Relationship of Serum Levels of Bone Specific Alkali Phosphatase (bALP) and Alkali Phosphatase (ALP) with Vascular Calcification in Chronic Kidney Disease Patient on Chronic Hemodialysis

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Abstract

Background: Vascular calcification is an important non-conventional for cardiovascular disease (CVD) in chronic kidney disease (CKD) patients with chronic hemodialysis (HD). Abdominal aortic calcification (AAC) is reported as an independent predictor for cardiovascular morbidity and mortality. Bone-specific Alkali Phosphatase (bALP) and Alkali Phosphatase (ALP) enzymes are produced and released when changes or disorders of bone and mineral metabolism occur. Given biomarker studies such as bALP and ALP which are more often associated with patient mortality, more research will be needed to assess whether these bALP and ALP biomarkers have a linear distribution of relationships with vascular calcification.

Objective: This study aimed to evaluate the serum biomarker to predict calcification and further can be one of diagnosis modality of calcification in hemodialysis patients.

Methods: a total of 75 chronic HD CKD patients were included in the study. bALP and ALP serum levels were measured with ELISA, as well as AAC measured by lateral abdominal radiographs (X-Ray).

Results: bALP and ALP are positively correlated with AAC scores (p value <0.001 and 0.045). Multivariate logistic regression analysis shows that history of diabetes, bALP levels, and parathyroid hormone (PTH) levels are independent risk factors for AAC in chronic HD CKD patients. Receiver Operating Characteristic (ROC) shows the area under the curve (AUC) of bALP and ALP for AAC prediction are 0.882 (95% CI: 0.801-0.962; p value: <0.001) and 0.634 (95% CI: 0.509-0.760; p value: 0.045).

Conclusion: ALP and especially bALP serum correlate closely with vascular calcification in chronic HD CKD patients accompanied by a superior diagnostic value of bALP biomarkers when compared to ALP.

Keywords: bone-specific alkaline phosphatase cardiovascular disease, chronic kidney disease

Introduction

The number of patients undergoing dialysis has increased dramatically over the past three decades. Several factors contribute to this increase including: an increase in the survival of the general population, a decrease in the number deaths of dialysis patients, increased incidence of chronic kidney disease (CKD), expansion of criteria for renal replacement therapy, and wider access to dialysis in low and middle-income countries.[1] According to the results of the Global Burden of Disease in 2010, CKD was the 27th leading cause of death in the world in 1990 and increased to 18th in 2010. Whereas in Indonesia, kidney disease treatment is the second largest funding after heart disease. The Indonesian Renal Registry (IRR) data from 249 reporting renal units, recorded 30,554 active patients undergoing dialysis in 2015, most were CKD patients on hemodialysis.[2,3]

Disturbances in mineral and bone metabolism are common complications in CKD and are a significant cause of morbidity and decreased quality of life. Previous studies have proven disorders of mineral and bone metabolism associated with an increased risk of calcification of soft tissues and heart blood vessels and renal osteodystrophy.[4] Cardiovascular disease is one of the main causes of death in patients with CKD who

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undergo hemodialysis (HD) or peritoneal dialysis (PD) therapy.[5]

Kidney Disease Improving Global Outcomes (KDIGO) has recommended alkali phosphatase (ALP) as an additional examination when evaluating Chronic Kidney Disease - Mineral and Bone Disorder (CKD-MBD).[6-8] However, various studies have shown that ALP measurements was not specific considering the broad distribution of ALP in various organs other than bone. Considering biomarker such as bone specific alkali phosphatase (bALP), ALP and PTH are more often associated with patient mortality, further and large studies are needed to assess whether these b-ALP and ALP biomarkers have a linear distribution of relationships with vascular calcification.[6,9] And whether the incorporation of bALP enzymes and ALP biomarkers into diagnostic standards as an additional examination can significantly improve the diagnosis of earlier vascular calcification so that it has a better repairing effect on the risk of cardiovascular and skeletal events in patients with disease chronic kidney. This study aimed to evaluate the serum biomarker to predict calcification and further can be one of diagnosis modality of calcification in hemodialysis patients.

Methods

This study was an observational cross-sectional study with analytical approach. This study was conducted at Haji Adam Malik General Hospital Medan during December 2018 with the approval from the Research Ethics Commission of Faculty of Medicine of Universitas Sumatera Utara. The informed consent was obtained from subjects who were willing to participate in this study. The number of samples were calculated using the proportion estimation formula with a minimum sample of 67.

Inclusion criteria in this study were: patients with CKD disease who have undergone routine hemodialysis for the past 12 weeks or more; age ≥18 years, and patients who were willing to participate and gave informed consent. The *exclusion criteria* in this study were: non-adherent hemodialysis patient; patient with acute infection, patient who were unstable hemodynamically, and patients with severe liver disease (according to liver enzymes).

The blood samples were collected at baseline prior to initiation of HD to examine bALP, ALP, serum calcium, phosphate, SGOT (AST), SGPT (ALT), Magnesium, PTH, routine blood, and serum electrolytes. All individuals underwent abdominal radiographs and interpretations of lateral abdominal radiographs were done by a radiologist. The data were collected at single point of time. Classification of the abdominal aorta was assessed using abdominal aortic calcification (AAC) score, which is divided into: mild (≤4), moderate (5-15), severe (≥16) based on CORD study.[10]

Descriptive statistics were used for basic characteristics. The relationship of two qualitative characteristics was verified based on the *Chi-square test* or *Fisher's exact test*. Comparison of two values were performed by independent *t-test* or *Mann-Whitney test*. Furthermore, multivariate analysis was performed with logistic

Table I Basic Characteristics of The Subjects

Characteristic	n=75 (%)211211211211
Sex, n (%)	
Male	47 (62.7)
Female	28 (37.3)
Age (years)[b]	54 (13-77)
Disease history, n (%)	
Hypertension	47 (62.7)
Diabetes mellitus	23 (30.7)
Coronary artery disease	3 (4.0)
Stroke	2 (2.7)
Urinary stone	1 (1.3)
>1 Disease	11 (14.7)
Smoking	29 (38.7)
History of drug use, n (%)	
Anti-hypertension	
Irbesartan/Valsartan/Candesartan	31 (41.3)
Amlodipine/Nifedipine	40 (53.3)
Bisoprolol	4 (5.3)
Phosphate binding	30 (40.0)
Classification CORD Study, n (%)	
Mild	37 (49.3)
Moderate	38 (50.7)
Hemodialysis, n (%)	
10 hours	75 (100)
12 hours	0
Duration of hemodialysis (month)	24 (3-132)
Serology, n (%)	
Hepatitis C	40 (53.3)
Negative	35 (46.7)
Laboratory	
Hb (g/dl)[a]	8.937 (1.478)
Hct (%)[a]	27.56 (4.633)
WBC (cells/mm^3)[a]	7471.47 (2901)
Platelet (cells/mm^3)[a]	228,802 (8794)
bALP (u/L)[b]	117 (6-448)
ALP (u/L)[b]	165 (9-636)
PTH (u/L)[b]	300 (78 -1873)
Ferritin[b]	311.3 (9,9-8675)
Serum iron[b]	43 (7-261)
TIBC[b]	191 (118-343)
SGOT (u/L)[b]	14.0 (3-96)
SGPT (u/L)[b]	16.0 (6-135)
BUN PreHD (mg/dl)[a]	125.17 (36.850)
BUN PostHD (mg/dl)[a]	40.88 (36.850)
URR[a]	66.67 (10.238)
Creatinine (mg/dl)[a]	12.72 (4.474)
Sodium, (mEq/l)[b]	136 (125-142)
Potassium (mEq/l)[b]	4.1 (2.9-6.7)
Chloride (mEq/l)[b]	104 (84—113)
Calcium (mg/dl)[b]	7.92 (0.716)
Phosphate (mg/dl)[b]	5.1 (1.4-10.60)
Magnesium (mg/dl)[b]	2.3 (1.18-5.85)

[a]normal distribution; mean±SD
[b]abnormal distribution; median (minimum-maximum)

Table II. Bivariate Analysis of predicting factors of vascular calcification degree in CKD patients with chronic HD

Variable	Mild Calcification	Moderate Calcification	p Value
Sex			
Female	10 (27)	18 (47.4)	0.069[c]
Male	27 (73)	20 (52.6)	
Age (years)	56 (37-77)	51 (13-68)	0.265[b]
Disease history			
Diabetes mellitus	6 (16.2)	17 (44.7)	0.007*[c]
Smoking	15 (40.5)	14 (36.8)	0.742[c]
Hemodialysis			
Twice a week	37 (49.4)	38 (50.6)	1.000[d]
SBP (Pre-HD)	130 (100-180)	145(100-190)	0.026*[b]
SBP (Post-HD)	130 (100-210)	150 (100-190)	0.090[b]
DBP (Pre-HD)	80(70-110)	80 (70-100)	0.860[b]
DBP (Post-HD)	80 (70-120)	90 (50-100)	0.266[b]
Duration of HD	24 (3-124)	24 (3-132)	0.828[b]
Serology			
Hepatitis C	19 (51.4)	21 (55.3)	0.734[c]
Negative serology	18 (48.6)	17 (44.7)	
Laboratory			
Hb (g/dl)	8.676(1.763)	9.192 (1.105)	0.135[a]
Hct (%)	26.84 (5.54)	28.26 (3.46)	0.188[a]
WBC (cells/mm^3)	7335 (2350)	7603 (3380)	0.693[a]
Platelet (cells/mm^3)	226.004 (85070)	231526 (91711)	0.788[a]
bALP (u/L)	102 (6-211)	189.5 (90-448)	<0.001*[b]
ALP (u/L)	160 (9-255)	169 (87-636)	0.045*[b]
PTH (u/L)	294 (78-500)	311.5 (114-1873)	0.066[b]
Ferritin	315.5 (29.86-8675)	310 (9.9-1869)	0.966[b]
Besi	48 (7-261)	42.5 (20-193)	0.452[b]
TIBC	188(118-343)	194 (122-303)	0.672[b]
SGOT (u/L)	16 (7-43)	13.5 (3-96)	0.185[b]
SGPT (u/L)	16 (6-49)	16 (6-135)	0.738[b]
Ur preHD (mg/dl)	125.40 (34.25)	124.94 (39.67)	0.958[a]
Ur postHD (mg/dl)	41.64 (14.589)	40.13 (17.302)	0.683[a]
URR	66.32 (10.055)	67.00 (10.536)	0.777[a]
Creatinine (mg/dl)	12.409 (4.11)	13.03 (4.83)	0.549[a]
Sodium, (mEq/l)	136 (129-142)	136 (125-141)	0.869[b]
Potassium (mEq/l)	4.2 (2.9-6.7)	4.1 (3.1-5.3)	0.848[b]
Chloride (mEq/l)	104 (93-109)	103 (84-113)	1.000[b]
Calcium (mg/dl)	8.11 (0.707)	7.73 (0.682)	0.021*[a]
Phosphate (mg/dl)	4.9 (1.4-8.8)	5.7 (2-10.6)	0.213[b]
Mg(mg/dl)	2.25 (1.54)	2.31 (1.18-3.23)	0.582[b]

[a]normal distribution; mean±SD; independent t-test
[b]abnormal distribution; median (minimum-maximum); mann-Whitney test
[c]chi-square test
[d]Fisher's exact test

regression tests to control for confounding factors, with *p<0.05* being considered significant.

Results

There were 75 subjects on chronic hemodialysis who met the inclusion criteria in this study.

The basic characteristics of the subjects are described in *Table I*. There was no loss to follow up. The average age was 54 years, with a male contribution of 62.7%. The median length of hemodialysis was 24 months with chronic hemodialysis frequency of twice a week. The comorbid factors were smoking, hypertension, diabetes mellitus, coronary heart disease and more than one disease with contributions of 38.7%, 62.7%, 30.7%, 4% and 14.7%.

Based on the CORD study criteria, 37 people (49.3%) were scored to have mild calcification and 38 people (50.7%) with moderate calcification. Serum bALP levels in chronic HD patients with a median (min-max) 117 u/L (6-448) and ALP of 165 u/L (9-636). Thirty-three patients (44%) had no calcification site at L1-L4 (AAC score = 0). There were 38 subjects (50.7%) with serum PTH ≥ 300pg/L.

Table II shows bivariate analysis of predicting factors of vascular calcification degree in CKD patients with chronic HD. There was a significant difference of serum bALP levels between mild and moderate AAC score among chronic HD CKD patients (*p<0.001*). Median serum bALP in the moderate group was 189.5 u/L (90-448) and in the mild group was 102 u/L (6-211).

Based on bivariate analysis, there was a significant difference between predictor factors: diabetes, systolic blood pressure before hemodialysis, serum bALP levels, ALP levels, and calcium levels with the degree of abdominal aortic calcification.

Table III shows multivariate analysis of predicting factors of vascular calcification degree in CKD patients with chronic HD. Multivariate analysis using logistic regression test showed that a history of diabetes, bALP levels, and PTH levels were risk factors that significantly influence the incidence of abdominal aortic vascular calcification.

The strength of the relationship of risk factors that influence the incidence of AAC can be seen in *Table IV* with a history of DM (OR: 19.228; 95% CI: 1.117-331, *p=0.042*), followed by bALP levels (OR: 1.058; 95% CI: 1.029-1.088; *p <0.001*) and PTH levels (OR: 1.009; 95% CI: 1.001-1.017; *p 0.022*).

Through logistic regression models or equations were derived to predict the occurrence of calcification of abdominal aortic vessels. The probability of vascular calcification can be predicted by the following equation: Events of calcification = -6.092 + (2.956 * history of diabetes) + (0.056 * bALP levels) + (0.009 * level of PTH)

To obtain the Area under the Curve (AUC) value, an analysis was performed using Receiver Operating Characteristics (ROC). The cut-off values of bALP and ALP

Table III. Multivariate Analysis of predicting factors of vascular calcification degree in CKD patients with chronic HD

Risk Factor	Coefficient	p	OR	95% CI
First step				
Diabetes	3.268	0.070	26.258	0.768-897.812
SBP pre-HD	0.10	0.827	1.010	0.922-1.107
bALP	0.054	0.001	1.055	1.023-1.088
ALP	0.007	0.558	1.007	0.985-1.029
Calcium	-1.504	0.180	0.222	0.025-2.006
Male sex	-1.154	0.481	0.315	0.013-7.823
SBP post-HD	0.035	0.390	1.036	0.956-1.122
Hb	0.478	0.822	1.612	0.025-102.483
Hct	-0.061	0.930	0.941	0.244-3.624
PTH	0.007	0.154	1.007	0.997-1.016
SGOT	-0.087	0.091	0.916	0.828-1.014
Phosphate	0.608	0.256	1.837	0.643-5.247
6th selection				
DM	2.956	0.042	19.228	1.117-331.116
bALP	0.056	<0.001	1.058	1.029-1.088
PTH	0.009	0.022	1.009	1.001-1.017
Cons	-6.092			

Table IV. The diagnostic value of bALP and ALP for the incidence of vascular calcification of AAC based on the study cut-off

Cut off	AUC	Sens (%)	*Spec (%)*	*PLR*	*NLR*	*PPV (%)*	*NPV (%)*	*Acc*
bALP ≥116.50 U/L	0.882	81.6	81.1	4.31	0.22	81.6	81.1	81.33
ALP ≥164.50 U/L	0.634	55.3	54.1	1.20	0.83	55.3	54.1	54.67

obtained with the highest sensitivity and specificity of ROC were 116.50 u/L (bALP) and 164.5 u/L (ALP), respectively (*Table IV*).

With an AUC value of 0.882 (95% CI: 0.801-0.962; $p < 0.001$) in bALP predictors, it can be stated that the levels of bALP in serum have a relatively good diagnostic value when compared to ALP levels with weak diagnostic values (AUC) 0.634; 95% CI: 0.509-0.760; $p = 0.045$) (*Figure 1*)

Furthermore, an analysis was performed to determine the diagnostic value of bALP and ALP on the occurrence

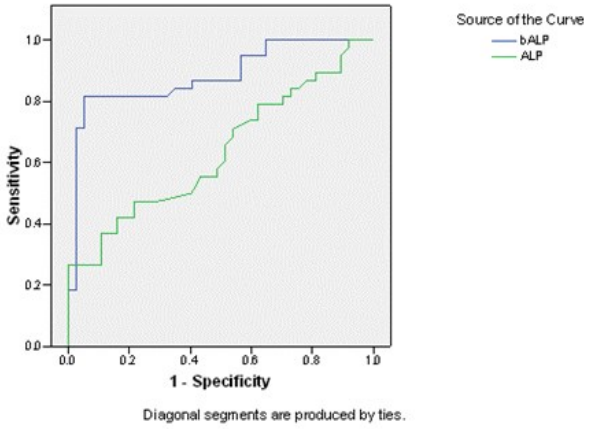

Figure 1 ROC curve to determine the cut-off values of bALP and ALP in predicting the incidence of vascular calcification in patients with CKD HD chronic

of blood vessel calcification in CKD patients with HD. Based on the cut-off levels determined by ROC (bALP ≥ 116.50 u/L and ALP ≥ 164.50 u/L), it was found that the sensitivity, specificity, and accuracy of bALP in predicting vascular calcification events were higher when compared to ALP (81.6% vs 55.3%, 81.1% vs 54.1%; and 81.33% vs 54.67%, respectively) (*Table IV*).

Discussion

This study showed that serum levels of bALP have a significant role in chronic hemodialysis patients. In addition, bALP is an independent risk factor for the incidence of AAC and has a relationship with a high predictive value for the incidence of AAC in CKD patients with chronic HD, thus showing a strong relationship between bALP and vascular calcification in the dialysis population.

Mineral and bone disorders occur in almost all dialysis patients, which can cause a decrease in their bone density, fracture, infection, immune dysfunction, accelerated incidence of atherosclerosis and blood vessel calcification. This significantly increases the incidence of vascular disease and affects the prognosis of dialysis patients. Many studies have recently reported this relationship between bone disorders and blood vessel calcification. Abnormal bone metabolism has also been considered an important risk factor for the occurrence of vascular calcification in patients with CKD.[10-12]

ALP is a group of isozymes that has ability to degrade phosphates in proteins and nucleic acids. In humans, ALP can be found throughout the body in the form of

isoenzymes, which originate not only from the liver and bones, but also the intestines, placenta, kidney, and leukocytes. It is not clear whether serum ALP in patients with renal impairment can mainly reflect changes in bone and mineral metabolism or other systemic processes.

Our study found an association between ALP levels and AAC in CKD patients with chronic HD through bivariate analysis. However, after multivariate analysis using logistic regression test, it was found that ALP did not significantly influence the incidence of abdominal aortic vascular calcification. This may be partly because ALP originates from a variety of different tissues and organs, which causes low sensitivity and specificity. In addition, it might also be due to the relatively small sample size of our study.

In our study, the results of serum bALP levels have better diagnostic value compared to ALP levels. Moreover, from the cut-off results determined by the ROC, it was found that bALP has a higher sensitivity, specificity, and accuracy in predicting the incidence of vascular calcification when compared to ALP so that it can be concluded that bALP can be a better predictor of blood vessel calcification. However, it must be recognized that the availability of ALP examination is more affordable than bALP.

The mechanism by which high levels of bALP drive vascular calcification can involve hydrolysis of pyrophosphates. In end-stage kidney disease, cells that resemble osteoblasts produce and release bALP. This will facilitate pyrophosphatase in the process of pyrophosphate hydrolysis, which to some extent will cause excessive deposition of hydroxyapatite crystals in the medial layer of the arterial wall, leading to vascular calcification.[13]

This study has the following limitations. First, because this is an observational study, the causal relationship between AAC and increased levels of bALP and ALP cannot be verified. The cross-sectional design of this study implies that the mechanism underlying the association cannot be explored. Second, although many risk factors (predictors) of vascular calcification, such as hypertension and diabetes, are tightly controlled, we cannot rule out the effects of involvement of several underlying diseases and drug use on the results of the study. Third, it is still necessary to design prospective experiments and establish specific clinical observation endpoints to clarify the role of bALP and ALP in the development of vascular calcification (AAC) in CKD patients with chronic HD. Finally, our sample size was small and vascular calcification values grouped into the severe AAC group have not been found in this study. Further investigation is needed to examine CKD patients with a larger sample size along with appropriate and quantitative assessment of vascular calcification.

Conclusion

Our study shows that the level of bALP in serum increases significantly and was closely related to the incidence of vascular calcification (AAC) in CKD patients with chronic HD. The multivariate analysis results showed that ALP was not an independent risk factor for the occurrence of vascular calcification in CKD patients with chronic HD.

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References

1. Chan C, Blankestijn P, Dember L, Gallieni M, Harris D, Lok C et al. Dialysis initiation, modality choice, access, and prescription: conclusions from a Kidney Disease: Improving Global Outcomes (KDIGO) Controversies Conference. Kidney International. 2019;96(1):37-47. https://doi.org/10.1016/j.kint.2019.01.017
2. PERNEFRI. 8 th Report Of Indonesian Renal Registry 2015.
3. InfoDATIN KKRI. InfoDATIN Pusat Data dan Informasi Kementrian Kesehatan RI : Situasi Penyakit Ginjal Kronis. Indonesia. 2007.
4. Kovesdy CP, Kalantar-Zadeh K. Bone and mineral disorders in pre-dialysis CKD. *International Urology and Nephrology*. 2008;40(2):427-440. doi:10.1007/s11255-008-9346-7.
5. Massy ZA, Drueke TB. Magnesium and outcomes in patients with chronic kidney disease: focus on vascular calcification, atherosclerosis and survival. *Clinical Kidney Journal*. 2012;5(Suppl 1):i52-i61. doi:10.1093/ndtplus/sfr167.
6. Kovesdy CP, Ureche V, Lu JL, Kalantar-Zadeh K. Outcome predictability of serum alkaline phosphatase in men with pre-dialysis CKD. *Nephrology Dialysis Transplantation*. 2010;25(9):3003-3011. doi:10.1093/ndt/gfq144.
7. Eckardt, K.-U., Kasiske, B.L. KDIGO clinical practice guideline for the diagnosis, evaluation, prevention, and treatment of Chronic Kidney Disease-Mineral and Bone Disorder (CKD-MBD). *Kidney International*. 2009;76. doi:10.1038/ki.2009.188
8. Ketteler M, Block GA, Evenepoel P, et al. Executive summary of the 2017 KDIGO Chronic Kidney Disease–Mineral and Bone Disorder (CKD-MBD) Guideline Update: what's changed and why it matters. *Kidney International*. 2017;92(1):26-36. doi:10.1016/j.kint.2017.04.006.
9. Regidor DL, Kovesdy CP, Mehrotra R, et al. Serum Alkaline Phosphatase Predicts Mortality among Maintenance Hemodialysis Patients. *Journal of the American Society of Nephrology*. 2008;19(11):2193-2203. doi:10.1681/asn.2008010014.
10. Verbeke F, Biesen WV, Honkanen E, et al. Prognostic Value of Aortic Stiffness and Calcification for Cardiovascular Events and Mortality in Dialysis Patients: Outcome of the Calcification Outcome in Renal Disease (CORD) Study. *Clinical Journal of the American Society of Nephrology*. 2010;6(1):153-159. doi:10.2215/cjn.05120610.
11. Peres LAB, Pércio PPV. Mineral and bone disorder and vascular calcification in patients with chronic kidney disease. *Jornal Brasileiro de Nefrologia*. 2014;36(2):201-207. doi:10.5935/0101-2800.20140031.
12. Shaheen FM, Kurpad R, Al-Sayyari A, et al. Multinational observational study on clinical practices and therapeutic management of mineral and bone disorders in patients with chronic kidney disease stages 4, 5, and 5D: The OCEANOS study. *Saudi Journal of Kidney Diseases and Transplantation*. 2016;27(2):290. doi:10.4103/1319-2442.178266.
13. Oneill WC, Sigrist MK, Mcintyre CW. Plasma pyrophosphate and vascular calcification in chronic kidney disease. *Nephrology Dialysis Transplantation*. 2009;25(1):187-191. doi:10.1093/ndt/gfp362.