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Role of Vitamin k 2 on Matrix Gla Protein and Vascular Stiffness in Hemodialysis Patients

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Abstract

Background: Matrix Gla protein (MGP) acts as a potent local inhibitor of vascular calcification. However, to be active, MGP must be phosphorylated and carboxylated; such carboxylation is vitamin K-dependent, and phosphorylation is necessary for the secretion of MGP. This study aimed to investigated the effect of vitamin K2 on MGP levels after 3 months' supplementation and the relationship between MGP level and vascular stiffness in hemodialysis patients. **Patients and methods:** This interventional, double armed, clinical trial (Single-blinded) study was conducted in the dialysis Unit in Al-Ahrar Teaching Hospital, Sharkia on 48 end-stage renal disease (ESRD) patients on regular hemodialysis divided into 2 groups; 24 patients receiving Mk -7 as 90 mcg/day and 24 patients receiving placebo. Carotid duplex ultrasound for right and left common carotid arteries was done to assess Peak Wave Velocity (PWV) and the presence or absence of atheromatous plaques, prior to administering MK-7 and again after 3 months, for both groups. **Results:** There was statistically non-significant difference between the studied groups regarding hemoglobin, serum calcium, phosphorus, potassium, sodium, alkaline phosphatase, Ktv and parathyroid hormone. There was statistically non-significant difference between the studied groups regarding baseline matrix Gla protein. There was statistically significant difference between the studied groups regarding matrix Gla protein after 3 months of administration of vitamin k2 supplementation and placebo within intervention and placebo groups respectively. **Conclusions**: Vitamin k2 supplementation to hemodialysis patients will reduce ucMGP, which in turn reduces calcification and stiffness of blood vessels and cardiovascular system which in turn reduces cardiovascular morbidity and mortality.

Keywords: Vitamin k2, Vascular Stiffness, matrix Gla protein (MGP), Hemodialysis, Chronic kidney disease (CKD).

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INTRODUCTION:

Hemoialysis patients show an increased cardiovascular morbidity and mortality. Cardiovascular calcifications are well-established mortality predictors in ESRD patients. Calcification is not only a passive but an actively regulated process dependent on calcification inhibitors. Vascular calcification is an important risk factor and predictor for cardiovascular mortality in ESKD patients. Even with the absence of traditional cardiovascular risk factors, vascular calcifications can occur in young to middle-aged hemodialysis patients[(1)].

Matrix Gla protein (MGP) is primarily secreted by chondrocytes and smooth vascular muscle cells, and acts as a potent local inhibitor of vascular calcification. However, to be active, MGP must be phosphorylated and carboxylated; such carboxylation is vitamin K-dependent, and phosphorylation is necessary for the secretion of MGP[(2)].

The vitamin K family includes phylloquinone (vitamin K1) and several menaquinones (vitamin K2). Notably, 72% of patients with chronic kidney disease (CKD) exhibit vitamin K intake lower than recommended levels[(3)].

A theoretical link exists among matrix Gla protein (MGP), vitamin K, vascular calcification, and cardiovascular disease (CVD); this link is more notable in CKD and HD patients. However, atherosclerotic calcification is more prevalent in elderly HD patients; thus, age is a primary risk factor for vascular calcification in such patients. Simultaneous assessment of matrix Gla protein (MGP) levels, vitamin K levels, and vascular calcification should be performed in age-matched populations[(4)]. The aim of the present study was to investigated the effect of vitamin K2 on MGP levels after 3 months' supplementation and the relationship between MGP level and vascular stiffness in hemodialysis patients.

PATIENTS and METHODS:

This interventional, double armed, clinical trial (Single-blinded) study was conducted in nephrology (dialysis) unit in Al-Ahrar teaching hospital - Sharkia from Septemper 2019 to February 2020 on 48 ESRD patients on regular HD were divided into 2 groups; first group 24 patients received Mk -7 as 90 mcg / day (**We used MENA-Q ,DEVARTLAB**) and second group received placebo, over period of 3 months. **Inclusion Criteria** were all patients with ESRD on regular HD in the hospital in a stable medical condition, receiving HD more than 3 months, aged 18 years or older, with secondary hyperparathyroidism. **Exclusion Criteria** were patients on Warfarin treatment, known intestinal malabsorption, short life expectancy and inability to provide informed consent were excluded.

Carotid duplex and laboratory tests were performed prior to administration of MK.7 and were repeated after 3 months of administration. Laboratory investigations included measurement of hemoglobin %, parathyroid hormone (PTH), calcium, phosphorus, sodium, potassium alkaline phosphatase, Uncarboxylated matrix Gla protein (ucMGP). The blood samples were collected after skin sterilization with the ethyl alcohol swap. 10 ml from peripheral venous blood was withdrawn from each patient using a disposable syringe under complete aseptic conditions. The minimum detectable dose of human ucMGP is typically less than 0.039 ng/ml. The sensitivity of this assay, or Lower Limit of Detection (LLD) was defined as the lowest protein concentration that could be differentiated from zero. It

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was determined the mean O.D value of 20 replicates of the zero standard added by their three standard deviations. No significant cross-reactivity or interference between human ucMGP and analogues was observed.

Carotid duplex ultrasound for right and left common carotid arteries was done to assess Peak Wave Velocity (PWV) and the presence or absence of atheromatous plaques, prior to administering MK-7 and again after 3 months, for both groups.

Calculation of the results was done by plotting the average OD for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis and draw a best fit curve through the points on the graph. These calculations can be best performed with computer-based curve-fitting software and the best fit line can be determined by regression analysis.

Statistical analysis

Data analysis was performed using the software SPSS (Statistical Package for the Social Sciences) version 20. Quantitative variables were described using their means and standard deviations. Categorical variables were described using their absolute frequencies and were compared using chi square test. Kolmogor¬ov-Smirnov (distribution-type) and Levene (homogeneity of variances) tests were used to verify assumptions for use in parametric tests. To compare quantitative data between two groups, independent sample t test was used to compare means when data was normally distributed and Mann Whitney test was used when data is not normally distributed. Paired t test was used to compare continuous normally distributed data within the same group at two points of time. Spearman rank and pearson correlation coefficients were used to assess length and direction of association between two continuous variables. Linear regression analysis was performed to measure associated independent factors for dependent factor. The level statistical significance was set at $P<0.05$.

RESULTS:

Table 1; showed that there are statistically non-significant differences between the studied groups regarding age, gender or smoking.

Table (1): Comparison between the studied groups regarding demographic data and special habits

Parameter	Groups		Test	
	Intervention group	Placebo group	t/χ^2	p
	N=24 (%)	N=24 (%)		
Age (year): Mean ± SD Range	 61.625 ± 6.099 50 – 74	 64.292 ± 5.449 54 – 72	-1.597	0.117
Gender: Male Female	 14 (58.3) 10 (41.7)	 13 (54.2) 11 (45.8)	0.085	0.771
Smoking: No Yes	 12 (50) 12 (50)	 13 (54.2) 11 (45.8)	0.083	0.773

χ^2 Chi square test t Independent sample t test

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Table 2; showed that there are statistically non-significant differences between the two groups regarding systolic or diastolic blood pressure, body mass index or duration of dialysis.

Table (2): Comparison between the studied groups regarding clinical data and duration of dialysis

Parameter	Groups		Test	
	Intervention group	Placebo group	t/Z	P
	N=24 (%)	N=24 (%)		
Systolic blood pressure (mmHg): Mean ± SD Range	140.21 ± 10.27 125 – 165	140.83 ± 13.57 120 – 165	-0.18	0.858
Diastolic blood pressure(mmHg): Mean ± SD Range	84.79 ± 7.44 75 – 100	84.375 ± 10.87 70 – 105	-0.155	0.878
BMI (kg/m^2): Mean ± SD Range	22.392 ± 1.921 19.8 – 25.6	22.204 ± 2.127 19.2 – 25.5	0.32	0.75
Duration of dialysis (year): Median Range	8.7 3.5 – 15.5	8.5 2.5 – 16	-0.61	0.542

t Independent sample t test Z Mann Whitney test

Table 3; showed that there are statistically non-significant differences between the studied groups regarding hemoglobin, serum calcium, phosphorus, potassium, sodium, alkaline phosphatase, Kt/v and parathyroid hormone.

Table (3) Comparison between the studied groups regarding laboratory data

Parameter	Groups		Test	
	Intervention group	Placebo group	t/χ^2	P
	N=24 (%)	N=24 (%)		
Hemoglobin (g/dL): Mean ± SD Range	10.7 ± 0.87 9.5 – 12.5	11.18 ± 0.93 9.8 – 12.5	-1.818	0.076
Serum calcium (mg/dL): Mean ± SD Range	8.375 ± 0.412 7.9 – 9.3	8.521 ± 0.252 8.1 – 9.1	-1.479	0.147

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Serum phosphorus (mg/dL): Mean ± SD Range	 5.008 ± 0.544 4.5 – 6	 5.317 ± 0.596 4.5 – 6	-1.871	0.068
Alkaline phosphatase:(IU/L) Median Range	 257.5 165 – 350	 205 150 – 310	-1.924	0.054
PTH: (Pg/ml) Median Range	 450 127 – 580	 450 230 – 560	 -0.28	 0.78
Serum potassium (mg/dL): Mean ± SD Range	 4.895 ± 0.337 4.1 – 5.5	 4.978 ± 0.334 4.36 – 5.6	0.099	0.922
Serum sodium (mg/dL): Mean ± SD Range	 135.92 ± 4.826 130 – 145	 135.38 ± 2.67 131 – 140	0.481	0.633
Kt/v: Mean ± SD Range	 1.339 ± 0.183 1.11 – 1.7	 1.416 ± 0.146 1.11 – 1.68	-0.366	0.716

t Independent sample t test Z Mann Whitney test

Figure 1; showed that there was statistically non-significant difference between the two studied groups regarding baseline MGP, while there is a statistically significant difference between the two groups regarding MGP after 3 months of administration of **vitamin k2 supplementation and placebo within intervention and placebo groups respectively**. There was a significant decrease in MGP level within the intervention group, while there is a non-significant decrease in its level within the placebo group.

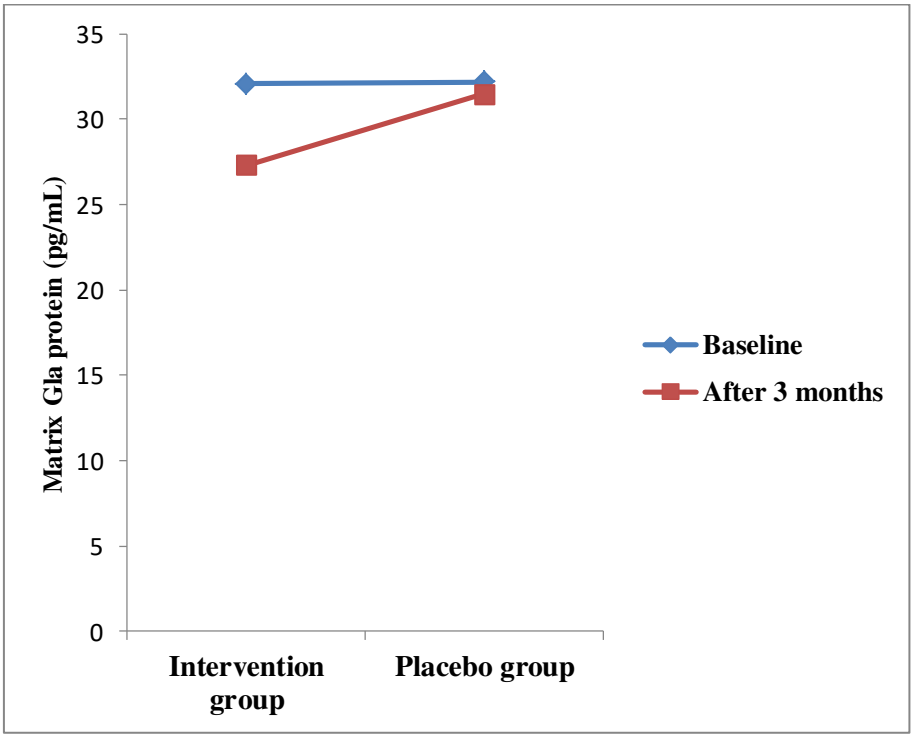

Figure (1): Multiple line graph showing matrix Gla protein level within the intervention and placebo groups, baseline and after 3 months.

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Table 4; showed that there was statistically significant difference between both groups regarding percent change in MGP (percent reduction was 16.7% in MGP and 14% in PWV within the intervention group compared to 0% and 0.388% respectively for the placebo group).

Table (4): Comparison between the 2 groups regarding percent change in MGP and PWV, 3 months after vitamin k2 supplementation (90 mcg / day MK-7)

Percent change	Groups		Test	
	Intervention group	**Placebo group**	**Z**	**p**
	N=24 (%)	**N=24 (%)**		
Matrix Gla protein: Median Range	-16.67% -41.349% – 71.429%	0% -46.429% – 44.928%	-4.063	<0.001**
PWV: Median Range	-14% -22.891% – (-4.557%)	-0.388% -14% - 9.804%	-5.752	<0.001**

Z Mann Whitney test **p≤0.001 is statistically highly significant

Figure 2; showed that there was statistically significant negative correlation between PWV and MGP level. On the other hand, there are non-significant correlations between PWV and the other parameters.

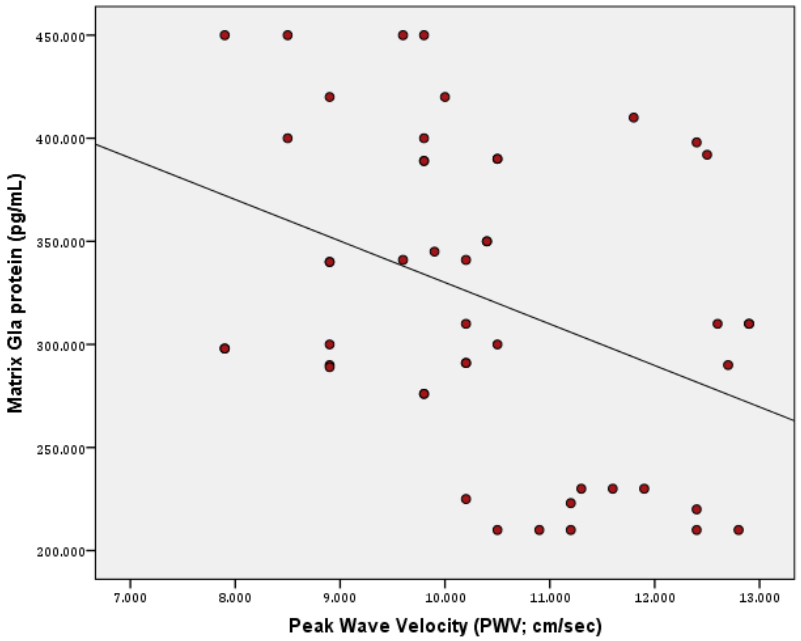

Figure (2): Scatter dot graph showing a significant negative correlation between PWV and MGP among the studied patients.

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Table 5; showed that there are statistically significant positive correlations between percent change in PWV and both hemoglobin and serum calcium. On the other hand, there are non-significant correlations between it and any other studied parameters.

Table (5): Correlations between percent change in PWV and the studied parameters

Parameter	PWV (% change)	
	r	p
Age (year)	0.057	0.702
BMI (kg/m2)	-0.005	0.975
SBP	0.092	0.535
DBP	0.097	0.512
Dialysis duration	-0.1	0.498
Hemoglobin	0.345	0.016*
Serum calcium	0.367	0.01*
Sodium	-0.057	0.702
Potassium	-0.083	0.573
Phosphorus	0.275	0.059
Alkaline phosphatase	-0.043	0.77
Parathyroid hormone	0.024	0.87
ktv	0.063	0.669

*p<0.05 is statistically significant r Spearman correlation coefficient

DISCUSSION:

In this study, there are statistically non-significant differences between the studied groups regarding age, gender, smoking and comorbidities.

Mizuiri et al. [5] also evaluated the association between serum MGP, plasma vitamin K1, and plasma vitamin K2 with coronary artery calcium score (CACS) and CV disease in maintenance HD patients. There were 152 subjects, including 40 healthy controls and 112 patients on maintenance HD. The two groups showed no significant differences in age, sex composition or body mass index.

In this study, there are statistically non-significant differences between the studied groups regarding systolic or diastolic blood pressure or body mass index.

In this study, there are statistically non-significant differences between both groups regarding hemoglobin, serum calcium, phosphorus, potassium, sodium, alkaline phosphatase, Kt/v and parathyroid hormone. In line with our study, there is a study performed by **Westenfeld et al.** [6], who gave vitamin K2 to 53 HD patients for 6 weeks, who were divided into 3 randomized groups, 1st group received 45 mcg, 2nd received 135 mcg and 3rd group received 360 mcg. They found that Vitamin K2 supplementation induced a dose and time-dependent decrease in circulating dephosphorylated-uncarboxylated MGP. Response rates in the reduction in dephosphorylated-uncarboxylated MGP levels were 77% and 93% in the groups receiving 135 μg and 360 μg of menaquinone-7, respectively. **Saad et al.** [7] found no significant differences in serum levels of PTH, calcium, phosphorus, sodium, potassium, hemoglobin, and alkaline phosphatase between group I and group II either at the start or after 3 months.

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Regarding MGP, in our study, there is a statistically non-significant difference between the studied groups regarding baseline MGP, but there is a statistically significant difference between both groups regarding MGP after 3 months of administration of vitamin k2 supplementation and placebo within intervention and placebo groups respectively. Also, there is significant decrease in MGP level within intervention group while there is non-significant decrease in its level within placebo group.

In the study done by **Caluwé**[8], 200 chronic HD patients randomly received 360, 720 or 1080µg of MK-7 thrice weekly for 8 weeks. Dp-uc-MGP was measured at baseline and after 8 weeks. He found that MK-7 supplementation dose dependently reduced dp-uc-MGP. The levels decreased by 17, 33 and 46% in the respective groups. **Neven and D'Haese**[9] stated that prevention of warfarin-induced medial calcification in rats could be obtained by vitamin K2, and regression of this vascular pathology in this rat model was found under high intake of both vitamins K1 and K2. Also, **Kurnatowska et al.**[10] obtained a significant change in serum levels of calcification promoters and inhibitors MGP, Osteocalcin (OC), and osteoprotegerin (OPG) after 270 days of supplementation with vitamin K2 in patients with CKD stages 3–5, concluding that supplementation with vitamin K2 may reduce the progression of atherosclerosis but does not significantly affect the progression of calcification.

Schlieper et al.[11] differentiated between desphospho-carboxylated MGP (dp-cMGP) and desphospho-uncarboxylated MGP (dp-ucMGP) and tested whether dp-cMGP and/or dp-ucMGP predict survival in a cohort of HD patients, 188 HD patients exhibited 3.3-fold elevated plasma levels of dp-cMGP and 6.5-fold elevated plasma levels of dp-ucMGP compared with 98 age matched healthy subjects with normal renal function. dp-cMGP exhibited an inverse correlation with dialysis vintage and a positive correlation with body mass index, whereas dp-ucMGP did not show such a relationship. Age, DM, and dialysis efficacy (*i.e.,* Kt/V) were not related to plasma levels of dp-cMGP. Patients with lower plasma levels of dp-cMGP had increased C-reactive protein (CRP) levels, whereas other serum parameters were not significantly related to dp-cMGP.

Saad et al.[7] found an elevation in the serum level of MGP in patients than in controls at the start of the study. After 3 months, they found significant elevation in MGP level in group I (after 3 months of vitamin K1) accompanied by a decrease in serum cholesterol level, compared with group II. **Mizuiri et al.**[5] found that serum total MGP levels were significantly higher in patients on maintenance HD than in healthy controls.

Regarding PWV, in our study, there is a statistically non-significant difference between the studied groups regarding baseline PWV. There is a statistically significant difference between both groups regarding PWV after 3 months of administration of vitamin k2 supplementation and placebo within intervention and placebo groups respectively. Also, there is a significant decrease in PWV level within intervention group while there is a non-significant decrease in its level within placebo group.

Pivin et al.[12] evaluated whether high levels of dp-ucMGP are associated with increased vascular stiffness as measured by aortic PWV. Their study included 1001 participants. They found that high levels of dp-ucMGP are independently and positively associated with arterial stiffness after adjustment for common CV risk factors, renal function, and age.

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In our study, there is a statistically significant difference between the studied groups regarding percent change in MGP (percent reduction was 16.7% in MGP and 14% in PWV within intervention group compared to 0% and 0.388% respectively for control group).

A positive has been reported between **VC** scores and dephosphorylated-uncarboxylated MGP in HD correlation patients, although an inverse correlation has also been reported between CACS and uncarboxylated MGP in HD patients. Notably, no correlation has been reported between CACS and uncarboxylated MGP in HD patients[13].

In this study, there is a statistically significant negative correlation between PWV and MGP level. On the other hand, there is a non-significant correlation between it and any other studied parameters.

Regression analysis revealed that MGP significantly independently associated with PWV. Also, there is a significant positive correlation between percent change in PWV and both hemoglobin and serum calcium. On the other hand, there is a non-significant correlation between it and any other studied parameters.

Pivin et al.[12] concluded that high levels of dp-ucMGP are independently and positively associated with arterial stiffness after adjustment for common CV risk factors, renal function, and age. Regarding MGP, there are non-significant correlations between percent change in MGP and any other studied parameters. But, there is a significant positive correlation between percent change in MGP level and percent change in PWV.

Pencak et al. [13] have shown that total MGP is not closely related with CACS in HD patients. **Fusaro et al.** [14] reported lower plasma vitamin K1 levels, lower plasma menaquinones (vitamin K2) levels, and increased levels of total MGP in HD patients, compared with healthy controls; they also found an association between the vitamin K system and VC in HD patients. Thus, they suggested that total MGP may not constitute a good marker of VC.

Schlieper et al. [11] reported that dephosphorylated, carboxylated MGP levels were lower in dialysis patients than in normal subjects, which increased risks of all-cause and CV mortality. They reported that the majority of dialysis patients exhibits pronounced vitamin K deficiency. Lower levels of circulating active MGP may serve as a predictor of mortality in dialysis patients. **Saad et al.** [7] found no correlation between MGP level and VC at the start or after 3 months in both groups of patients and concluded that vitamin K supplementation may be essential for ESRD patients on regular HD. Vitamin K can increase the level of active MGP and decrease cholesterol level.

We concluded that the ESRD patients on regular HD have high level of Uncarboxylated matrix Gla protein (ucMGP) which is associated with the presence of CV disease in HD patients.

Vitamin K2 supplementation reduces ucMGP level and cosequently reduce vascular stiffness so reduce cardiovascular morbididty and mortality .

REFERENCES:

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1- Jablonski KL, Chonchol M. Vascular calcification in end-stage renal disease. Hemodial Int. 2013 Oct;17 Suppl 1(0 1):S17-21. doi: 10.1111/hdi.12084. PMID: 24134325; PMCID: PMC3813300.

2- Wuyts J, Dhondt A. The role of vitamin K in vascular calcification of patients with chronic kidney disease. Acta Clin Belg. 2016 Dec;71(6):462-467. doi: 10.1080/17843286.2016.1180770. Epub 2016 May 25. PMID: 27254816.

3- Barrett H., O'Keeffe M., Kavanagh E. Is matrix Gla protein associated with vascular calcification? A systematic review. Nutrients. 2018; 10: 415. doi: 10.3390/nu10040415.

4- Ruderman I, Holt SG, Hewitson TD, et al., Current and potential therapeutic strategies for the management of vascular calcification in patients with chronic kidney disease including those on dialysis. *Semin Dial*. 2018; 31: 487–499.

5- Mizuiri, Yoshiko Nishizawa, Kazuomi Yamashita, Relationship of matrix Gla protein and vitamin K with vascular calcification in hemodialysis patients, (2019) Renal Failure, 41:1, 770-777, DOI: 10.1080/0886022X.2019.1650065.

6- Westenfeld R, Krueger T, Schlieper G, Effect of vitamin K2 supplementation on functional vitamin K deficiency in hemodialysis patients: a randomized trial. Am J Kidney Dis. 2012 Feb;59(2):186-95. doi: 10.1053/j.ajkd.2011.10.041. Epub 2011 Dec 9. PMID: 22169620.

7- Saad, A.A.E.A., Kenawy, E.M., Elashakr, A.M. *et al*. Effect of vitamin K_1 supplementation on matrix Gla protein level and vascular calcification in hemodialysis patients. *Egypt J Intern Med* **29,** 5–10 (2017).

8- Caluwé R, Vandecasteele S, Van Vlem B, Vermeer C, & De Vriese AS. (2014). Vitamin K2 supplementation in haemodialysis patients: a randomized dose-finding study. Nephrology Dialysis Transplantation, 29(7), 1385-1390.

9- Neven E, De Schutter TM, Dams G, Gundlach K, et al. (2014) A Magnesium Based Phosphate Binder Reduces Vascular Calcification without Affecting Bone in Chronic Renal Failure Rats. PLoS ONE 9(9): e107067.

10- Kurnatowska I, Grzelak P, Masajtis-Zagajewska A, Effect of vitamin K2 on progression of atherosclerosis and vascular calcification in nondialyzed patients with chronic kidney disease stages 3-5. Pol Arch Med Wewn. 2015;125(9):631-40. doi: 10.20452/pamw.3041. Epub 2015 Jul 15. PMID: 26176325.

11- Schlieper G, Westenfeld R, Krüger T. Circulating nonphosphorylated carboxylated matrix gla protein predicts survival in ESRD. J Am Soc Nephrol. 2011 Feb;22(2):387-95. doi: 10.1681/ASN.2010040339. PMID: 21289218; PMCID: PMC3029911.

12-Pivin E, Ponte B, Pruijm M, et al., Inactive matrix Gla-protein is associated with arterial stiffness in an adult population-based study. *Hypertension*. 2015; 66: 85–92.

13-Pencak P, Czerwieńska B, Ficek R, Wyskida K, Kujawa-Szewieczek A, Olszanecka-Glinianowicz M, Chudek J. (2013). Calcification of coronary arteries and abdominal aorta in relation to traditional and novel risk factors of atherosclerosis in hemodialysis patients. BMC nephrology, 14(1), 1-9.

14- Fusaro M, Noale M, Viola V, Galli F, Tripepi G, Vajente N, Vitamin K Italian (VIKI) Dialysis Study Investigators. (2012). Vitamin K, vertebral fractures, vascular

ISSN: 0975-3583, 0976-2833 VOL 12, ISSUE 03, 2021

calcifications, and mortality: VItamin K Italian (VIKI) dialysis study. Journal of Bone and Mineral Research, 27(11), 2271-2278.