SERUM VITAMIN D_2, GROWTH HORMONE, ALKALINE PHOSPHATASE AND CALCIUM PHOSPHATE PRODUCT IN PATIENTS WITH END STAGE RENAL DISEASE

Santosh Chaudhary,[1] Narayan Gautam,[2] Manoj Karki,[3] Sunkeshari Deshar,[2] Archana Jayan,[2] Amit Chandra Jha,[2] Binaya Tamang,[2] Buddhi Raj Pokhrel,[2] Jharana Shrestha,[2] Raju Kumar Dubey[4]

ABSTRACT

INTRODUCTION

The chronic kidney disease (CKD) patient's calcium phosphate product, alkaline phosphatase (ALP), vitamin-D_2 and human growth hormone (hGH) are altered under haemodialysis. This study aimed to evaluate these biochemical variables in conjunction with haemoglobin and blood pressure to find out their association in End Stage Renal Disease (ESRD) patients.

MATERIAL AND METHODS

This cross-sectional study comprised of 104 patients with ESRD undergoing haemodialysis. The estimated glomerular filtration rate (eGFR) was calculated by Cockcroft-Gault (CG) equation and calcium, phosphorus, ALP were measured by fully automated analyzer whereas vitamin-D_2 and hGH were measured by sandwich and competitive enzyme linked immune sorbent assay (ELISA) techniques.

RESULTS

The mean age of patients was 53.12 ±16.37 years comprising 68% male. The hypovitaminosis D was 57.7% deficiency and 23.1% insufficiency states whereas hGH insufficiency was 22.1%. The calcium phosphate product was found to be increased in only 39.9% cases. The increased ALP level was observed in 64.4% cases. There was statistically significant association between hGH and Hb status (p=0.03). The significant difference in mean sodium and Ca×P of ESRD cases was observed with hypertension status (p=0.03 and p=0.01) respectively. Moreover, the significant difference in mean eGFR and hGH was observed with haemoglobin status (p=0.0001and p=0.01) respectively.

CONCLUSION

Increased level of ALP and hypovitaminosis-D was very common in ESRD patients undergoing dialysis with less prevalence of hGH insufficiency and calcium phosphate product increment. The anaemia and hypertension status can be pre-existing condition with ESRD which are cumbersome to control if not monitor in these patients.

KEYWORDS

Alkaline phosphatase, Calcium phosphate product, End stage renal disease, Human growth hormone.

1. District Hospital Taplejung, Fungling, Taplejung, Nepal
2. Department of Biochemistry, UCMS, Bhairahawa, Nepal
3. Department of Internal Medicine, UCMS, Bhairahawa, Nepal
4. Department of Biochemistry, Maharajgunj Medical Campus, Maharajgunj

DOI: http//doi.org/10.3126/jucms.v9i01.37979

For Correspondence
Mr. Narayan Gautam
Department of Biochemistry
Universal College of Medical Sciences
Bhairahawa, Nepal
Email: ng_bp22@yahoo.com

INTRODUCTION

Chronic kidney disease (CKD) stages IV and V are classified as End Stage Renal Disease (ESRD).[1,2,3] It is a worldwide health problem associated with cardiovascular morbidity and mortality in 10-16% of adult populations.[4,5,6,7] As kidney function declines, mineral homeostasis is altered with alteration in serum concentration of calcium, phosphorous, alkaline phosphatase (ALP), and vitamin-D.[2,8]

After renal failure, excretion of phosphorous by kidney is reduced which decrease the 1-α hydroxylase activity with lesser synthesis of calcitriol leading to hypocalcaemia. As a result, parathyroid hormone (PTH) secretion stimulates bone demineralization and high bone turnover with production of ALP by osteoblast.[2,3] Hyperphosphatemia and poor control of Ca×P cause vascular calcification leading to left ventricular hypertrophy (LVH) which is common in ESRD patients followed by diastolic dysfunction.[5,9] Growth hormone insensitivity is seen in advanced CKD, where functional insulin like growth factor-1(IGF-1) is deficient.[10,11]

This study aims to estimate the serum calcium phosphate product, ALP, Vitamin-D and hGH in CKD patients with IV and V stage ESRD and to find out association with CKD variables.

MATERIAL AND METHODS

It was a hospital based cross sectional and analytical study conducted at Universal College of Medical Sciences (UCMS), Bhairahawa, Nepal from March 2019 to August 2019 that was approved by Institutional Review Committee with registration number UCMS/IRC/029/19 prior to the study. Both verbal and written consents were taken from the participants. Total 104 ESRD patients more than 15 years of age under haemodialysis were included. General characteristics of the patients like name, age, sex, body weight, haemoglobin level and blood pressure were noted. eGFR was calculated by CG equation and staging was done according to Kidney Disease Outcomes Quality Initiative (KDOQI) guideline. Serum level of calcium, phosphorous, alkaline phosphatases using standard protocol in fully automated analyzer (HUMAN 600) whereas vitamin-D_2 and hGH were measured by sandwich and competitive ELISA technique respectively. The categorization variables were done as per manufacturer's instructions: ALP >11 KAU high, 4-11 KAU normal and <4 KAU decreased (ARKRAY Healthcare Pvt. Ltd; India), hGH <9.1 µIU/ml insufficient and 9.1-55 µIU/ml sufficient (Diametra ELISA; Italy), Vitamin D_2 <10 ng/ml deficient, 10-30 ng/ml insufficient and 30-100 ng/ml sufficient (Diametra ELISA; Italy), the Ca×P product <40 group I, 40-55 group II and >55 group III (Erba; Germany).

The patients with liver disease, dwarfism or acromegaly, bone lesions, acute kidney disease and less than 15-years cases were excluded. The consent was taken from patients or their guardians and pre-defined questionnaire was filled up.

All the data from cases were fed in Microsoft (MS) Excel office and then analyzed by Statistical Package for Social Service (SPSS) for window version 22, Inc., Chicago, IL. The data were expressed in terms of percentage frequency and categorical data were analyzed by Chi-square test. The difference of the mean was compared by student's t-test. *p*-value <0.05 was considered to be statistically significant.

RESULTS

Table 1. Demographic variables of the ESRD cases (N=104)

Variables	Status	ESRD stages (n) IV	ESRD stages (n) V	Total (N)
Gender	Male	15 (78.9%)	56 (65.9%)	71 (68.26%)
	Female	4 (21.1%)	29 (34.1%)	33 (31.73%)
	Total	19	85	104
Age	≤45	6 (13.95%)	30 (49.18%)	36 (34.6%)
	>45	37 (86.05%)	31 (50.82%)	68 (65.4%)
	Total	43	61	104
Age (years) mean ± SD		53.94 ±11.14	52.94 ±17.37	53.12 ±16.37
Weight (Kg) mean ± SD		60.63 ±8.81	55.88 ±10.87	56.75 ±10.64

Out of the total 104 cases, 71 (68.26%) were males. The age >45-years cases were maximum with frequency 68 (65.4%). The mean age of the case was 53.12±16.37 years and mean body weight was 56.75±10.64 Kg.

Table 2. Association between ESRD, haemoglobin and hypertension status with ALP, hGH, vitamin D_2 and Ca×P

Variables	Status	ESRD stage IV	ESRD stage V	Hb level (gm/dl) <10	Hb level (gm/dl) ≥10	HTN status (mmHg) ≥140/90	HTN status (mmHg) (<140/90)	Total
ALP	High	14	53	57	10	40	27	67
(KAU)	>11	73.7%	62.4%	67.1%	52.6%	58.8%	75%	64.4%
	Normal	4	27	24	7	24	7	31
	4-11	21.1%	31.8%	28.2%	36.8%	35.3%	19.4%	29.8%
	Low	1	5	4	2	4	2	6
	<4	5.3%	5.9%	4.7%	10.5%	5.9%	5.6%	5.8%
hGH	Insufficient	6	17	15	8	15	8	23
(µIU/ml)	<9.1	31.6%	20%	17.6%	42.1%	22.1%	22.2%	22.1%
	Sufficient	13	68	70	11	53	28	81
	9.1-55	68.4%	80%	82.4%	57.9%	77.9%	77.8%	77.9%
Vitamin D	Deficient	9	51	49	11	41	19	60
(ng/ml)	<10	47.4%	60%	57.6%	57.9%	60.3%	52.8%	57.7%
	Insufficient	5	19	20	4	16	8	24
	10-30	26.3%	22.4%	23.5%	21.1%	23.5%	22.2%	23.1%
	Sufficient	5	15	16	4	11	9	20
	30-100	26.3%	17.6%	18.8%	21.1%	16.2%	25%	19.2%
Ca×P	Group I	12	45	46	11	36	21	57
(mg^2/dl^2)	<40	63.2%	52.9%	54.1%	57.9%	52.9%	58.3%	54.8%
	Group II	3	30	26	7	26	7	33
	40-55	15.8%	35.3%	30.6%	36.8%	38.2%	19.4%	31.7%
	Group III	4	10	13	1	6	8	14
	>55	21.1%	11.8%	15.3%	5.3%	8.8%	22.2%	13.5%
Total		19	85	85	19	68	36	104

The frequency distribution of ALP shows increase level in 67 (64.4%) cases. The distribution of hGH shows insufficient level in 23 (22.1%) cases and vitamin D_2 deficiency was observed in 60 (57.7%) and insufficient vitamin D_2 in 24 (23.1%) cases. The increased Ca×P product was observed in 14 (13.5%) cases.

Table 3. Mean ± SD of ALP, hGH, vitamin D_2, Ca×P, urea, creatinine, sodium, potassium, eGFR, Hb level based on ESRD, Hb and HTN status

Variables	ESRD stage		Hb level (gm/dl)		HTN status (mm Hg)	
	IV	V	<10	≥10	(≥140/90)	(<140/90)
ALP (KAU)	20.4±15.45	18.15± 16.6	19.26±17.51	15.43±9.24	18.04±14.26	19.55±19.88
	p=0.93		p=0.20		p=0.92	
hGH (µIU/ml)	16.42±10.41	14.88±8.19	15.02±7.86	15.78±11.60	14.86 ±8.72	15.75±8.47
	p=0.08		p=**0.01**		p=0.97	
Vit.D_2 (ng/ml)	23.39 ±26.99	16.07±20.6	17.42±21.96	17.36±22.45	16.63±18.88	22.13±21.81
	p=0.12		p=0.95		p=0.95	
Ca×P (mg^2/dl^2)	39.92 ±23.67	38.96±19.14	40.0 ±20.99	35.26 ±14.0	37.69 ±15.52	41.86± 26.36
	p=0.58		p=0.38		p=**0.01**	
Urea (mg/dl)	113.73±43.33	152.47±55.26	147.42±54.65	136.31±58.11	144.96±54.17	146.22±57.79
	p=0.59		p=0.79		p=0.64	
Creatinine (mg/dl)	4.34±1.41	8.57±3.53	7.95±3.67	7.12±3.47	8.17±3.55	7.09±3.73
	p=**0.002**		p=0.99		p=0.88	
Na (mmol/l)	136.62±4.86	135.99±4.14	135.99 ±4.16	136.62± 4.81	135.75 ±3.67	136.77±5.21
	p=0.58		p=0.40		p=**0.03**	
K (mmol/l)	4.61±0.92	4.66 ±0.76	4.7±0.72	4.43±1.03	4.58±0.80	4.77 ±0.75
	p=0.06		p=0.31		p=0.78	
eGFR (ml/min)	18.22± 3.70	8.79±2.92	10.10± 4.05	12.35± 7.02	9.97± 4.24	11.54± 5.54
	p=0.44		p=**0.0001**		p=0.14	
Hb (gm/dl)	8.8± 1.80	7.51± 2.06	7.10± 1.57	10.65± 1.40	7.92± 2.04	7.42± 2.09
	p=0.55		p=0.11		p=0.98	

There was significant decrease in hGH in cases with haemoglobin level <10 gm/dl as compared to 10 gm/dl (p=0.01). eGFR was observed significantly higher in Hb 10 gm/dl as compared to <10 gm/dl (p=0.0001). The Ca×P and sodium was significantly different in hypertension group (p=0.01 and p=0.03). Serum creatinine was statistically different in ESDR IV and ESDR V group (p=0.002).

DISCUSSION

Different pathophysiological processes like abnormal kidney function and decreased GFR is commonly seen in patients with CKD which become symptomatic at stage IV and V called End Stage Renal Disease (ESRD).[3,4] Anaemia, bone turnover and mineralization, abnormal calcium, vitamin D, sodium, potassium, water and acid homeostasis are the other complications seen in CKD patients besides abnormal mineral homeostasis.[3] However, hyperphosphatemia is more prevalent in CKD.[2]

CKD was more prevalent in male patients (68.3%) which correspond with the result reported by Mishra D et al[12] who showed (64.6%) cases were male. However, Khan YH et al[7] and Verma PP et al[13] reported higher prevalence of CKD among female patients. The age categorization ≤45 years and >45 years based on CKD complication where maximum prevalence occurs in age >45 years. The present study has shown high prevalence of hypovitaminosis-D_2 (80.8%), hGH insufficiency (22.1%), hypocalcaemia (58.6%), hyperphosphatemia (71.1%), high ALP level (64.4%) in ESRD patients. This study is in congruence to the study done by Rajbhandari A et al[8] who reported hypocalcaemia in 60.6% patients. Hypocalcaemia is caused because of decreased intestinal absorption of calcium, calcium phosphorus product (Ca×P) deposition due to vitamin D deficiency.[14] Moreover, the patients in our study were under maintenance haemodialysis in both stages of CKD. According to Freethi R et al as CKD stage increases calcium level is reduced.[2]

Rajbhandari A et al[8] found hyperphosphatemia in 63.6% patients which is similar to our study. Likewise, other studies also shown significant increment of serum phosphorus in CKD patients with 69.4% in Nigeria[8,15] and 64.1% in India.[8,16] Although other studies reported gradual increment of serum phosphorus with the rise of CKD stage.[12,14] There was no significant association between phosphorus and CKD stage. Hyperphosphatemia can be independent predictor of increased cardiovascular mortality.[2,5,8] Elevated phosphorous accelerate the effect of coronary atherosclerosis via vascular calcification and proliferation of smooth muscle. Vascular calcification reduce vessel wall elasticity, increase thickness of ventricular wall (>12 mm) and often linked with left ventricular hypertrophy (LVH).[2,5] Very few studies were conducted to find out relation of serum phosphorous and blood pressure (BP). None of the studies yet found association between Ca×P product and BP in haemodialysis patients.[6] Similarly, in the present study there was no significant association was observed between Ca×P product and HTN status (p=0.054). Sixty-eight (65.3%) patients had hypertension which is similar to result of Shrestha TM et al[17] and Shivendra S et al[5] who observed hypertension in 50.7% and 77.14% patients respectively. We found significant difference in Ca×P product with HTN status (p=0.01). The poorly controlled Ca×P and high phosphorous is mainly associated with diastolic dysfunction due to altered mineral metabolism.[5,9]

ALP produced by osteoblast is a biochemical marker of bone turnover and help in monitoring metabolic bone disease associated with renal insufficiency.[2,3] Several studies reported increased level of ALP in CKD including our study which shows high prevalence of raised ALP level in 64.4% patients. Hypocalcaemia stimulate increased PTH secretion. As PTH level is elevated, it stimulates bone demineralization leading to high bone turnover. Thus, ALP production by osteoblast is accelerated which contribute its high level in serum in response of low calcium level.

Low haemoglobin levels (<10 gm/dl) were observed in 85

(81.7%) patients. Similarly, Shivendra S et al[5] observed anaemia in all patients and Hb less than 10 gm/dl was seen in 60 % of them. Gupta V et al[11] has shown that repeated blood sampling, surgical intervention, blood loss via use of dialyzer, shortened RBC life span are the cause of anaemia in CKD patients.

Hypovitaminosis D is result of reduced 1-α hydroxylation of vitamin D in CKD patients due to reduced renal mass.[18] Different studies held at different geographical regions reported low vitamin D. Hypovitaminosis D is 100% in Malaysia,[19] 72.2% in Thailand,[14] 86% in South India[16] and 73.6% in Brazil.[20] These results are consistent with our study which shows high prevalence of hypovitaminosis D in 80.8% patients with deficiency states in 57.7%, insufficiency states in 23.1%. Low vitamin D observed in general population due to low intake of vitamin D in diet and inadequate exposure to sunshine.[21] However, in case of CKD patients with hypovitaminosis D, other factors like, female sex, proteinuria, diabetes, low physical activity and adiposity are associated condition.[22] According to Canakya E et al vitamin D level is lower in hemodialysis patients.[23] Guesseous I et al observed vitamin D deficiency in CKD patients and general population was quiet similar.[24] Vitamin D deficiency is more severe in V stage CKD.[14,16] But some study reported that vitamin D level is not influenced by eGFR.[20]

There was no association between vitamin D and CKD stage which is similar to result of Rajbhandari A et al[8] but some study noted decrease in vitamin D level with progression of CKD.[2] Vitamin D is believed to be inhibitor of renin-angiotensin mechanism and high vitamin D concentration has inverse relation with rennin activity.[25]

Renal dysfunction directly affects the production of erythropoietin.[11,26] Increased renal sodium loss is secondary cause of reduced erythropoietin production. CKD patients having reduced GFR has reduced sodium absorption and a net relative excess of oxygen which serves as signal to decreased erythropoietin production.[11] The hGH affects renal function and renal growth. There was significant association between hGH and Hb status (p=0.03). There was significant difference in mean hGH levels in Hb status (p=0.01). GFR is increased by hGH like insulin-like growth factor-1 (IGF-1).[11,26] In our study, the prevalence of hGH insufficiency was less i.e, 22.1%. Some study showed that kidney plays vital role in metabolic clearance of hGH, as a result CKD patients hGH level is increased.[26] Kidney helps in excretion of hGH metabolites but in CKD cases kidney can't function properly so less excretion of hGH. Therefore, hGH in blood is increased and this could be one reason for less prevalence of hGH insufficiency. Although some other study says that insufficient hGH level in CKD patients is main reason for growth retardation. Gupta V et al reported that 23% children (>12 years) with chronic renal failure exhibit growth failure.[11]

CONCLUSION

Increased level of ALP and hypovitaminosis D are more prevalent whereas of hGH insufficiency and increased calcium phosphate product are less prevalent in ESRD patients undergoing dialysis. Moreover, the significant association of hGH, eGFR with haemoglobin level and electrolytes like sodium, Ca×P with HTN may exacerbate cardiovascular problem associated in ESRD patients which need to be monitored in well clinical set-up.

ACKNOWLEDGEMENT

We would like to acknowledge our gratitude to all of the participants of this study.

REFERENCES

1. Karki KB, MJ GM PA, Makai P, Subedi R, Poudyal A, Aryal KK. Assessment of chronic kidney disease support program of Government of Nepal, 2016. Kathmandu: Nepal Health Research Council. 2017.
2. Freethi R, Raj AV, Ponniraivan K, Khan MR, Sundhararajan A. Study of serum levels of calcium, phosphorus and alkaline phosphatase in chronic kidney disease. International Journal of Medical Research & Health Sciences. 2016;5(3):49-56.
3. Nirmala Devi K, Dr. Begum AA, Sathiya K, Deepa Laksmi P, et al. Study of serum magnesium, calcium, phosphorous and alkaline phosphatase in chronic kidney disease. National Journal of Basic Medical Sciences. 2017;8(1):16-20.
4. George C, Mogueo A, Okpechi I, Echouffo-Tcheugui JB, Kengne AP. Chronic kidney disease in low-income to middle-income countries: the case for increased screening. BMJ Global Health. 2017;2(2): e000256.
5. Shivendra S, Doley PK, Pragya P, Sivasankar M, Singh VP, et al. Echocardiographic changes in patients with ESRD on maintenance hemodialysis-A single centre study. Journal of Cardiovascular Disease and Diagnosis. 2014;2:4.
6. Ashkar ZM. Association of calcium phosphorus product with blood pressure in dialysis. The Journal of Clinical Hypertension. 2010;12 (2):96-103.
7. Khan YH, Mallhi TH, Sarriff A, Khan AH, Tanveer N. Prevalence of chronic kidney disease in Asia: A Systematic review of population-based studies. Journal of the College of Physicians and Surgeons Pakistan. 2018;28(12):960-6.
8. Rajbhandari A, Agrawal RK, Baral A, Pokhrel A, Shrestha D, Hada R. Estimation of serum vitamin d, calcium and phosphorus in chronic kidney disease. Medical Journal of Shree Birendra Hospital. 2017;16(1):30-6.

9. Achinger SG, Ayus JC. Left ventricular hypertrophy: is hyperphosphatemia among dialysis patients a risk factor? Journal of the American Society of Nephrology. 2006;17(12 suppl3): S255-61.

10. Drube J, Wan M, Bonthuis M, Wühl E, Bacchetta J, Santos F, Grenda R, Edefonti A, Harambat J, Shroff R, Tönshoff B. Clinical practice recommendations for growth hormone treatment in children with chronic kidney disease. Nature Reviews Nephrology. 2019;15(9):577-89.

11. Gupta V, Lee M. Growth hormone in chronic renal disease. Indian Journal of Endocrinology and Metabolism. 2012;16(2) :195-203.

12. Mishra D, Koirala P. Status of chronic kidney disease patients registered in National Kidney Center, Banasthali, Kathmandu. Journal of Manmohan Memorial Institute of Health Sciences. 2015;1(4):19-23.

13. Varma PP, Raman DK, Ramakrishnan TS, Singh P, Varma A. Prevalence of early stages of chronic kidney disease in apparently healthy central government employees in India. Nephrology Dialysis Transplantation. 2010;25(9):3011-7.

14. Satirapoj B, Limwannata P, Chaiprasert A, Supasyndh O, Choovichian P. Vitamin D insufficiency and deficiency with stages of chronic kidney disease in an Asian population. BMC Nephrology. 2013;14(1):206.

15. Okoye JU, Arodiwe EB, Ulasi II, Ijoma CK, Onodugo OD. Prevalence of CKD-MBD in pre-dialysis patients using biochemical markers in Enugu, South-East Nigeria. African Health Sciences. 2015;15(3):941-8.

16. Arulanantham R, Mariappari S, Radhakrishnan S. Prevalence of vitamin D deficiency in chronic kidney disease: A single centered study from a rural tertiary care hospital in South India. Journal of Evidence Based Medicine and Healthcare. 2016;3(22):978-82.

17. Shrestha TM, Prasad PN, Neupane RP, Bhusal L, Ghimire R. Clinical presentation in chronic kidney disease patients on regular haemodialysis attending in Tribhuvan University Teaching Hospital Emergency Services. Journal of Karnali Academy of Health Sciences. 2019;2(2):138-43.

18. Okunola OO, Akinsola A. Chronic kidney disease, mineral bone disorder: review and appraisal. Tropical Journal of Nephrology. 2010;5(1):5-16.

19. Rozita M, Afidza MN, Ruslinda M, Cader R, Halim AG, Kong CTN, et al. Serum vitamin D levels in patients with chronic kidney disease. EXCLI Journal 2013;12:511-20.

20. Diniz HF, Romão MF, Elias RM, Júnior JER. Vitamin D deficiency and insufficiency in patients with chronic kidney disease. Brazilian Journal of Nephrology. 2012;34(1):58-63.

21. Bhatta MP. Prevalence of vitamin D deficiency among adult population of Western Region of Nepal. International Journal of Medicine & Biomedical Sciences. 2016;1(2):7-12.

22. Jean G, Souberbielle JC, Chazot C. Vitamin D in chronic kidney disease and dialysis patients. Nutrients. 2017;9(4):328.

23. Çankaya E, Bilen Y, Keleş M, Uyanık A, Akbaş M, Güngör A, Arslan Ş, Aydınlı B. Comparison of serum vitamin D Levels among patients with chronic kidney disease, patients in dialysis, and renal transplant patients. In Transplantation Proceedings 2015;47(5):1405-1407.

24. Guessous I, McClellan W, Kleinbaum D, Vaccarino V, Zoller O, Theler JM, Paccaud F, Burnier M, Bochud M. Comparisons of serum vitamin D levels, status, and determinants in populations with and without chronic kidney disease not requiring renal dialysis: a 24-hour urine collection population-based study. Journal of Renal Nutrition. 2014;24(5):303-12.

25. Cervellin G, Salvagno G, Bonfanti L, Bonelli P, Guidi GC, Lippi G. Association of hyponatremia and hypovitaminosis D in ambulatory adults. Journal of Medical Biochemistry. 2015;34(4):450-4.

26. Ogle GD, Rosenberg AR, Kainer G. Renal effects of growth hormone. I. Renal function and kidney growth. Pediatric Nephrology. 1992;6(4):394-8.