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Valvular Calcification in Hemodialysis Cases: Relation to Functional Deficiency of Vitamin K and 25-Hydroxyvit-D Serum Level

Sherif El Sayed Mansour*, Ghada Mohamed Hasan Elkanishy, Tarek Medhat Abbas, Ahmed Bahy Eldeen Ibrahim

Internal Medicine and Nephrology Department, Faculty of Medicine, Mansoura University, Egypt

***Corresponding Author:** Sherif El Sayed Mansour**, Mobile:** (+20) 1091900828, **E-mail:** sherif_mansour1@yahoo.com

ABSTRACT

Background: Elevated incidence of death in hemodialysis (HD) cases is frequently accompanying with quicker atherosclerosis and increased vascular calcification.

Objective: The current study aimed to determine the relation between valvular calcification in HD cases and functional vitamin-K (Vit-K) as presented by serum level of uncarboxylated matrix Gla protein (ucMGP) and 25(OH) vitamin-D (Vit-D) levels.

Patients and Methods: This work was conducted over six months and included 90 HD cases and 20 apparently healthy adults with normal kidney function (to establish normal range of ucMGP); age and gender matched to the HD cases.

Results: About one-third of the patients (41.1%) had calcifications on the aortic valve, and about one-quarter had calcifications on the mitral valve (27.8%). Non-significant association was noted between MGP and vit-D (P = 0.439).

Conclusion: We suggested that the end-stage renal disease (ESRD) in HD is accompanied by a shortage levels of vit-K and vit-D built in present work. The most important result of the present work was the significant difference in MGP between patients and controls, suggesting a correlation between MGP level and aortic valve calcifications.

Keywords: Hemodialysis, Serum Level, Vit-K, 25-Hydroxyvit-D.

INTRODUCTION

Functional shortage of proteins concerned in the regulating calcium metabolism is perhaps a crucial mechanism for this procedure [(1)]**.** A straight connection amid the reduced accessibility of vit-K and vascular calcifications had been proposed by many researchers[(2)].

Vit-K intake is influenced by reduced-potassium and reduced-phosphorus-suggested diet in HD cases. Shortage of vit-K, either because of reduced intakes or the usage of coumarin derivatives, causes below carboxylation of vit-K-depending proteins (VKDPs)[(3)].

The buildup of ucMGP in atherosclerotic lesions and zones of calcifications had been stated in many reports [(4,5)]. Generally, cases with chronic kidneys diseases, the shortage of active vit-D is very frequent [(6,7)]. Low level of 25-hydroxyvit-D is very clear in HD cases, and is accompanied with increased mortality rate [(8)].

Vit-D could raise the calcium and phosphorus absorptions in the gastro-intestinal tract, thus elevating the calcium-phosphorus products and vascular calcifications, but in contrast, vit-D has positive straight impact on the vascular wall [(9)].

It was revealed that the serum vit-D levels in HD cases is harmfully associated with vascular sclerosis [(10)] and vascular calcifications grade [(11)]. This proposes that, vit-D can be a calcification inhibitor, and low levels of 25-hydroxyvit-D is strictly linked with cardio-vascular conditions and cardio-vascular death in HD cases [(12, 13)]**.**

Clinically, the active vit-D is frequently utilized in HD cases on the foundation of high levels of parathyroid hormone, and 25-hydroxyvit-D isn't regularly determined. Consequently, the impact of 25-hydroxyvit-D levels on vascular and valvular calcifications isn't very obvious.

The current study aimed to determine the relation between valvular calcification in HD cases and functional vitamin-K (Vit-K) as presented by serum level of uncarboxylated matrix Gla protein (ucMGP) and 25(OH) vitamin-D (Vit-D) levels.

PATIENTS AND METHODS

This study was done over six months and included 90 HD cases, and 20 healthy controls of comparable age to the HD cases, with ordinary kidney functions (to establish normal range for ucMGP). Ninety HD cases were included in this work to assess the presence of valvular calcification, and cases were allocated according to the incidence of valvular calcifications to case group or controls and to evaluate the relation with functional vit-K as presented by serum level of uncarboxylated matrix Gla protein (ucMGP) and 25(OH) vit-D levels in these cases.

Sample size:

The needed sample size was determined via the Med Calc statistical software. The primary outcome measure is the correlation of valvular calcification with functional deficiency of vit-K and serum 25-hydroxyvit-D level in a cohort of HD cases. Sample size was estimated by using results from a preceding studies [(14)]**.** So, it was reported that a suitable sample size was of **90** HD cases and **20** apparently healthy adults of comparable ages to the HD cases, with normal kidneys functions (to establish normal range for ucMGP). The power of 80% (type-II error, 0.2) chi-squared test with a confidence of 95% (two-sided type I error, of 0.05). This variance reflects the small

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effect size (w) of 0.07. The effect size (w) is assessed by the following formula [15]:

$w = \sqrt{\chi^2/N}$, Where χ^2 is the chi-squared statistic and **N** is the total sample size.

Exclusion criteria:

Patients younger than 18 years, patients with liver disease, cases on hemodialysis duration of less than6 months, patients taking vit-K supplements or vit-K antagonists for last 6 months, patients had preceding coronary artery bypass grafting, patients with history of rheumatic valve disease, patients had coronary stents, prosthetic or mechanical heart valve, patients taking any native form of vit-D for last 6 months and patients unable to provide signed informed consent.

Methods:

History taking and revision of patient files: with emphasis on age, gender, hypertension, diabetes, smoking, history of cardiovascular disease, cause of the original kidney disease, disease duration, medication history. Clinical examination: blood pressure measurement, full clinical examination of all systems and measurement of BMI. Laboratory investigations include serum calcium, phosphorus, hemoglobin level, serum albumin, lipid profile, C-reactive protein serum parathyroid hormones level, alkaline phosphatase. Undercarboxylated matrix Gla protein (ucMGP), and 25(OH) vit D levels were assessed by commercially available ELISA kits.

Cardiac echo study. The extent of valvular calcification (VC) was done and interpreted by a single qualified cardiologist blinded to cases data. VC was defined as bright echoes of more than 1 mm on 1 or more cusps of the aortic and the mitral valves. Patients were assessed non-invasively by means of echo-cardiogram. Valvular calcifications definite as bright echoes of >1 mm on 1 or more cusps of the aortic and the mitral valves [16]. In accordance to the guidelines of Kidney Diseases Outcome Quality Initiative (KDOQI)[12], 25-hydroxyvit-D deficiency was defined as (serum level <30 ng/ml).

Primary outcome:

Study the relation between valvular calcifications and functional vit-K as presented by serum level of uncarboxylated matrix Gla protein (ucMGP), and the deficiency of 25(OH) vit-D HD cases.

Secondary outcome: Study the correlation amid valvular calcification and other clinical, demographic and laboratory parameters within HD cases.

Ethical consent:

An approval of the study was obtained from Mansoura University Academic and Ethical Committee. Every patient signed an informed written consent for acceptance of participation in the study. This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Statistical Analysis

Collected data were analyzed via IBM-SPSS 25. (USA). Quantitative data were tested for normality by means of the tests of Kolmogorov–Smirnov, the Shapiro-Wilk, and direct data visualization approaches. In accordance to normality, quantitative data were presented as means and SD or medians (range). Qualitative data were presented as number and percent. Quantitative data were *compared* amid 2 groups by means of independent t-test or Mann-Whitney U test for normal and non-normal distribution numerical data, correspondingly. Qualitative data were matched by means of the Chi-square testing. Correlations were assessed via Spearman's correlation. All tests were 2-sided. At P values <0.05 results had significance.

RESULTS

The median MGP was significantly higher in cases than controls **(Table 1).**

Table (1): MGP level in cases and controls

		Cases (n = 90)	**Controls (n = 20)**	**P-value**
MGP (Pg/ml)	Mean ±SD	588 ± 138.65	118 ± 27.51	<0.001

About one-third of the patients had calcifications on the aortic valve, and about one-quarter had calcifications on the mitral valve **(Table 2).**

Table (2): Valvular calcification in aortic and mitral valves

	n (%)
VC of aortic valve	37 (41.1)
VC of mitral valve	25 (27.8)

Non-significant differences were noted among those with and with no valve calcifications as regard age, gender, BMI, and disease duration **(Table 3).**

Table (3): General characteristics in accordance to the existence of valve calcifications

		Valve calcifications		
		Yes (n = 46)	**No (n = 44)**	**P-value**
Age (years)	Mean ±SD	50 ±14	50 ±14	0.884
Gender	Males n (%)	20 (43.5)	24 (54.5)	0.294
	Females n (%)	26 (56.5)	20 (45.5)	
Body mass index (kg/m^2)	Mean ±SD	26 ±6	26 ±5	0.984
Hemodialysis duration (years)	Median (range)	4.5 (0.3 - 18)	4 (0.3 - 11)	0.318

Triglycerides were significantly lower in those with valve calcifications than those without **(Table 4).**

Table (4): Laboratory findings in accordance to the existence of valve calcifications

		Valve calcifications		
		Yes (n = 46)	**No (n = 44)**	**P-value**
Hemoglobin (g/dL)	Mean ±SD	9 ±2	10 ±2	0.286
Albumin (g/L)	Mean ±SD	3 ±0.4	3 ±0.4	0.188
PTH (pg/mL)	Mean ±SD	337±76.32	315±72.32	0.850
Ca^{++} (mg/dL)	Mean ±SD	8 ±1	8 ±1	0.274
Vit-D (ng/mL)	Mean ±SD	21±4.31	25±5.42	0.305
PO_4 (mg/dL)	Mean ±SD	5 ±1	5 ±1	0.585
Alkaline phosphatase (IU/L)	Mean ±SD	374 ±39	377 ±10	0.905
Positive CRP (mg/dL)	n (%)	21 (45.7)	17 (38.6)	0.501
Cholesterol (mg/dL)	Mean ±SD	213 ±39	211 ±39	0.771
Triglycerides (mg/dL)	Mean ±SD	126 ±6	151 ±6	0.047
HDL (mg/dL)	Mean ±SD	43 ±6	42 ±6	0.482
LDL (mg/dL)	Mean ±SD	142 ±37	132 ±16	0.211
MGP (Pg/ml)	Mean ±SD	653 ± 159.64	499 ± 121.12	0.066

HDL: High density lipoprotein, LDL: Low density lipoprotein

Non-significant association was noted among MGP and other parameters, including age, BMI, hemoglobin, albumin, PTH, Ca, vit-D, PO4, and alkaline phosphatase **(Table 5).**

Table (5): Correlation among MGP and other parameters

	MGP	
	r	**P**
Age (years)	0.038	0.725
Body mass index (kg/m^2)	0.133	0.223
Disease duration (years)	0.208	0.055
Hemoglobin (g/L)	-0.09	0.417
Albumin (g/L)	0.075	0.496
PTH (pg/mL)	0.043	0.695
Ca^{++} (mg/dL)	-0.068	0.534
Vit-D (ng/dL)	0.085	0.439
PO_4 (mg/dL)	0.034	0.757
Alkaline phosphatase (IU/L)	0.147	0.177
Cholesterol (mg/dL)	0.018	0.872
Triglycerides (mg/dL)	-0.137	0.208
HDL (mg/dL)	-0.072	0.511
LDL (mg/dL)	0.062	0.574

r: Correlation coefficient, HDL: High density lipoprotein, LDL: Low density lipoprotein

DISCUSSION

Vit-K is enrolled in vascular calcifications through its function as a co factor in the carboxylation, that is, stimulation, of the calcification inhibitors MGP (matrix Gla protein). Under-carboxylated or non-active MGP, counted as high dp-ucMGP levels, is a marker of low vit-K levels. High dp-ucMGP level was accompanying with elevated vascular calcifications and cardio-vascular diseases (CVD) risk, but not in all cases [17]**.** The function of vit-D in this procedure is contentious. However, vit-D shortage was stated to elevate cardio-vascular morbidities and death both in the normal people and in CKD cases. The impacts of vit-D on vascular calcifications look to trail a biphasic pattern, with excess as well as shortage indorsing its advance [18]**.**

The aim of this work was to find out the relation between valvular calcification in HD cases and functional vit-K as presented by serum level of uncarboxylated matrix Gla protein (ucMGP) and 25(OH) vit-D level.

This study was conducted over six months and included 90 HD cases and 20 apparently healthy adults with normal kidney function age and gender matched to the HD cases (to establish normal range for ucMGP).

Our results revealed that the median MGP was higher significantly in patients (588) than control group (118). In agreement with our results the study by **Mosa and Harfoosh** [19] reported that MGP was significantly higher in chronic HD cases than control group ($P < 0.005$). Our findings were in line with **Aoun *et al.*** [20] who revealed that dp-ucMGP levels (reflecting Vit-K deficiency), was significantly higher in HD cases in comparison to control group. In agreement with our results **Caluwé *et al.*** [2] reported that chronic HD cases having elevated level of inactive MGP, maybe connected to a low dietary vit-K intake.

However, in disagreement with our findings **Cranenburg *et al.*** [21] reported that the mean ucMGP levels in HD cases (193 ± 65 nM) was significantly lower in comparison with control group of comparable age (441 ± 97 nM; p value< 0.001). As well the study by **Hermans *et al.*** [16] revealed that ucMGP levels were significantly low in HD cases in comparison with controls (173±70 vs. 424±126 nmol/l; p value<0.0001). In addition, the study by **Schlieper *et al.*** [14] stated that ucMGP levels were significantly low in HD cases in comparison with controls.

Regarding valvular calcifications, we found that about one-third of the patients (41.1%) had calcifications of the aortic valve, and about one-quarter had calcifications on the mitral valve (27.8%). Nonsignificant variances were noted amid those with and with no aortic valve calcifications as regard age, gender, BMI, and disease duration.

We also found that triglycerides were significantly low in those with aortic valve calcifications (122) than those without (149). The median MGP was significantly high in those with aortic valve calcification (705) than those with no calcification (499). Nonsignificant changes were noted among those with and with no aortic valve calcification as regard the rest of laboratory parameters.

The present study also revealed that nonsignificant differences were noted among those with and with no mitral valve calcification as regard age, gender, BMI, and disease duration. We also found that cholesterol was significantly higher in those with mitral valve calcification (229) than those without (205). And nonsignificant changes were noted among those with and with no mitral valve calcification concerning the rest of laboratory parameters. In agreement with this result **Ma *et al.*** [15] reported that there was nonsignificant change among calcific aortic valve cases and control group regarding age and sex. While they reported that there was nonsignificant difference among calcific aortic valve cases and control group as regard leukocyte and LDL-C but there was significant difference regarding neutrophil, platelet, monocyte, lymphocyte and LMR.

The most important result of the present work was the significant difference in MGP between patients and controls, suggesting a correlation between MGP level and aortic valve calcifications. This was supported by **Thamratnopkoon *et al.*** [22] who concluded that plasma dp-ucMGP level rises with the severity of CKD. Plasma dp-ucMGP was positively correlated with vascular calcifications and may be assed an early biomarker for vascular calcifications in cases with CKD. This also further supported by **Brandenburg *et al.*** [23] who reported that vit-K supplementations can denote an effective and harmless treatment in CVD connected to ectopic calcifications like calcific aortic stenosis. Furthermore, the study by **Caluwé *et al.*** [2] revealed that there was significant association amid levels of MGP and vascular calcification.

This result was in disagreement **Koos *et al.*** [24] who reported that nonsignificant association was found amid serum levels of t-ucMGP and Agatston aortic valve calcifications scores in the cases group. Furthermore, the study by **Mosa and Harfoosh** [19] reported that Vit-K supplementations couldn't stop vascular calcification but significantly reduced their progressions.

Kraus *et al.* [25] reported that vascular and valvular calcifications were more dominant in the HD individuals. Peripheral vascular calcifications associate significantly with raised pulse. Pressure and can be evaluated simply by means of side lumbar X-ray.

Finally, the present study showed that nonsignificant association was noted among MGP and other parameters, including age, BMI, Disease duration, hemoglobin, albumin, PTH, Ca, vit-D, PO4, alkaline phosphatase. While the study by **Caluwé *et al.*** [2] reported that there was highly significant association among dialysis period and base-line dp-ucMGP was

detected (P value< 0.001, r = 0.29). Baseline dp-ucMGP wasn't related with other base-line parameters. In addition, **Plytzanopoulou *et al.* [(26)]** enrolled 42 cases out of them 50% had mitral calcifications, 38 % had aortic valve calcifications, and 16.7% had calcifications in the two. ROC curve analysis showed that older age (p value=0.011), elevated CRP (p value=0.038) and reduced value of serum albumin to total proteins ratio (p value=0.012) were positive predictive factors for moderate to severe degrees of cardiac valve calcifications. Low phase angle was as well related to CVC, but with moderate specificity.

CONCLUSION

Our study revealed that there was a functional deficiency of vit-K among the HD cases, aortic valve calcification was the most prevalent valvular calcification, and was associated with significantly higher MGP as well as triglycerides. In addition, the mitral valve calcification was significantly associated with higher cholesterol and LDL levels. We found no correlation between MGP and demographic data and laboratory results.

Conflict of interest: The authors declare no conflict of interest.

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Author contribution: Authors contributed equally in the study.

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