A Multivariate Analysis-Factors Related To Vascular Calcification On Regular Hemodialysis Patient; First In Indonesia

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Abstract

Introduction: Cardiovascular disease is one of the main causes of death in patients with chronic kidney disease (CKD) undergoing hemodialysis or peritoneal dialysis therapy. This is related to calcification in large blood vessels such as the aorta. Although some known coronary risk factors, such as age, dyslipidemia, diabetes and smoking, play an important role in cardiovascular disease in patients undergoing hemodialysis, but several other risk factors such as anemia, uremic toxins, oxidative stress, and disorders of mineral and bone metabolism also associated with CKD. **Method:** This study was an analytical cross-sectional study with subjects were from CKD patients who underwent regular hemodialysis (for at least 3 months) in hemodialysis unit of RSKG Rasyida Medan from May 2018 and aged > 18 years. Interviews were conducted regarding age, duration of hemodialysis. Laboratory examination of calcium, phosphate and fetuin-A levels and lateral abdominal X-ray examination were conducted, then a statistical analysis was performed to determine the relationship of these factors with blood vessel calcification in regular hemodialysis patients. **Results:** Of 113 subjects, duration of hemodialysis in no calcifications group was 52.09 ± 21.725 , while mild grade calcification underwent HD for 51.81 ± 29.115 , and severe calcification group underwent HD for 69.06 ± 36.030 . A total of 10 patients without calcification had a history of DM, which were total 14 patients with calcification (mild and severe group) had history of DM. The mean serum calcium level in the group without calcification was $9,467 \pm 0,843$ mg / dl, $9,638 \pm 0,708$ mg / dl in mild group, while the average calcium level was $5,412 \pm 0,645$ mg / dl in severe group. **Conclusion:** Duration of hemodialysis, diabetes mellitus, calcium levels and fetuin-A significantly influence the occurrence of calcification in the abdominal blood vessels. ($p = 0.004$, $p = 0.049$, $p = 0.005$ and $p = 0.004$)

Background

Cardiovascular disease is one of the main causes of death in patients with chronic kidney disease (CKD) undergoing hemodialysis or peritoneal dialysis therapy. Increased mortality caused by cardiovascular disease, associated with calcification in large blood vessels, such as the aorta, where this can cause stiffness in the arteries and increase pulsation pressure and decreased myocardial perfusion at diastole.¹

Therefore, prevention of cardiovascular disease is important to reduce the incidence of morbidity and mortality. Although some known coronary risk factors, such as age, dyslipidemia, diabetes and smoking, play an important role in cardiovascular disease in patients undergoing hemodialysis, but several other risk factors, which are associated with chronic kidney disease are also involved, such as anemia, uremic toxins, oxidative stress, and mineral and bone metabolic disorders.² For this reason, many researchers are now finding out the relationship between a disorder of mineral metabolism and bone and vascular calcification.

The mechanism of ectopic calcification is very diverse and is not clearly known. The most studied is the pathology of vascular calcification as a major risk factor for cardiovascular mortality. Basically,

regulation of calcification is regulated by maintaining extracellular calcium and phosphate concentration.³

There are many methods that can be use to diagnose a vascular calcification such as ultrasound mostly for superficial vascular, multi-slice computed tomography (MSCT) but the downside is it cannot differentiate a intima or media tunica calcification, and lateral lumbal X-ray. There are no any gold standard to diagnose a vascular calcification. Although it is still a controversy, The Kidney Disease Improving Global Outcome (KDIGO) suggest a lateral abdominal radiography can be use for detection of vascular calcification.⁴

Methods

Study Samples

Samples were from populations of CKD patients who underwent regular hemodialysis in the hemodialysis unit of RSKG Rasyida Medan from May 2018 (for at least 3 months) and aged > 18 years. The subject had received information and gave consent to participate in informed and voluntary research. Patients who underwent irregular hemodialysis or were unstable were excluded from this study.

Study Design

This study is an analytical cross-sectional study. The proportion formula is used to determine the number of samples. After obtaining approval from the ethics committee, the subjects who met the inclusion and exclusion criteria were given an explanation and asked to provide informed consent to take part in the study. Interviews were then conducted to determine the age, history of diabetes mellitus and the duration of the patient's hemodialysis. Then blood sampling is performed to determine serum calcium levels and serum phosphorus, as well as lateral abdomen x-ray. After the data collected, data processing and data analysis were then conducted.

Statistical Analysis

Univariate analysis was conducted to obtain an overview of each variable studied. Bivariate analysis is used to state the analysis of two variables, which are the dependent variable and the independent variable. To see the strength of the relationship between the dependent and independent variables, the value of the Prevalence Ratio (RP) is used. In bivariate analysis anova test is used because the independent variable is numerical data and the dependent variable is ordinal data.

Multivariate analysis is used to see the relationship of several independent variables with the dependent variable by connecting independent variables with the dependent variable at the same time. In this analysis, the variables with the greatest influence can be known, the form of relationships between variables, related directly or indirectly to other variables.

Results

Research Subjects Characteristic Distribution

This study was followed by 113 patients as research subjects who had met the inclusion and exclusion criteria. The majority of the study subjects were men (59.3%) with a median age of 57 years. Based on the results of laboratory tests, the median serum calcium level was 9.8 mg / dl, the mean serum phosphate level was 5.39 mg / dl. From the results of lateral abdominal X-ray examination in this study the majority of patients having severe calcification of 43.4%, no calcifications of 38.1%, and only 18,6% having mild calcification. (Table 1)

Relationship between Research Subject Characteristics with the Degree of Blood Vessel Calcification

Man having more severe calcification (47.8%), compared with women who showed the same results in severe calcification and no calcification (each of 37.0%). Based on the statistical test, it was concluded that there was no significant difference between gender and the degree of calcification ($p = 0.211$). (Table 2)

Subjects with no calcification were 49.0 ± 13.020 years of age, lower than the average age of mild and severe degrees (54.81 ± 9.19 and 59.47 ± 8.615). Based on the statistical test, it was concluded that there were significant differences between mean of age to the degree of calcification ($p = 0.001$) (Table 2). In Table 3 there was a significant difference in age at normal levels compared to severe degrees ($p = 0.001$).

The subjects who have no calcifications had underwent hemodialysis for 52.09 ± 21.725 , the average HD duration of mild calcification degree was 51.81 ± 29.115 , while the average length of HD degrees of severe calcification was 69.06 ± 36.030 . It was concluded that there was a difference in the average duration of HD to the degree of calcification ($p = 0.004$). In table 3 there is a significant difference between the no calcification and severe calcification ($p = 0.007$) and between mild calcification and severe calcification ($p = 0.007$).

10 subjects with no calcification had a history of DM more than of mild and severe group (8 and 6 people) so it was concluded that there was a correlation between the history of DM and the degree of calcification ($p = 0.049$).

Subjects who did not have calcifications had an average serum calcium level of $9,467 \pm 0,843$ mg / dl, the average level of mild calcium was $9,638 \pm 0.708$ mg / dl, while the average level of calcium in severe calcification group was 5.412 ± 0.645 mg / dl. It can be concluded that there are significant differences between the average calcium level and the degree of calcification ($p = 0.005$). In table 3 there is a significant difference between the degree of absence of calcification and severe calcification ($p = 0.002$) and between mild calcification and severe calcification ($p = 0.063$).

The serum phosphate levels of no calcification group of patients were 5.419 ± 0.626 mg / dl, level of 5.290 ± 0.656 mg / dl in mild calcification group, and level of 10.047 ± 0.774 mg / dl in severe calcification

group. Based on the statistical test it was concluded that there was no difference in the average phosphate level to the degree of calcification ($p = 0.723$).

Correlation Between Calcium Serum Level and Phosphate Level

From this analysis it was found that serum calcium and serum phosphate had a significant relationship ($p = 0.001$) with a fairly strong relationship strength ($r = 0.324$) (Table 4)

Multivariate Analysis

Variables with bivariate test results with p values <0.25 were included in further multivariate analysis. The multivariate analysis test used was a multinomial logistic regression test using the Backward method. Analysis was conducted by determining the degree of no calcification as a comparison reference

The results in table 5 show that none of the variables had a statistically significant effect on the predictions of mild calcification compared with no calcification ($p = 0.346$). As for the prediction of severe calcification compared with no calcification, age had a significant effect ($p = 0.001$). The duration of hemodialysis, calcium level, age and Fetuin-A have correlation significantly ($p = 0.005$; 0.018 ; 0.001 and 0.004 respectively).

$$Ln = -12,812 + 0,091(\text{age}) + 0,025(\text{duration of HD}) + 0,825(\text{calcium level}) - 0,005(\text{Fetuin-A level})$$

(Please see equation in Supplemental Files)

Discussion

In this study the majority of the study subjects were male (59.3%), this result was consistent with the research report of Honkanen E et al. and IRR (Indonesian Renal Registry) data, The incidence of male calcification was greater than women with accompanying risk factors such as smoking history, diabetes, and coronary heart disease.¹

In this study there are more severe degree of calcification found in patients with chronic renal failure (43.4%) because the study population was patients with stage V CKD with dialysis. From Kimura's research, et al. stated that one of the factors in severe blood vessel calcification is affected by the severity of CKD and also the duration of dialysis experienced by a person.⁴ This is also in line with the theory described by Herrmann.²

Based on the results of a comparison of the mean age, there were significant mean differences in severe calcification compared to normal blood vessels, the mean age of patients having severe calcification was aged 59.47 years while those who did not experience calcification were 49 years old. Research by Turkmen, et al in Turkey get more or less the same results where the average patient who has severe

calcification degree is 56 years.⁶ Krauss, et al. explained that increasing age, the degree of calcification is increasingly severe in patients with chronic kidney disease.⁷ Research conducted by Barreto et al on 101 routine hemodialysis patients, stated that there was a positive correlation between age and coronary artery calcification which was statistically significant with moderate correlation strength ($r = 0.57$; $p = 0.001$).⁸⁻⁹ In line with the study Coll et al stated that there was a significant relationship between age and calcification in regular HD patients ($p = 0.001$).¹¹

This is because with increasing age, the risk of increased blood vessel calcification coupled with decreased kidney function. Including the role of the kidney as a calcification regulation, where maintaining extracellular calcium and phosphate concentration and its inhibitory factors.² this is aggravated by chronic kidney disease where the process of calcification has occurred since the onset of chronic kidney disease.¹

Based on the statistical test it was concluded that there was a difference in the average duration of HD to the degree of calcification. In the study, the duration of hemodialysis significantly affected the occurrence of calcification in the abdominal blood vessels. In accordance with the CORD Study (Calcification Outcome in Renal Disease) study conducted by Honkanen, et al. Showed that in 933 study samples with dialysis duration ≥ 38 months found calcification (AAC (Abdominal Aortic Calcification) score ≥ 1) through lateral lumbal radiology occurred in 81% of patients; the severity increased significantly from L1 to L4 ($P < 0.0001$) and affected all segments in 51% of patients. And the annual increased risk of calcification of dialysis patients reaches 11%.¹⁰

Based on the statistical test, it was concluded that there was a relationship between the history of DM and the degree of calcification ($p = 0.049$). The oxidation process and oxidative stress of the tissue formed as a product of increased glucose levels are a risk factor for increasing the degree of calcification. This situation also occurs in the study of Joachim, et al.^{10,11} The results of this study are in line with the research conducted by Coll et al where there was a significant relationship between DM patients with the occurrence of vascular calcification ($p = 0.007$).⁹

In this study, it was found that serum calcium had a significant relationship with the occurrence of vascular calcification, in severe and without calcification. Calcium plays a major role in regulating contraction and relaxation of vascular smooth muscle by maintaining extracellular calcium and phosphate concentration.¹³ In patients with chronic kidney disease, there is a relationship between impaired mineral metabolism (serum calcium and phosphorus levels), bone abnormalities (renal osteodystrophy) and vascular calcification.¹ Disorders of mineral and bone metabolism are the underlying factors in the progression of vascular calcification in patients with chronic kidney disease.¹

Based on the statistical test it was concluded that there was no difference in mean phosphate levels to the degree of calcification ($p = 0.723$). The results of this study are in line with Coll et al's research ($p = 0.66$).⁹ although different from the research by Distanbanchong et al, which states that phosphorus affects calcification due to changes in calcium and phosphate balance in patients with chronic kidney

disease, causing vascular calcification.¹⁴ This result occurs because the population of the study sample has been using phosphate binders. So that the phosphate level in the blood is not too high (5.290 ± 0.656 in mild calcification, 5.412 ± 0.774 in severe calcification). As concluded by Chan, et al., Phosphate binders can reduce serum phosphorus levels, but do not change the risk of death caused by vascular disease, one of which is vascular calcification.¹⁵

There are not many studies that multivariately review the risk factors associated with the occurrence of calcification in addition to serum calcium, serum phosphate and Fetuin-A levels but in this study it was found that age, duration of hemodialysis, calcium levels and Fetuin-A were risk factors that significantly affected the occurrence of severe calcification compared with no calcification. ($p = 0.001$). None of the risk factors had a significant effect on the occurrence of mild calcification compared with no calcification ($p = 0.180$).

This study is in line with theory telling that Fetuin-A is a systemic inhibitor of ectopic or calcification dystrophy. The involvement of Fetuin-A in pathologic calcification has been confirmed in large amount of studies involving patients suffering from calcification dystrophy.^{2,16}

Advice on Tables

Table 1: Subjects Characteristics

Characteristic	n=113
Sex, n (%)	
Male	67 (59,3%)
Female	46 (40,7%)
Age (years), Median (Min-Max)	57 (22 – 78)
Duration of HD, Median (Min-Max)	47 (27 – 229)
Diabetes History	
Yes	24 (21,2%)
No	89 (78,8%)
Serum Calcium,mg/dl Median (Min-Max)	9,8 (8,0 – 12,0)
Serum phosphate, mg/dl Mean $\pm$ S.D	5,392 $\pm$ 0,636
Classification degree	
No calcification	43 (38,1%)
Mild calcificatio	21 (18,6%)
Severe calcification	49 (43,4%)

Table 2 Correlation between Study Subjects Characteristics and Blood Vessel Calcification Degree

Characteristic	Classification degree			<i>p</i>
	No calcification	Mild	Severe	
Sex, n(%)				0,211
Male	26 (38,8)	9 (13,4)	32 (47,8)	
Female	17 (37,0)	12 (26,0)	17 (37,0)	
Age, years	49,0±13,020	54,81±9,179	59,47±8,615	0,001
Duration of HD	52,09±21,725	51,81±29,115	69,06±36,030	0,004
Diabetes Melitus, yes n(%)	10 (41,7)	8 (33,3)	6 (25,0)	0,049
Serum Calcium	9,467±0,843	9,638±0,7081	10,047±0,774	0,005
Serum Phosphate	5,419±0,626	5,290±0,656	5,412±0,645	0,723

Table 3 Post Hoc Mann Whitney Test, Degree of Calcification in Research Subjects with Significant Difference of Means

Age	P*
No calcification vs mild calcification	0,066
No calcification vs severe calcification	0,001
Mild calcification vs severe calcification	0,065
Duration of HD	
No calcification vs mild calcification	0,360
No calcification vs severe calcification	0,007
Mild calcification vs severe calcification	0,007
Serum calcium	
No calcification vs mild calcification	0,363
No calcification vs severe calcification	0,002
Mild calcification vs severe calcification	0,063

*Mann-Whitney Test

Table 4 Spearman Correlation Test for Calcium and Phosphate Level

Variable		<i>r</i>	<i>P</i>
Serum Calcium	Serum Phosphate	0,324	0,001

Table 5. Multivariate Analysis of Multinomial Logistic Regression of Calcification Degree

Variable	Coefisien	<i>p</i>	OR	IK95%
Mild calcification, <i>Intercept</i>	-3,461	0,346		
Severe calcification, <i>Intercept</i>	-12,812	0,001		
Fetuin-A Levels	-0,005	0,004	0,995	0,991 – 0,998
Duration of HD	0,025	0,005	1,026	1,008 – 1,044
Age, years	0,091	0,001	1,096	1,039 – 1,155
Calcium level	0,825	0,018	2,281	1,151 – 4,522

Reference category : No calcification.

Conclusions

The conclusion of this study is that the duration of hemodialysis, diabetes mellitus, and calcium levels significantly influence the occurrence of calcification in the abdominal blood vessels. The prevalence of vascular calcification in hemodialysis patients who experienced severe calcification from this study was 49 people (43.4%), there were no calcifications as many as 43 patients (38.1%), and only 21 people who experienced mild calcification (18.6%).

Abbreviations

CKD: Chronic Kidney Disease, HD: Hemodialysis, MSCT: Multi-Slice Computed Tomography, KDIGO: The Kidney Improving Global Outcome, DM: Diabetes Mellitus, IRR: Indonesian Renal Registry, AAC: Abdominal Aorta Calcification.

Declarations

Ethics Approval and Consents

This research was approved by Health Research Ethical Committee Medical Faculty of Universitas Sumatera Utara with Approval number 314/TGL/KEPK FK USU-RSUP HAM/2018. All patients that participate in this research were explained about the steps of the research. And it was written in a informed consent form that should be signed by participants.

Conflict of Interest

The authors report no conflicts of interest in this study.

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Authors Contributions

All authors contributed toward data analysis, drafting and revising the paper, Riri A. Muzasti and Syafrizal Nasution gave a final approval of the version to be published and agree to be accountable for all aspects of the work.

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