05	83,12
Male_high_PTH	23	41,08	67,33
BAP_normal_PTH: Total	45	22,60	29,12
Female__normal_PTH	20	21,35	12,96
Male _normal_PTH	25	23,56	37,30

On the Fig.3 the mean values ± St. Error of BAP and its 95% confidence intervals in the two researched groups are presented.

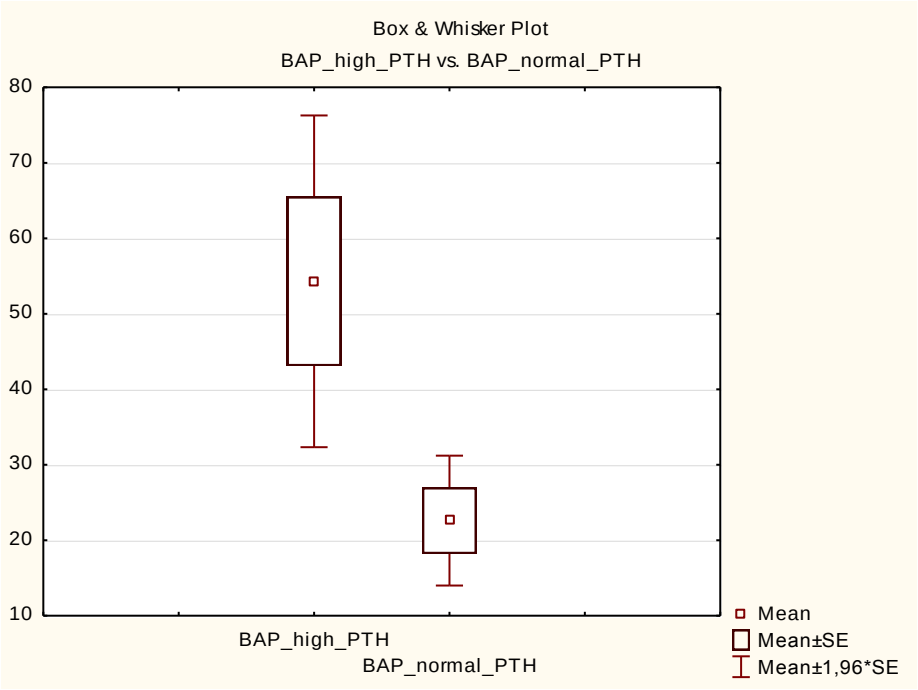

Fig.3. Box & Whisker plot of the mean values of BAP and its 95% confidence intervals in the HD patients.
On Fig. 4 the mean values ± Std. Dev. of BAP measured in female and male patients are presented.

Authors in [13] propose the use of bone-specific alkaline phosphatase as an alternative to parathyroid hormone because this enzyme is directly related to the bone reorganization, reflects its histomorphometry and can predict the results of hemodialysis treatment in patients with chronic kidney disease and disorders of bone mineral metabolism. Bone-specific alkaline phosphatase and intact parathyroid hormone values vary within the widest range. The levels of specific protein biomarkers - bone alkaline phosphatase and vitamin D are very low in patients on chronic hemodialysis and in patients in the pre-dialysis period [12].

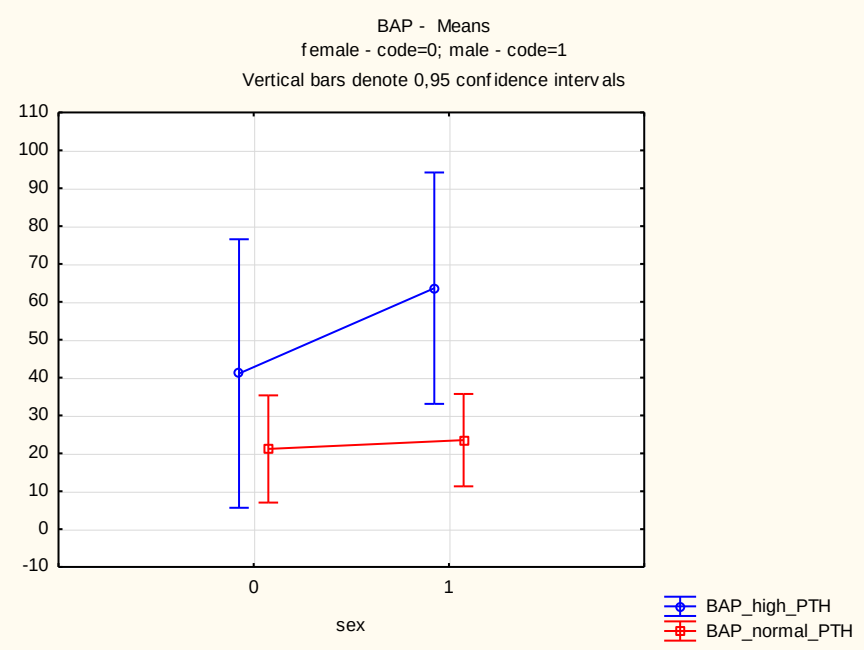

Fig. 4. The mean values and its 95% confidence intervals of BAP measured in female (coded by 0) and male (coded by 1) patients in the two researched groups.

IV. Conclusion

The treatment of bone and mineral disorders requires continuous monitoring of protein bone markers - PTH, serum Vitamin D levels., osteocalcin and bone alkaline phosphatase. Our results show a significant difference in the serum BAP, osteocalcin and bone alkaline phosphatase in the studied groups of patients - with high PTH and normal PTH on HD, which is consistent with studies in the foreign literature.

In conclusion, the findings of the present work prove that the simultaneous control of bone biomarkers results in a higher survival rate than control of only one or two of these parameters. Long-term and consistent control is associated with longer survival and better quality of life.

Acknowledgements

This research is partially supported by project DN12/11/dec. 20. 2017 of Ministry of Education and Science of Bulgaria.

References

[1] B. D. Reams, Dluzniewski P.J., Do T.P., Dynamics of cinacalcet use and biochemical control in hemodialysis patients: a retrospective New-user cohort design. *BMC Nephrol,16*, 2015, 16-175.

[2] T. B. Drueke , Massy Z.A., Circulating Klotho levels: clinical relevance and relationship with tissue Klotho expression, *Kidney Int., 83*, 2013, 13–15.

[3] R. D. Kilpatrick, Danese M.D., Belozeroff V., The association of vitamin D use with hypercalcemia and hyperphosphatemia in hemodialysis patients: a case-crossover study, *Pharmacoepidemiol Drug Saf, 20,*. 2011, 914-921.

[4] J. Michaud, Naud J., Ouimet D., Demers C. Petit J.L., Leblond F.A, Reduced hepatic synthesis of calcidiol in uremia,. *J. Am. Soc. Nephrol., 21*, 2010, 488–1497.

[5] Arbor Research Collaborative for Health: , *Annual Report of the Dialysis Outcomes and Practice Patterns Study: Hemodialysis Data 1997-2011*,2012.

[6] STATISTICA-*Manual Data analysis software system*, Version 10 (StatSoft Inc. USA, 2010).

[7] S. M. Moe, Thadhani R., What have we learned about chronic kidney disease-mineral bone disorder from the EVOLVE and PRIMO trials?, *Curr Opin Nephrol Hypertens,22*, 2013, 651–655.

[8] M. Cozzolino, Tomlinson J., Walsh L., Emerging drugs for secondary hyperparathyroidism, *Expert Opin Emerg Drugs*, *20*, 2015, 197-208.

[9] F. Ahmadi, Damghani S.; Lessan-Pezeshki M.; Razeghi E.; Maziar S.; Mahdavi-Mazdeh M., Association of low vitamin D levels with metabolic syndrome in hemodialysis patients, *Hemodial. Int., 20*, 2016 261–269.

[10] G. L. Liu, Pi H.C.; Hao L.; Li D.D.; Wu Y.G.; Dong J. ,Vitamin D Status Is an Independent Risk Factor for Global Cognitive Impairment in Peritoneal Dialysis Patients, *PLoS ONE, 10,* 2015,e0143782.

[11] F. Bienaime; Girard D., Anglicheau D., Canaud G.; Souberbielle J.C., Kreis, H., Noel L.H., Friedlander G., Elie C., Legendre C., Vitamin D status and outcomes after renal transplantation, *J. Am. Soc. Nephrol., 24*, 2013, 831–841.

[12] E. Ishimura, Okuno S, Okazaki H, Norimine K, Yamakawa K, Yamakawa T, Shoji S, Nishizawa Y, Inaba M, Significant association between bone-specific alkaline phosphatase and vascular calcification of the hand arteries in male hemodialysis patients, *Kidney Blood Press Res.*, 39(4), 2014, 299-307.

[13] S. Sardiwal, Magnusson P, Goldsmith DJ, Lamb EJ. Bone alkaline phosphatase in CKD-mineral bone disorder, *Am J Kidney Dis., 62(4)*, 2013, 810-822

Sv. Staykova "Comparison of Specific Bone Biomarkers in Cronic Kidney Disease Bulgarian Patients with secondary hyperparathyroidsm." IOSR Journal of Dental and Medical Sciences (IOSR-JDMS) , vol. 17, no. 2, 2018, pp. 85-90.