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Short communication

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Associations of vascular calcification, calcium phosphate disturbances, FGF 23 and Matrix Gla protein with mortality of hemodialysis patients: one center cohort study

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Abstract

***Background and objectives**: Vascular calcification (VC) is one of the factors associated with mortality in hemodialysis (HD) patients. The purpose of the study was to assess associations between prevalent VC and disturbances of calcium-phosphate metabolism as well as changes in vitamin D (25(OH)D), FGF 23 and MGP levels and to evaluate the possible impact of VC and changes of these biomarkers on survival in HD patients.* ***Methods**: The study population consisted of 81 prevalent patients in the hemodialysis unit of Hospital of Lithuanian University of Health Sciences Kaunas Clinics. A simple vascular calcification score (SVCS) was evaluated as it is described by Adragao et al. 25(OH)D (nmol/L), FGF 23 (ng/L) and MGP (ng/mL) were measured and analysed.* ***Results**: Patients were divided into two groups: SVCS<3 (31 patient (38.3%) and SVCS ≥3 (50 patients (61.7%)). In multivariate logistic regression, age (odds ratio 1.062, 95% CI [1.024-1.1] p=0.001) and diabetes (odds ratio 6.9, 95% CI [1.5-31], p=0.012) were associated with SVCS ≥3. The multivariate logistic regression revealed the highest negative impact of SVCS ≥3, age and 25(OH)D level for death risk.* ***Conclusion**: VC in HD patients is highly influenced by age and presence of diabetes and associated with higher risk of death. No significant association was found between MGP and FGF 23 and VC as well as between these two biomarkers and risk of death. Lower 25(OH)D levels were associated with mortality in this dialysis patients cohort.*

***Keywords**: mineral metabolism disorders; FGF 23; MGP; hemodialysis, survival*

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Introduction

Chronic kidney disease is an independent risk factor for cardiovascular diseases even from its early stages (1). Cardiovascular diseases dependent death risk in dialysis patients is significantly higher compared to general population (2,3). This increased risk can not be attributed only to traditional cardiovascular risk factors (4-6). One of the specific factors associated with mortality in hemodialysis (HD) patients in particular is vascular calcification (VC) (7,8). Despite the well known negative impact of atherosclerosis causing intima lesions, media calcification is known to be a specific injury for HD patients affecting hemodynamics significantly and contributing to negative outcomes as well (9).

Media calcification, as well as increased cardiovascular risk, has long been related to calcium (Ca)-phosphorus (P) metabolism disorders. Hyperphosphatemia is important in process of osteoblastic transformation of smooth muscle cells in the vessel wall as well as mineral deposition (8,10). In addition, vitamin D (25(OH)D) deficiency together with secondary hyperparathyroidism and hyperphosphatemia were considered as the main factors contributing to cardiovascular risk in renal patients (11-13). VC used to be described only as a passive process earlier. Lately many studies proved that it is an actively regulated process dependent on calcification promoters and inhibitors. One of the pivotal calcification inhibitors is Matrix Gla protein (MGP). An inverse association between total uncarboxylated MGP and VC in HD patients was demonstrated (14,15). Independent associations of MGP and arterial calcification as well as carotid atherosclerosis in patients without kidney disease were also presented in a study of Sitar Taut A. and coauthors (16). Moreover, lower levels of circulating MGP were found to be a predictor of mortality in HD patients (17). In addition to that fact, several studies revealed an independent association between mortality of chronic kidney disease patients and increased levels of another biomarker-Fibroblast Growth Factor 23 (FGF 23) (18,19). FGF 23 was also presented as an independent biomarker of VC, it‘s progression and cardiovascular diseases of HD patients (20,21). Though the golden standard for detection of VC is electron beam or multislice computed tomography (22), the use of plain radiography is recommended in KDOQI guidelines for VC assessment as an adequate replacement (23). After introducing to practice the VC evaluation for prevalent HD patients, we investigated relationships of VC with disturbances of Ca-P metabolism and levels of 25(OH)D, FGF23 and MGP.

We hypothesized that higher FGF 23, lower MGP would be associated with more pronounced VC and mortality of HD patients. In order not to underestimate the role of Ca-P disorders, vitamin D deficiency, these parameters were also evaluated in complex.

Methods

The observational cohort study population consisted of prevalent patients (n-95) on maintenance HD in the dialysis unit of Hospital of Lithuanian University of Health Sciences Kaunas Clinics. The study was approved by Kaunas Regional Biomedical Research Ethics Committee (protocol number BE-2-9, 2013 12 02) and the informed consent was obtained from all study participants. 14 patients were excluded from analysis. Their blood samples after evaluation of VC were not obtained, as they moved to continue HD in other center or were transplanted. Data of 81 patient was analysed.

The collection and processing of demographic, clinical and laboratory data is described in details in previous published work of our group (24).

Simple vascular calcification score (SVCS) was evaluated as it is described by Adragao et al. with SVCS ≥3 considered as cut off value (25) and the methodology is presented in details in our previous research article as well (24).

Blood samples for measurements of FGF 23, MGP and 25(OH)D were collected as SVCS was evaluated. Patients were observed until the end of May 2017. Mean follow up – 1058.8 days (23-1560 days).

Human FGF 23 ELISA Kit (Sunlong Biotech, Zhejiang China) and Human MGP ELISA Kit (Sunlong Biotech, Zhejiang China) were used for analysis of FGF 23 and MGP accordingly. Measurement and analysis of both results were done using immunoassay ELISA analyzer Gemini (Stratec Biomedical GmbH, Birkenfeld, Germany). Methods of measurements of serum biochemical parameters are presented in previous work of the group (24).

For discrete factors variables were expressed as frequencies, percentages. Based on the normality of data distribution of continuous factors, median [minimal-maximal values] or means with standard deviation were used. We performed statistical comparison using the two-tailed chi-square test for categorical variables and two-tailed Student‘s T-test or Mann-Whitney test for continuous variables were appropriate. For survival analysis, the Kaplan-Meier survival curves of patients with SVCS ≥3 and <3 were compared by log-rank test.

To identify clinical factors and laboratory changes that may be important for severe VC (SVCS≥3), odds ratios obtained from univariate and multivariate binary logistic regression were used. Independent variable was severe VC (SVCS≥3), dependent variables were age, gender, diabetes, HD vintage, 25(OH)D, FGF 23, MGP, biochemical paramethers reflecting Ca-P disturbances at the start of dialysis and at the moment of evaluation of SVCS. Regressors that statistically significantly prognosed severe VC in univariate analysis, were included in a multivariate binary logistic model. To assess possible associations between survival rate and VC as well as age, presence of diabetes, HD vintage, FGF23 and MGP levels as well as 25(OH)D we also performed univariate and multivariate binary logistic regression analysis. For comparison a P value <0.05 was considered significant. IBM SPSS statistics 24.0 software package for statistical analysis was used.

Results

SVCS was evaluated in 44 men (54.3%) and 37 women (45.7%). 17 (21%) had diagnosed diabetes. The mean age was 60.9±16.01 (22-86) and the mean HD duration of all patients included 39.26±46.24 months (1-182). 23 patients (28.4%) were treated with alfacalcidol. 49 (60.5%) received Ca acetate/Mg subcarbonate (Osvaren) and 30 (37%) Ca carbonate as a phosphate binder.

SVCS <3 was observed in 31 patient (38.3%) and SVCS ≥3 in 50 patients (61.7%). Comparison of clinical and demographical profiles is presented in Table 1. The prevalence of diabetes was significantly higher in the group with SVCS ≥3. Patients in this group were significantly older as well (Table 1).

There was a trend to higher values of serum P, alkaline phosphatase (ALP) and C reactive protein (CRP) in the group with SVCS ≥3 at the start of HD treatment, but the differences didn‘t reach the level of significance. At the moment of evaluation of SVCS, patients with SVCS ≥3 had statistically significant lower Kt/V value, but other biochemical parameters didn‘t differ significantly, though higher levels of serum P and ALP remained observed. No statistically significant differences were observed comparing FGF23 and MGP levels between the groups with different SVCS (Table 2).

Table 1. Demographic and clinical information in groups with different simple vascular calcification score

	SVCS<3	SVCS≥3	Significance (p)
Participants (n)	31	50	
Age, mean (SD), years	53.97 (16.2)	65.2 (14.4)	0.002
HD vintage, mean (SD), months	34.23 (33.19)	42.38 (52.8)	p>0.05
BMI, mean (SD), kg/m²	24.7 (4.7)	25.8 (5.0)	p>0.05
Diabetes, n (%)	3 (9.6)	14 (28)	0.05
Hypertension, n (%)	29 (93.5)	45 (90)	p>0.05
Cardiovascular Disease, n (%)	13 (41.9)	29 (58)	p>0.05
Gender, n (%)			
Female	14 (45.16)	23 (46)	p>0.05
Male	17 (54.8)	27 (54)	p>0.05
Treatment			
Alfacalcidol, n (%)	12 (38.7)	11 (22)	p>0.05
Calcium carbonate, n (%)	14 (45.2)	16 (32)	p>0.05
Ca acetate/Mg subcarbonate, n (%)	18 (58.1)	31 (62)	p>0.05

Results in mean values (SD) or n (%); SVCS-simple vascular calcification score; Two-tailed chi-square test for categorical variables and two-tailed Student's T-test for continues variables was used

Table 2. Biochemical parameters of the hemodialysis patients begining hemodialysis and at the moment of radiological evaluation

	SVCS <3 (n-31)	SVCS ≥3 (n-50)	Significance (p)
Begining of hemodialysis			
Ca (mmol/L)	2.09 (0.24)	2.09 (0.21)	p>0.05
P (mmol/L)	1.59 (0.59)	1.71 (0.58)	p>0.05
Ca×P (mmol/L²)	3.34 (1.37)	3.59 (1.3)	p>0.05
iPTH (pmol/L)	40.9 (35.2)	44.8 (33.7)	p>0.05
ALP (U/L)	78.8 (42.8)	108.76 (125.9)	p>0.05
Kt/V	1.28 (0.28)	1.24 (0.32)	p>0.05
Hb (g/L)	91.45 (16.8)	90.61 (13.9)	p>0.05
CRP (mg/L)	9.31 (19.32)	20.39 (46)	p>0.05
Cholesterol (mmol/L)	5.01 (1.32)	4.9 (1.52)	p>0.05
Albumin (g/L)	34.6 (5.6)	33.0 (5.9)	p>0.05
Radiological evaluation moment			
Ca (mmol/L)	2.21 (0.18)	2.19 (0.21)	p>0.05
P (mmol/L)	1.67 (0.55)	1.88 (0.54)	p>0.05
Ca×P (mmol/L²)	3.7 (1.3)	4.06 (1.3)	p>0.05
iPTH (pmol/L)	46.8 (39.48)	47.45 (44.60)	p>0.05
ALP (U/L)	77.96 (35.5)	106.8 (193.2)	p>0.05
Kt/V	1.52 (0.27)	1.37 (0.24)	0.02
Hb (g/L)	110 (14.2)	108 (13.2)	p>0.05
CRP (mg/L)	8.11 (12.5)	12.7 (24.4)	p>0.05
Cholesterol (mmol/L)	4.9 (1.47)	5.1 (1.2)	p>0.05
Albumin (g/L)	35.5 (3.7)	34 (3.6)	p>0.05
25(OH)D (nmol/L)	46.45 (19.15)	37.94 (17.69)	0.045
FGF 23 (ng/L)	33.34 [3.36-199.79]	44.76 [0.00-788.99]	p>0.05
MGP (ng/mL)	1.66 [0.7-5.96]	1.6 [0.41-27.43]	p>0.05

SVCS -simple vascular calcification score; results presented as mean values (SD); Two-tailed Student's T-test for comparison was used. Results of FGF 23 and MGP presented as median [minimal-maximal values]; Mann-Whitney test used for comparison

The mean 25(OH)D value was 41.2±18.6 nmol/L and ranged from 10 to 101.9 nmol/l. 56 patients (69.1%) had severe 25(OH)D deficiency with 25(OH)D levels less than 50 nmol/L. 19 patients (23.5%) were vitamin D insufficient with 25(OH)D values between 51 and 69 nmol/L. Only 6 patients (7.4%) had 25(OH)D values over 70 nmol/L. 25(OH)D values within the groups with different SVCS are presented in Table 2. Patients with severe VC had significantly lower 25(OH)D levels.

In univariate logistic regression, age and diabetes but not 25(OH)D, FGF23, MGP or Ca-P disturbances were associated with SVCS ≥3 (Table 3). For multivariate binary logistic regression, only statistically significant factors were included. The influence of age, for severe VC remained stable with OR 1.062 [1.025-1.1], p=0.001 and the importance of diabetes even increased with OR 6.9 [1.5-31], p=0.012.

During the observational period, 22 deaths occurred. The Kaplan - Meier analysis revealed a tendency for higher risk of death for patients with more severe VC (SVCS ≥3 vs SVCS <3 (40% vs 6.5%, log rank 10.96, p=0.001)) (Fig 1).

To assess possible associations between survival rate and VC, age, the presence of diabetes, HD vintage as well as FGF 23, MGP and 25(OH) D levels we performed univariate and multivariate logistic regression analysis and results are presented in Table 4 and Table 5. For multivariate binary logistic regression, only statistically significant factors were included and it revealed the highest negative impact of severe VC with SVCS ≥3 for death risk of studied HD patients. Age and 25(OH)D, but not HD vintage remained important as well.

Discussion

The results of the study confirm the high prevalence of VC in HD patients. It also demonstrates the contribution of VC on mortality of HD patients.

Remarkably, in our cohort, only age and presence of diabetes were associated with higher scores of VC, but neither biomarkers reflecting

Table 3. Risk factors for vascular calcification: univariate analysis

Independent variable	OR	95 proc. CI	Significance (p)
Age, years	1.062	1.024-1.1	0.001
Male	1.07	0.469-2.46	0.866
Diabetes mellitus	5.4	1.17-24.9	0.03
HD vintage, month	1.007	0.99- 1.01	0.071
FGF 23 (ng/L)	1.004	0.996-1.013	0.321
MGP (ng/mL)	1.105	0.854-1.430	0.447
25(OH)D (nmol/L)	0.975	0.951-1.0	0.51
Begining of hemodialysis			
Ca (mmol/L)	0.99	0.137-7.2	0.998
Ca ion (mmol/L)	0.233	0.61-5.9	0.373
P (mmol/L)	1.437	0.647-3.19	0.373
Ca×P (mmol/L²)	1.16	0.895-1.7	0.184
iPTH (pmol/L)	1.003	0.99-1.017	0.617
Radiological evaluation moment			
Ca (mmol/L)	0.671	0.07-6.397	0.729
Ca ion (mmol/L)	0.055	0.001-4.9	0.205
P (mmol/L)	1.681	0.725-3.89	0.226
Ca×P (mmol/L²)	1.209	0.816-1.69	0.273
iPTH (pmol/L)	1.0	0.99-1.01	0.957

OR- odds ratio, CI- confidence interval

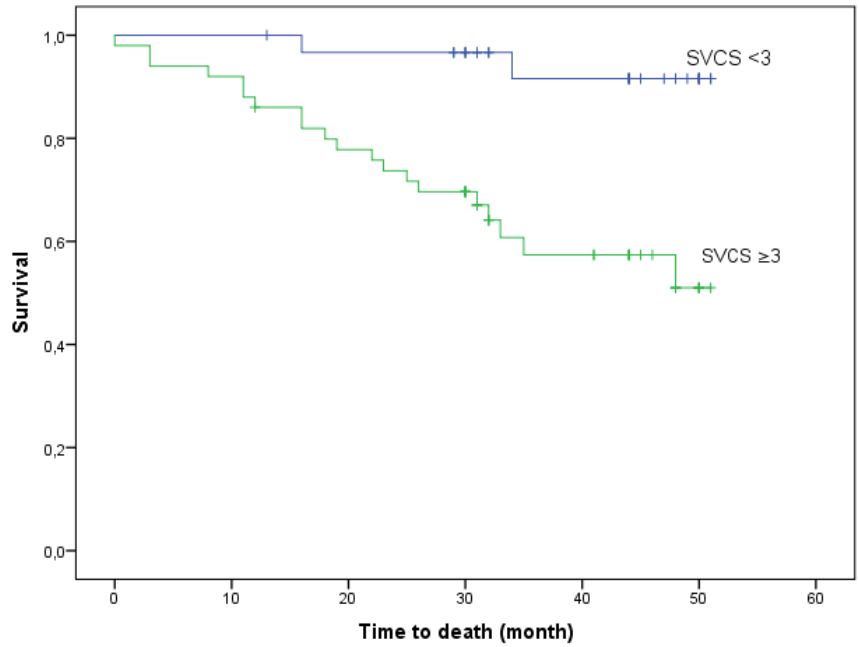

Figure 1. Survival in patients with simple vascular calcification score <3 and ≥3

Table 4. Risk factors for death: univariate analysis

Independent variable	OR	95 proc. CI	Significance (p)
SVCS ≥3	8.804	2.763-28.05	0.0001
Age	1.064	1.028-1.101	0.0001
Diabetes mellitus	2.56	0.919-7.1	0.072
HD vintage	1.011	1.011- 1.021	0.02
FGF 23	0.991	0.978-1.004	0.172
MGP	0.532	0.279-1.016	0.056
25(OH)D	0.950	0.917-0.983	0.04

OR-odds ratio, CI- confidence interval, SVCS -simple vascular calcification score

Table 5. Risk factors for death: multivariate analysis

Independent variable	OR	95 proc. CI	Significance (p)
SVCS ≥3	4.676	1.1-19.88	0.037
Age	1.053	1.009-1.1	0.018
HD vintage	1.05	0.993- 1.0108	0.426
25OHD	0.930	0.869-0.996	0.038

OR-odds ratio, CI- confidence interval, SVCS -simple vascular calcification score

Ca-P metabolism disturbances nor calcification biomarkers, such as FGF 23 and MGP, were not. The fact that patients with higher VC scores were older and more often had diabetes probably supports the possibility of intima and media calcification co-existence, which is described by previous works (8,26). Both intima and media calcification are associated with poor prognosis

in HD patients and the principal question would be whether these different forms of vascular injury require a different treatment in everyday practice.

Media calcification, specific for dialysis patients and patients with chronic kidney disease has long been associated with disturbances of Ca- P metabolism presenting as hyperphoshatemia, hypercalcemia and higher levels of Ca×P product (27,28). Still, recent scientific data regarding these associations remains controversial. Some authors presented the results reflecting associations between higher serum phosphate levels and calcification lessions in the arteries, the others, including author of the method of evaluation SVCS, did not (8,25,29). We did not find any associations of Ca-P disturbances neither analyzing laboratory data at the start of the treatment of HD nor at the moment of evaluation of SVCS though one of our hypothesis was that patients who start treatment with severe disturbances of Ca-P metabolism present with more severe VC later on.

VC is no longer considered only as a passive degenerative process, but an actively regulated one with several biomarkers suggested as calcification promoters and inhibitors (30). MGP is a potent inhibitor of extra osseous Ca-P precipitation. It is a vit K dependent cyclin and it‘s activation requires γ carboxylation. MGP acts as calcification inhibitor in vivo inhibiting Ca precipitation and crystallization in the vessel wall (31).

Data on the role of MGP changes in a uremic state is still inconclusive. Several studies have reported significantly lower serum levels of uncarboxylated MGP in dialysis patients than healthy controls (32). In others, markedly elevated plasma levels of MGP were found as compared to healthy controls probably due to impaired MGP activation, increased production and release as well as decreased clearance of MGP in HD patients (17). In our study, the levels of MGP were higher compared to those presented in the study of Xiao et al. (32) though both studies evaluated overall serum MGP. We did not find any significant difference in MGP levels of patients with different VC severity. It is demonstrated in the literature that higher FGF 23 levels and lower uncarboxylated MGP levels are associated with mortality and cardiovascular events in patients without end-stage renal disease (32). We also expected to find this type of associations in HD patients, but our study did not succeed to prove it and we can only speculate about the underlying mechanism of these observations. The possibility, that higher levels of MGP in HD patients are due to impaired activation in vessel wall because of deficit of vit K, may be one of the explanations. Results of the observational study of prevalent HD patient performed by Delanaye et al. would not contradict the hypothesis. Higher concentrations of inactive dp-ucMGP were found in hemodialysis patients as compared to healthy controls. A significant correlation between inactive MGP level and calcification score was confirmed both by univariate and multivariate analysis (33). In a study performed by Aoun M et al. reported that vit K deficiency, as assessed by high dp-ucMGP levels, is profound in HD patients of their study population and it is significantly correlated with VC. Daily supplementation of menaquinone-7 effectively reduced dp-ucMGP in this population (34).

In our study, we failed to confirm the correlation of MGP levels and VC. A limited number of patients in the study and possibility to measure only overall MGP levels could be one of the explanations.

FGF 23 is known to be elevated in patients with chronic kidney disease and is strongly associated with mortality and cardiovascular diseases (19,35-37) . In our study, we found relatively low values of FGF23 as compared to Xiao et al study (32) and this probably needs more detailed

investigation to clarify the reasons. Our study failed to show statistically significant differences in FGF 23 levels in patients with different VC scores. Scialla et al. in both clinical and experimental analysis proved that elevated FGF 23 levels were not consistently associated with VC in CKD patients. They suggest that the strong associations of elevated FGF 23 with cardiovascular events and mortality may be explained by other forms of cardiovascular injury such as FGF23 induced left ventricular hypertrophy and klotho–independent effects on cardiac myocytes, but not calcification of vessels (38). In a study by Moldovan et al. FGF-23 did not correlate with VC score as well. Interestingly, the lower FGF23 levels were identified as risk factors for cardiovascular diseases (39).

Our study also confirms the importance of vit D for death risk for dialysis patients but not for the presence of vascular calcification. Vitamin D deficiency is well known to be associated with high mortality as well as with several other unfavorable adverse effects in general population (40). But data about negative impact of vitamin D defficiency for HD patients is still inconclusive and there is no precise recommendations for treatment of the condition. Our study is consistent with previously published systematic review and meta-analysis of clinical studies by Zhang Y, which revealed positive associations of higher serum 25(OH)D level with lower all-cause mortality and lower cardiovascular mortality in dialysis patients (41).

Limitations of our study are relatively small sample size and observational structure. We also did not have a possibility to discriminate active and inactive forms of MGP measuring only overall values. Additional evaluation of vitamin K status could have been interesting and important.

As it is agreed that VC is responsible for many negative effects for HD patients, it is crucial to find reliable tools to prevent this disorder. For years, it was believed that optimal long-term control of Ca-P balance would play the major role in the prevention of VC. We did not find any significant associations between Ca-P metabolism disorders and present VC. Changes of novel biomarkers such as FGF 23 and MGP do not answer questions too.

Conclusions

In conclusion, the present study showed that vascular calcification in our cohort hemodialysis patients is highly influenced by age and presence of diabetes but neither disturbances of calcium-phosphate metabolism nor levels of FGF23, MGP or 25(OH)D. Age, vascular calcification and lower levels of 25(OH)D but not FGF 23 or MGP were major factors associated with higher risk of death.

Author Contributions

VP- study design, data collection and interpretation, writing the first draft, preparation of final manuscript.
RV- data collection, statistical analysis, preparation of final manuscript.
VK- study design, data interpretation, preparation of final manuscript.
EZ- study design, data collection, preparation of final manuscript.
SG- data collection, statistical analysis, preparation of final manuscript.
EJ- radiological data analysis, preparation of final manuscript.
ES- performed imunoassays, preparation of final manuscript.
IAB- study design, data interpretation, preparation of final manuscript.

Conflicts of Interest

The authors declare that they have no conflicts of interests.

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