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Vitamin k deficiency related some biochemical parameters in women with cardiovascular disease in Kirkuk city

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Abstract

The current study was conducted in order to find out the changes in a number of biochemical parameters, including, Matrix G1a protein (MGP), osteocalcin hormone levels , and calcium concentration that are associated with vitamin K deficiency in women with cardiovascular disease(CVD) (myocardial infarction and atherosclerosis) in Kirkuk city. The study was designed with 60 women attending Kirkuk General Hospital and Azadi Teaching Hospital in Kirkuk city, whose ages ranged between (45-65) years, and were distributed among 20 women in the control group, 20 women in the myocardial infarction disease and 20 women in the atherosclerosis disease. Blood samples were collected from patients attending the hospital for the period from December 2022 to February 2023.The results of the study showed a significant decrease ($p \leq 0.05$) in the concentration of vitamin K in the blood serum of the CVD patients compared to the healthy control group. There was also a decrease ($P \leq 0.05$) in the matrix Gla protein (MGP) in CVD in compared to the healthy group. The myocardial infarction patients showed an increase in osteocalcin, concentration in blood serum but atherosclerosis patients did not show any difference in osteocalcin concentration. Calcium concentration decreased in CVD in compared to the healthy group.

Key Words : vitamin k , Matrix Gla protein , Osteocalcin, Myocardial infarction, Atherosclerosis

Introduction :

There is no doubt that a vitamin deficiency can have serious health consequences. Vitamin K is a fat-soluble vitamin that was first identified in a study on blood clotting by Dane Carl Peter Henriken. Vitamin K plays a large role in biological activities including blood clotting .regulation of calcium metabolism in tissues, cell growth and proliferation, oxidative stress, inflammatory reactions (Hamidi *et al*., 2013) The involvement of vitamin K in vascular calcifications, cardiovascular and bone diseases has been discovered in the past two decades, therefore, many articles have analyzed the effects of vitamin K on both the physiological and therapeutic levels (Fusaro *et al.,* 2022a).

Vitamin K exists in 3 main forms, which share a basic structure of two naphthoquinone aromatic rings .Vitamin K1 is more commonly found in green leafy vegetables, while k2 (MKn) is derived from fermented foods and intestinal bacteria (Simes *et al.,* 2020)and K3 which is a synthetic form (Fusaro *et al*., 2017).

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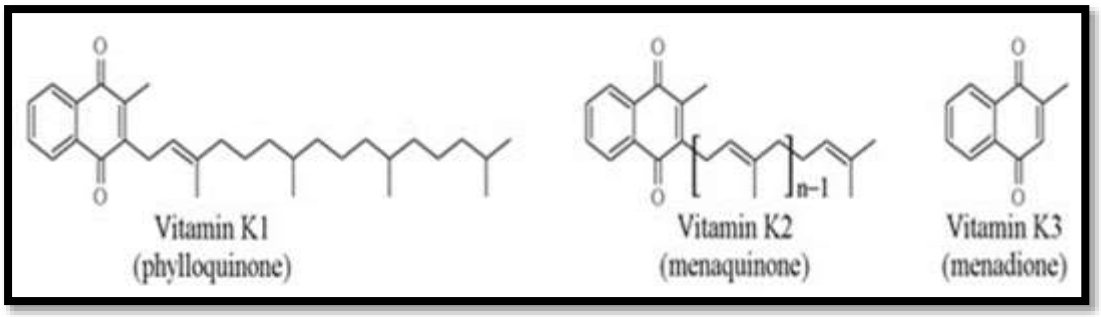

Figure 1. Chemical structures of different vitamin K forms

Hydroquinone is the active form of vitamin K. It is created from quinone under the co ntrol of an enzyme found in the endoplasmic reticulum membrane called vitamin K ep oxide reductase (VKOR) or cytoplasmic quinone reductase (QR.The cofactor for glut amylcarboxylase is hydroquinone (GGCX), that are involved in processes such as regulation of blood coagulation, prevention of vascular calcification, bone metabolism and modulation of cell proliferation (Beulens *et al.*, 2013).

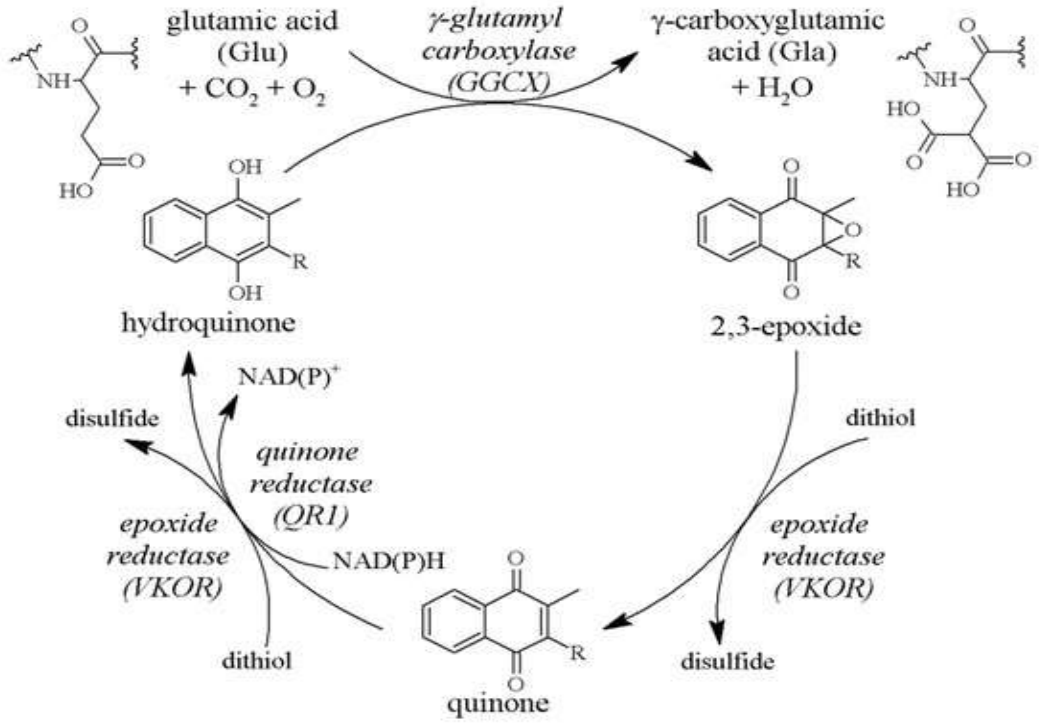

Figure 2. The vitamin K cycle. Vitamin K (quinone) is reduced to hydroquinone by QR1 or VKO

The main proteins involved in vascular and bone function are matrix Gla protein (MGP) and osteocalcin (OC), which belong to a large and distinct group of vitamin K-dependent proteins. MGP (Matrix Gla protein) prevents calcium deposition in the inner wall of blood vessels and may also reverse abnormal deposition to a certain extent to promote calcium entry into bone.it is secreted into the extracellular interstitial substance mainly by vascular smooth muscle cells (VSMCs) and chondrocytes, Osteocalcin (OC)

may attract carboxylate and bind calcium ions to transport calcium ions to the bone ,it is produced by osteoblasts (Fakhree *et al*., 2021).
Cardiovascular disease (CVD) currently ranks first in global morbidity and mortality and is the most common cause of death among females (Ciumărnean *et al.*, 2022). Vascular calcium deposition is an established feature of vascular disease and is predictive of cardiovascular events. It has been hypothesized that circulating calcium levels directly promote vascular calcification(Reid *et al*., 2017)

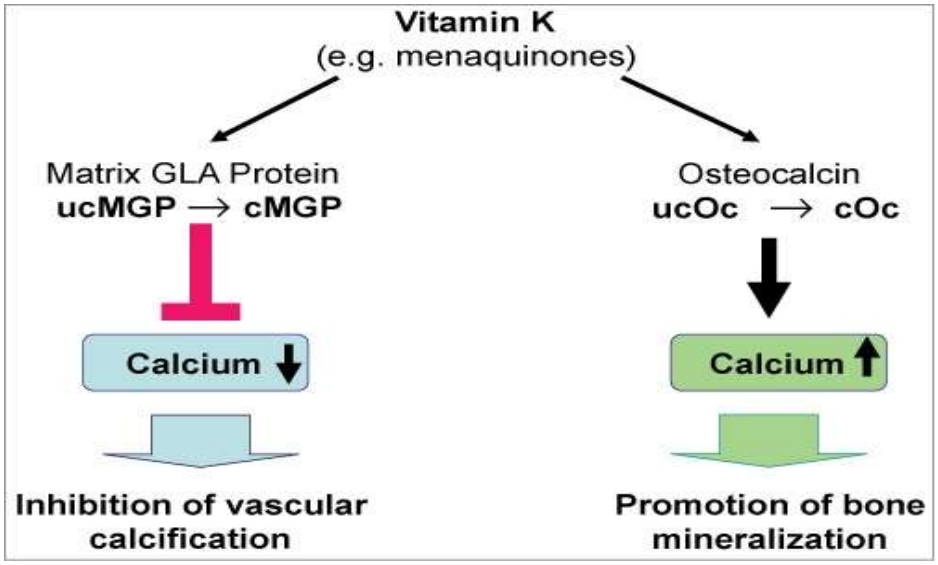

Figure3. The interactions of calcium on both vascular calcification and MGP.

Material & Methods :

Blood samples were collected from women with CVD disease attending Azadi Teaching Hospital and Kirkuk General Hospital, aged between 45-65, for the period from Desember2022 to February 2023. The blood was separated to obtain blood serum using a centrifuge and the serum was kept at -20 degrees Celsius for biochemical analysis later. An analysis of the concentration of vitamin K in the blood serum of the studied samples was carried out using the ELISA kit with the immunoassay of the enzyme competitive inhibition. The analysis of the Matrix Gla protein and the osteocalcin hormone was performed for the studied samples performed by using the ELISA kit on the principle of the enzyme sandwich immunoassay, where the concentrations were determined by comparing the OD of the samples with the standard curve. The calcium concentration was carried out for the studied samples by using a spectrophotometer.

Result & Discussion :

1- The results of the current study as shown in Figure (4) , there was a significant decrease at ($p<0.05$)level in the concentration of vitamin K1 in the blood serum of infected patients in myocardial infarction and atherosclerosis (1708.45±1.76 pg/ml) (1805.46 pg/ml ±3.33) compared in healthy control group (1838.97 ± 307.61pg/ml).

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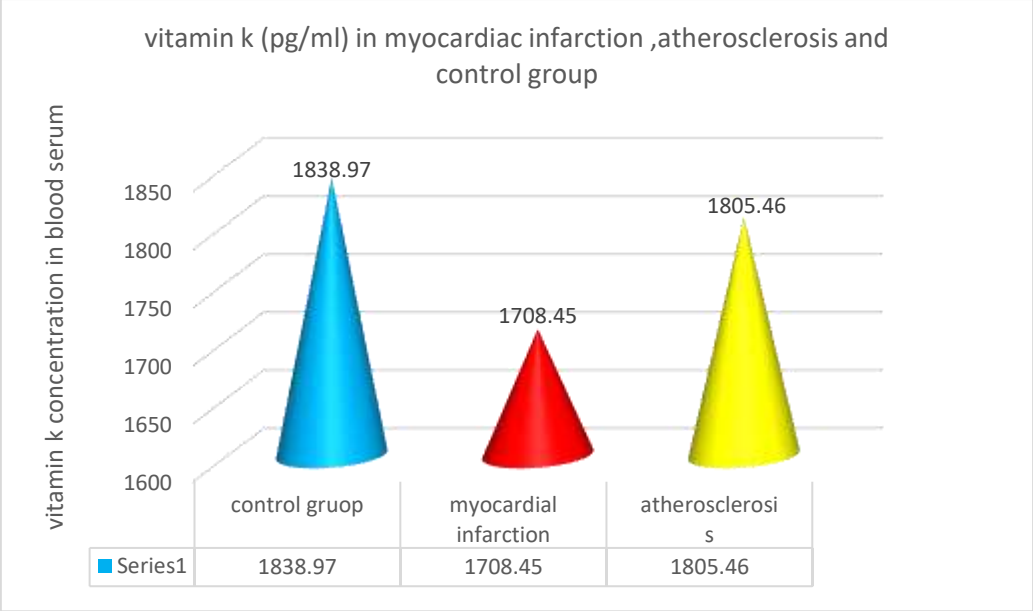

Figure (4) The average concentration of vitamin K in the serum of the studied samples

The low concentration of vitamin K in cardiovascular patients in this study agreed with the findings of Xu *et al* (2022) when they studied the relationship between vitamin K level and coronary artery calcification in heart patients, where the concentration of vitamin K in blood serum was found to be significantly lower in Patients with coronary heart disease compared with other groups.

The decrease in vitamin K concentration may be due to treatment with anticoagulants. Oral anticoagulants are a matter of debate for cardiovascular safety, and in particular concerns have been raised about the risk of myocardial infarction (MI).

In patients treated long-term with oral anticoagulants (vitamin K antagonists) (VKA) (Tornyos *et al.*, 2016).

Vitamin K deficiency may lead to suboptimal carboxylation of these proteins and impair their function, which may ultimately lead to an imbalance between promotion and inhibition of calcium deposition in the vascular wall, arterial calcification, and other related disorders (Xu *et al*., 2022).

2- The results of the current study as shown in Figure (5) a significant decrease ($P<0.05$) in the concentration of matrix Gla protein (MGP) in the blood serum of myocardial infarction(6.64± 2.72 ng/ml) when compared with the healthy control group (7.05± 3.89 ng/ml. As for the patients with atherosclerosis (7.59 ± 3.57ng/ml), there was no difference in the concentration of the baseline protein when compared with the healthy control group.

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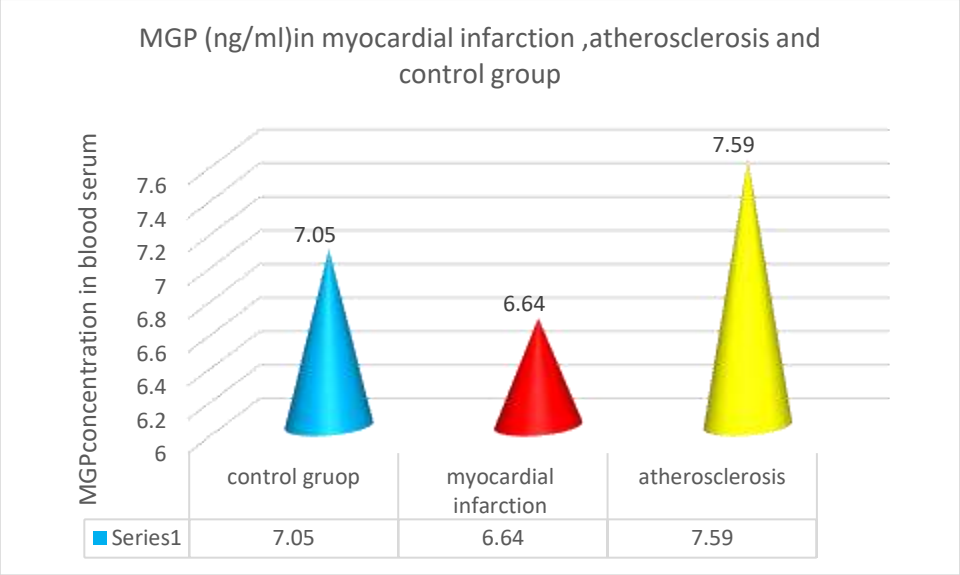

Figure(5) The average concentration of matrix Gla protein in the serum of the studied samples

The decrease in the concentration of Matrix Gla protein (MGP) in CVD patients agreed with the findings of Kiselova-Kaneva *et al.* (2021) who observed a clear trend of low MGP expression in all studied groups with high CVD risk. Other studies have indicated that in atherosclerotic plaques, the γ-carboxylation of MGP is insufficient or ineffective. The reasons for this may be that the enzyme γ-carboxylase is not expressed in sclerotic plaques or that the plaque environment (calcification) does not favor the active reduced form of vitamin K that activates MGP to its carboxylated form (Proudfoot & Shanahan, 2006).

MGP concentration in cardiovascular patients when compared with healthy subjects using measurement of serum MGP levels by enzyme-linked immunosorbent assay, and based on these data they concluded the possible role of MGP in the development of vascular calcification. Since MGP requires a carboxylate-dependent vitamin K for activation, MGP needs to undergo posttranslational γ-glutamyl carboxylation to achieve its biological activity (Jono *et al.*, 2004). It plays a pivotal role in bone metabolism and vascular health (Roumeliotis et al., 2004). ., 2020), it is speculated that subclinical vitamin K deficiency or poor tissue vitamin K status may play an essential role in the deficiency of MGP in its active form (Jono *et al.*, 2004).

3-The results of the current study as shown in Figure (6) ,there was a significant increase at ($P<0.05$) level in the concentration of osteocalcin in the blood serum of patients With myocardial infarction (1.36± 0.19 ng/ml) in compared with the healthy control group (0.61± 0.04ng/ml). for patients with atherosclerosis, there was no difference in the concentration of osteocalcin (0.61 ± 0.01 ng/ml) in their blood serum compared to the healthy control group.

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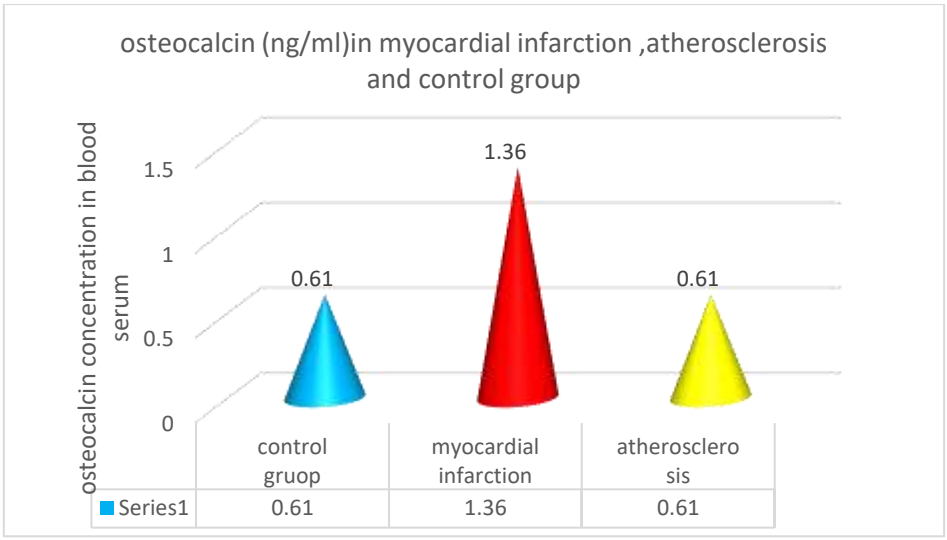

Figure (6) The average concentration of Osteocalcin in the serum of the studied samples

The results of our current study were identical to the findings of Luo *et al.* (2015). In our study, we also found that there was a significant difference in blood osteocalcin levels between men and women, which is consistent with previous findings that it may be the difference in bone turnover rates between men. And women, especially postmenopausal women, is the main cause of the gender difference in osteocalcin. Deficiencies of calcium and phosphorus may result in a decreased rate of formation of hydroxyapatite crystals, which is considered a condition of reduced bone mineralization.

In this case, free osteocalcin may be released into the circulation, which may cause an increase in the serum osteocalcin concentration of postmenopausal women, which in turn may explain the reason for the difference between the sexes, especially in postmenopausal women (Jagtap *et al.*, 2011)(Jung et al., (2016). Also, the increase in the concentration of osteocalcin (OC) in cardiovascular patients agreed with the findings of Holvik *et al.* (2014), where they indicated that the elevated concentration of OC in plasma was associated with an increased risk of cardiovascular disease in women.

4- The results of the current study shown in Figure (7) showed a significant decrease at ($p<0.05$) in the serum calcium concentration of patients with myocardial infarction (mg/dl 8.07 ± 1.14), and there was no significant difference in patients with atherosclerosis (9.47 ± 1.65 mg/dl) compared to the healthy group (10.33 ± 1.94 mg/dl).

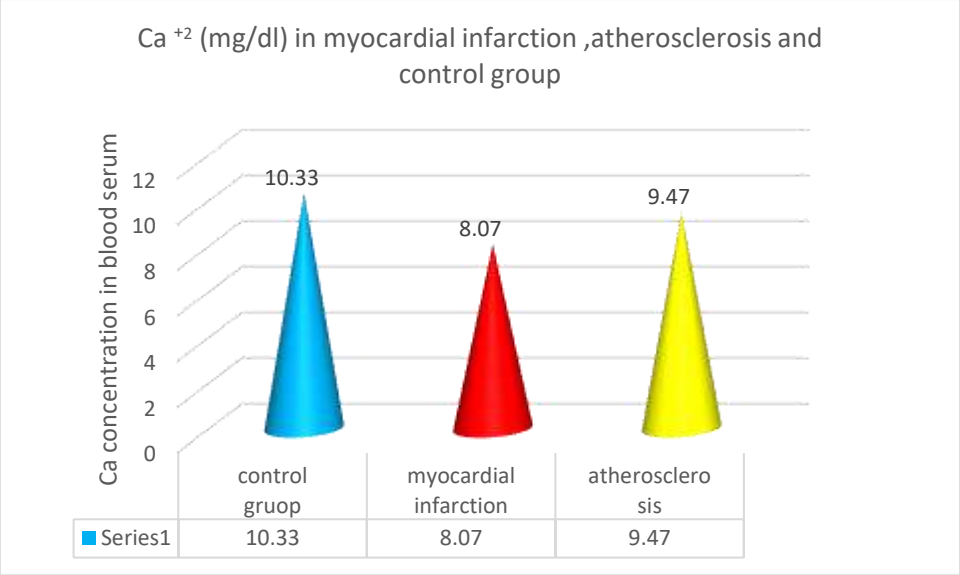

Figure (7) The average concentration of Ca^{+2}in the serum of the studied samples

Low level of calcium in cardiovascular patients agreed with the study Pravina *et al.* (2013) that indicated to a decrease in the concentration of calcium in the blood in patients with cardiovascular disease, may occur from treatment with drugs, such as diuretics. As a possible mechanism, the Parathyroid hormone associated with cardiovascular events has a role in the regulation of serum calcium. Parathyroid hormone is thought to be associated with the renin-angiotensin-aldosterone system. Also may affects the adrenal glands and thus increases aldosterone secretion. It is known that aldosterone increases urinary calcium excretion and thus reduces blood calcium levels (Fujii, 2018).

A recent study indicated that vitamin K may be involved in bone metabolism and the regulation of free calcium, by activating vitamin-dependent proteins and binding these proteins to calcium to carry out their functions (Roumeliotis *et al.,* 2020).

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