



IS UNDERCARBOXYLATED MATRIX GLA PROTEIN A RELIABLE BIOMARKER OF VITAMIN K2 STATUS IN PATIENTS WITH CHRONIC KIDNEY DISEASE?

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ABSTRACT:

Vitamin K2 deficiency is associated with arterial calcification, which is a significant problem in patients with chronic kidney disease. It occurs more rapidly and earlier and appears to be an independent risk factor for higher morbidity and mortality. The ucMGP is considered to be a biomarker for indirect measurement of vitamin K2 status.

Purpose: Our study aimed to measure ucMGP levels in patients at different stages of CKD and to evaluate its potential role as a biomarker of vitamin K2 status.

Material/Methods: Sixty-seven patients aged between 31 – 87 years took part in the study. They were divided into groups according to glomerular filtration rate: Group I, with GFR ≥ 90 ml/min, Group II, with GFR 89 – 45 ml/min, Group III, with GFR 44 – 15 ml/min. Venous blood was tested for calcium, phosphate, creatinine and ucMGP.

Results: Serum calcium and phosphate levels were within the reference ranges across all groups. Phosphate levels showed a progressive increase, with significantly lower levels in Group III. Mean ucMGP levels decreased from Group I to Group III, although these differences did not reach statistical significance. In Group I, women had significantly lower ucMGP concentrations compared to men. The incidence of CVD increased significantly with the progression of renal failure. Participants with CVD in Group I demonstrated significantly higher ucMGP concentrations compared to Group III.

Conclusions: Uncarboxylated MGP can provide information about vitamin K2 and its carboxylation, but it is not a specific or reliable marker of vitamin K2 status.

Keywords: CKD, non-dialysis patients, uncarboxylated MGP, vitamin K2,

INTRODUCTION

Vitamin K is a fat-soluble vitamin that encompasses a family of compounds sharing a common chemical structure known as 2-methyl-1,4-naphthoquinone. This family includes vitamin K1 (phylloquinone) and various forms of vitamin K2 (menaquinones), which differ in their side chain lengths. Vitamin K2 is an essential nutrient crucial for maintaining normal human health, with numerous clinical studies highlighting its significant role in bone development, vascular protection, and the management of metabolic, hepatic, and renal diseases [1].

The broad physiological functions of vitamin K2 are primarily associated with calcium homeostasis through vitamin K-dependent proteins (VKDPs). As a cofactor for the enzyme α -glutamyl carboxylase, vitamin K2 facilitates the post-translational carboxylation of glutamic acid residues in these proteins. Notable VKDPs include osteocalcin (OC) and matrix Gla protein (MGP). Osteocalcin, secreted by osteoblasts, is essential for bone mineralization, while MGP, produced by chondrocytes and vascular smooth muscle cells, plays a critical role in vascular cell migration, angiogenesis, and vascular calcification. Recent research indicates that MGP inhibits calcification through mechanisms that are not yet fully understood [2].

Clinical studies reveal that modern diets are associated with a significant decline in vitamin K2 intake, with even well-balanced diets proving insufficient to meet physiological requirements [3]. Poor dietary intake of vitamin K2 leads to the production of inactive matrix Gla protein (MGP), termed undercarboxylated MGP (ucMGP), which enters systemic circulation. Emerging evidence indicates paradoxically elevated ucMGP levels in otherwise healthy adults, suggesting widespread subclinical vitamin K2 deficiency in the general population [4].

Accurately assessing vitamin K2 status poses challenges in both research and clinical settings. Due to its low molecular weight and rapid clearance, direct measurement of circulating vitamin K2 is technically limited and often detectable only at very high concentrations. Consequently, researchers increasingly rely on quantifying the undercarboxylated fractions of vitamin K-dependent pro-

teins (VKDPs), such as osteocalcin (OC) or MGP, as functional biomarkers of deficiency. Notably, vitamin K2 insufficiency correlates strongly with elevated ucMGP levels, making this biomarker a more reliable indicator of the vitamin's biological activity than direct serum measurements [5].

Patients with chronic kidney disease (CKD), especially as the disease progresses, are often deficient in vitamin K2 due to strict dietary restrictions, medication regimens, and impaired intestinal absorption [6]. As CKD progresses, the risk of developing cardiovascular disease and skeletal fragility increases, leading to heightened morbidity and mortality rates. Insufficient vitamin K2 status is a significant factor in both the progression of CKD and the development of associated skeletal and cardiovascular complications [1]. Despite its importance, there is a notable lack of data regarding vitamin K2 levels in patients with early-stage CKD. Furthermore, the common practice of administering high-dose calcium and active vitamin D supplements to these patients coupled with existing vitamin K2 deficiency may exacerbate vascular calcification and elevate cardiovascular risk.

Our study aimed to obtain data on serum levels of ucMGP in patients with different stages of CKD and its potential role as a biomarker for vitamin K2 deficiency.

MATERIALS AND METHODS:

Participants: We studied 67 adult Caucasian patients aged between 31–87 years (46 women and 21 men) in non-dialysis CKD stages. All participants have signed an informed consent. The Kidney Disease Outcomes Quality Initiative (KDOQI) defines CKD in 5 stages depending on the level of kidney function and glomerular filtration rate (GFR) [7]. The patients in our study were divided into three groups according to plasma creatinine concentration and GFR – Group I, with GFR \geq 90 ml/min (I KDOQI), Group II, with GFR 89–45 ml/min (II and IIIa KDOQI),

Group III, with GFR 44–15 ml/min (IIIb and IV KDOQI).

The GFR was calculated using MDRD GFR Calculator based on age, race, gender, and serum creatinine (SCr) concentration.

Blood samples collecting: Biochemical tests on the venous blood were made to measure levels of calcium, phosphate, and creatinine. Blood samples were collected in the morning after overnight fasting. The biochemical tests for calcium, phosphate, and creatinine were made using the biochemical analyzer Cobas E 311, Roche Diagnostic. Blood samples for measurement of ucMGP, after centrifugation and plasma separation, were frozen and stored at -80°C. UcMGP concentrations were measured with the Human Undercarboxylated Matrix Gla Protein (ucMGP) ELISA Kit (Abbeva, UK), with a detection range of 0.156 ng/ml – 10 ng/ml and sensitivity of < 0.1 ng/ml. The test was performed according to the manufacturer's instructions.

Statistical analysis: Statistical analysis was performed with SPSS v23.0 and Microsoft Office Excel 2003. Data were assessed for normality of distribution through the Kolmogorov-Smirnov and Shapiro-Wilk tests. The parametric t-test for independent samples, one-way analysis of variance (ANOVA) and nonparametric Kruskal-Wallis and Mann-Whitney tests were used for group comparisons. Pearson correlation analysis and Spearman's rank correlation were used to determine the relationship between parameters.

Patient Data Collection: Data about accompanying cardiovascular diseases (CVD) – hypertension, coronary artery disease, cerebrovascular disease and atherosclerosis, were collected from participants' medical documentation.

RESULTS:

The mean age of the participants, the concentrations of Ca, inorganic phosphate (Pi), SCr, GFR and ucMGP in the studied groups are presented in Table 1:

Table 1. Clinical characteristics of the groups

Parameter	Group I n=26	Group II n=22	Group III n=19	p-value
Age (years)	60.0 \pm 15.76	64.77 \pm 11.2	65.74 \pm 10.76	p=0.705
Ca (mmol/l)	2.46 \pm 0.11	2.48 \pm 0.11	2.58 \pm 0.41	p=0.469
Pi (mmol/l)	1.12 \pm 0.17	1.14 \pm 0.15	1.24 \pm 0.2	p=0.031*
SCr (μ mol/l)	66.38 \pm 11.9	102.41 \pm 16.29	181.37 \pm 43.27	p<0.001***
GFR (ml/min)	97.54 \pm 11.47	57.41 \pm 9.71	31.58 \pm 7.78	p<0.001***
ucMGP (ng/ml)	2.01 \pm 0.83	1.98 \pm 0.47	1.78 \pm 0.64	p=0.543

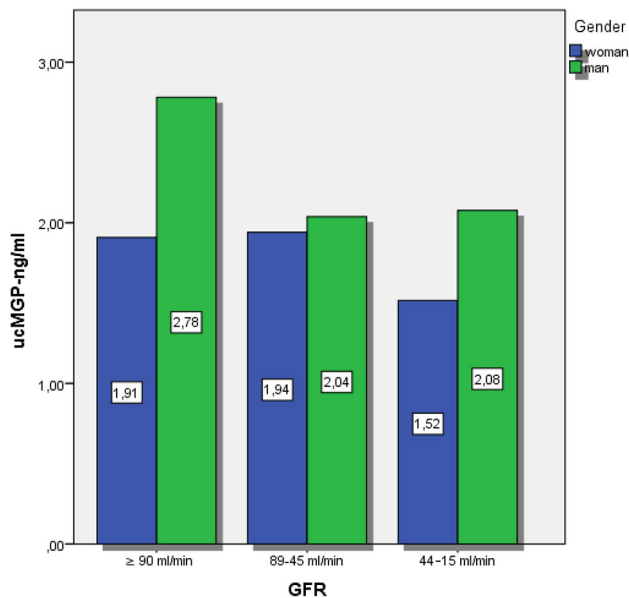
Serum concentrations of calcium and phosphates in all three studied groups were in the reference ranges of the commercial kits used. We found a gradual increase in the concentrations of calcium with a decrease in glomerular filtration. The differences between groups were not statistically significant. Phosphate levels showed a progressive increase, and comparison between groups showed statistically significant difference between group I and

group III. The concentration of creatinine increases progressively in the groups, and differences are statistically significant. The mean levels of ucMGP showed a gradual decrease from group I to III. Comparison between groups showed no significant differences.

The mean serum concentration of ucMGP in the groups divided by gender is shown in Fig. 1. We found lower concentrations of ucMGP †in women than in men

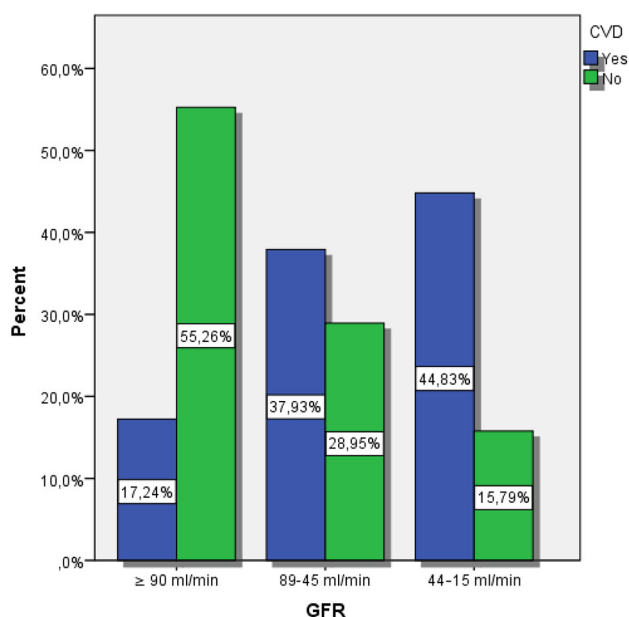
in the three groups. In group I, the differences were statistically significant ($p=0.024$). No significant differences were found in II and III groups ($p>0.05$).

Fig. 1. Mean serum concentrations of ucMGP in ng/ml in GFR groups divided by gender. In group I, differences are statistically significant ($p<0.05$).



The percentage of patients with cardiovascular disease increases in the groups with the decrease in GFR. The incidence of cardiovascular disease increases with the progression of renal failure. Comparison between groups showed a statistically significant difference ($p=0.003$). Post-hoc tests showed a significant difference between groups I and III ($p=0.002$) (Figure 2).

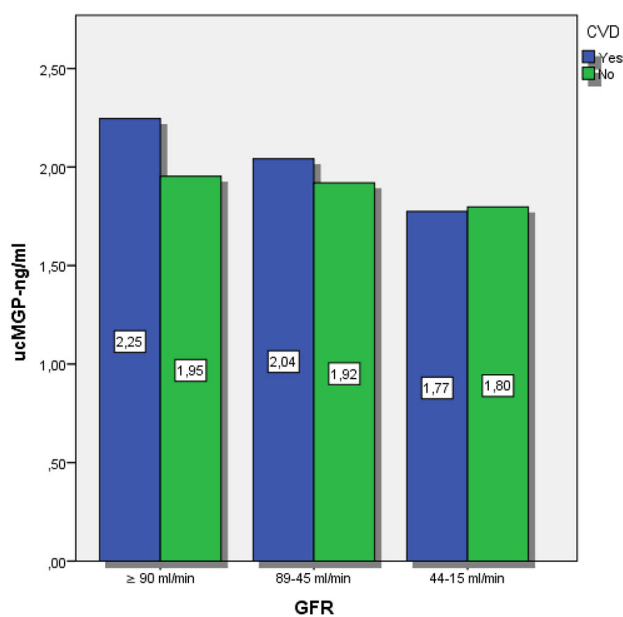
Fig. 2. Cardiovascular disease in percentage in GFR groups. The differences between groups are statistically significant ($p<0.05$).



The mean serum concentration of ucMGP in the groups divided by the presence of cardiovascular disease is shown in Figure 3. Serum ucMGP gradually decrease with a decrease in GFR. Participants with CVD and normal kidney function (group I) had significantly higher concentrations of ucMGP compared to participants with CVD in group III ($p=0.040$).

No significant correlations were observed between ucMGP levels and any demographic, clinical or biochemical characteristic in the three studied groups.

Fig. 3. Mean serum concentrations of ucMGP in ng/ml in GFR groups divided by the presence of cardiovascular disease. Participants with CVD and normal kidney function (group I) had significantly higher concentrations of ucMGP compared to participants with CVD in group III ($p<0.05$).



DISCUSSION:

Chronic kidney disease-mineral and bone disorder (CKD-MBD) is a complex syndrome characterized by multiple disturbances in mineral metabolism regulation and bone remodeling, leading to impaired mineralization and the deposition of calcium in blood vessels and soft tissues. In patients with CKD, vascular calcification occurs more rapidly and at earlier stages, serving as an independent risk factor for increased morbidity and mortality [8].

Abnormal mineral metabolism, marked by hypocalcemia and hyperphosphatemia plays a key role in the pathogenesis of vascular calcification. Elevated serum phosphate levels are strongly associated with heightened CVD risk [9]. Notably, even serum phosphate concentrations within the reference range correlate with increased CVD risk and mortality, underscoring phosphate's toxicity beyond overt hyperphosphatemia. As kidney function declines, elevated serum phosphate levels contribute to sys-

temic complications, including hypertension, vascular and cardiac valvular calcification, atherosclerosis, left ventricular hypertrophy, and myocardial fibrosis [10]. These findings align with our study results, which demonstrated a gradual increase in serum phosphate concentrations alongside declining GFR across patient groups despite levels remaining within the reference range. A statistically significant difference in phosphate concentrations was observed between Group I (early-stage CKD) and Group III (advanced CKD), mirroring the progressive rise in CVD incidence as renal failure advances (Figure 2).

Global evidence indicates that CKD patients suffer from subclinical vitamin K2 deficiency because of low intake associated with dietary restrictions, use of calcium phosphate binders with food, drugs such as omeprazole and antibiotics that contribute to dysbiosis and reduce vitamin K2 production by intestinal bacteria [11]. Several studies have suggested that serum ucMGP levels can be used as an indirect marker of vitamin K2 deficiency and increased risk of vascular calcification. Theuwissen et al. [12] and Cranenburg [13] et al. recommended measurements of circulating ucMGP levels as a more effective method of assessing vitamin K2 status. To date indirect measurement by undercarboxylated protein fractions is still not well standardized both in terms of reference values in healthy individuals and in terms of their levels, which may be associated with deficiency. Despite numerous reviews on the topic, there are insufficient randomized clinical trials of ucMGP levels in healthy individuals and in patients with CKD. Most studies have been conducted in hemodialysis patients, and there are a very limited number of studies on levels in patients in the predialysis stages of CKD. The manufacturer of the ELISA kit we used in our study reports concentrations of ucMGP below 1 ng/ml in healthy adults. Higher ucMGP levels we found in CKD patients, possibly reflecting vitamin K2 deficiency. However, this association requires further investigation in light of recent data that suggests alternative explanations.

The physiology and biochemistry of matrix Gla protein (MGP) remain an active area of investigation. Undercarboxylated MGP has emerged as the most extensively studied VKDP in vascular calcification regulation. Emerging evidence indicates that ucMGP levels are modulated not only by vitamin K2 status but also by multiple interrelated factors, including changes in mineral metabolism and vitamin D status, renal function, inflammatory conditions, and metabolic disorders. This complicates the interpretation of results and makes it difficult to associate them with vitamin K2 alone, especially in CKD patients.

The gender differences in ucMGP concentration we found need to be confirmed in larger groups and explained in detail, but cannot be related only to dietary intake of vitamin K2 (Figure 1).

Vitamin D directly regulates the synthesis and activity of VKDPs, including MGP. Specifically, vitamin D upregulates MGP gene expression by binding to a vitamin D response element within the gene's promoter region. This interaction increases MGP mRNA production, leading to a several-fold rise in MGP synthesis and secretion [14]. However, the MGP produced under vitamin D's influence remains inactive (undercarboxylated MGP, ucMGP) until vitamin K2-dependent γ -glutamyl carboxylase modifies its structure. This creates a critical interdependency: while vitamin D drives MGP production, vitamin K2 is essential for its activation. The interplay highlights how deficiencies in either nutrient can disrupt vascular calcification regulation, particularly in populations prone to vitamin K2 insufficiency, such as CKD patients [15].

Phosphate, calcium and magnesium are involved in bone mineralization and play an important role in vascular calcification. Phosphate and calcium can simultaneously induce an upregulation of MGP protein and gene expression, which possibly inhibits calcification [16].

Whereas the carboxylase reaction is complex, using multiple substrates and cofactors, such as carbon dioxide, oxygen, energy [17]. Oxidative stress, hypoxia, pH, the availability of substrates may affect the carboxylation process. Variations in the activity of genes involved in vitamin K2 metabolism and MGP synthesis between individuals are also important [18].

In summary, while ucMGP can indicate a deficiency in vitamin K, its lack of specificity and functional impairment limits its utility as a marker for vitamin K2 status.

The discovery of matrix Gla protein (MGP) as a local tissue inhibitor of vascular calcification has fundamentally transformed the understanding of this process and paved the way for identifying novel biomarkers for CVD [19, 20]. Undercarboxylated MGP has been linked to several markers of cardiovascular pathology, including increased arterial stiffness, vascular and valvular calcification, insulin resistance, and heart failure indices, all of which contribute to elevated cardiovascular mortality [21]. Low, rather than high, circulating ucMGP levels are a powerful predictor of cardiovascular calcification, and ucMGP serum levels are decreased in CKD patients. In patients in the initial stages of kidney damage, compensatory increased serum levels of ucMGP are observed, which subsequently, with the progression of CKD, begin to decrease. One possible explanation for the reduced circulating levels of ucMGP is its accumulation in calcified vessel walls [22]. Our results confirm the findings above. We found the highest mean concentrations in group I and a gradual decrease in groups II and III. However, the differences were not significant (Table 1). Mean serum concentrations of ucMGP in GFR groups divided by the presence of cardiovascular disease show statistically signifi-

cant differences. Participants with CVD and normal kidney function (group I) had higher concentrations compared to participants with CVD in group III (Figure 3).

CONCLUSIONS:

In non-dialysis CKD patients, circulating serum undercarboxylated MGP cannot be an accurate marker of vitamin K2 status due to multiple factors that affect its levels. It can provide information partly about vitamin K2 deficiency and the carboxylation process. To date, ucMGP appears to be a better marker of vascular calcification and cardiovascular risk, but the data remains to be confirmed with studies of a larger number of participants.

LIMITATIONS: Our study has several limitations. The sample size is relatively small, which limits the generalizability of the conclusions. Additionally, the study design is cross-sectional, which does not allow for causal inferences to be drawn. Future studies with a larger sample size and a prospective design may confirm our findings and provide a deeper understanding of the relationship between

levels of and CVD risk in patients with CKD.

In 2002, K/DOQI proposed a substantially new concept and definition of CKD, grouping it into five stages, according to the calculated glomerular filtration – eGFR. Since 2004, the Kidney Disease Improving Global Outcomes (KDIGO) has made some changes that essentially change parts of the philosophy of the previous classification – limiting the independent role of eGFR and including as an additional factor the degree of albuminuria. The latter is especially important in comparing glomerular filtration and various concomitant events, for example – cardiovascular. Our study undoubtedly suffers from a lack of information about the extent of albuminuria in the subjects studied. We are using the KDIGO classification and not the K/DOQI, because in terms of eGFR, they have no difference. The study was funded by Medical University – Pleven.

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