REVIEW

DOI: 10.4274/ijca.2024.83703
Int J Cardiovasc Acad 2024;10(4):91-101

Prognostic Value of Circulating Osteogenic Proteins for Stratifying Coronary Artery Calcification Risk

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Abstract

Increasing evidence suggests a common physiological process for bone and coronary artery calcification (CAC), implying the role of bone metabolism markers in subclinical atherosclerosis development. However, the association between bone turnover markers and the development of CAC has remained controversial, as seen in various studies. Because CAC measurement has both financial burden and radiation exposure risk in individuals with suspected cardiovascular disease (CVD), applying the diagnostic role of osteogenic markers in predicting abnormal CAC would improve treatment adherence and reduce the rate of CVD mortality. In this review, we begin by describing the current understanding of the molecular mechanisms of bone markers in the etiology of CAC. Furthermore, we summarize bone-associated regulatory factors at the molecular level as novel therapeutic targets for CAC. In addition, we focused on the current results on the prognostic role of novel mediators of osteogenic activity in determining the risk of CAC as a preclinical factor of atherosclerotic CVD. Accumulating evidence suggests the role of bone marker-mediated pathways in the progression of CAC, which may lead to early diagnosis of CVD complications and the establishment of innovative targets for pharmacological therapy. Indeed, miRNAs and lncRNAs, as novel therapeutic interventions, can be a research priority in regulating bone metabolism at the gene expression level to attenuate high CAC and improve CVD outcomes.

Keywords: Bone marker, osteoprotegerin, RANKL, fetuin-A, calcium score, non-coding RNAs

INTRODUCTION

Coronary artery calcification (CAC) is a prominent feature of atherosclerosis and is not the only principal cause of coronary artery disease (CAD) but also leads to increased mortality and atherosclerosis outcomes.[1] The baseline coronary artery calcium score is the most robust marker in the subclinical prediction of calcium in the walls of the heart's arteries, which is a significant factor in increasing the risk of CAD.[2,3]

To cite this article: Prognostic Value of Circulating Osteogenic Proteins for Stratifying Coronary Artery Calcification Risk. Int J Cardiovasc Acad. 2024;10(4):91-101

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Received: 12.10.2024
Revised: 29.11.2024
Accepted: 03.12.2024
Published Online: 16.12.2024

Using the coronary artery calcium score, individuals can be appropriately classified based on CAC categories that predict atherosclerotic cardiovascular disease in both sexes, ages, and races with similar magnitude impacts. Hence, preventive therapy may be beneficial for patients with elevated risk of CAC.[4-6] Accordingly, the potential role of CAC in the early diagnosis of patients with CAD burden will substantially reduce mortality and provide suitable care. Clinical studies have confirmed that artery mineralization is an active, complex process similar to bone formation, as both appear to share common signaling pathways, transcription factors, and extracellular matrix mediators. An emerging body of evidence suggests that bone markers, including matrix Gla protein (MGP), osteocalcin (OCN), osteoprotegerin (OPG), receptor activator of NF-kB ligand (RANKL), osteopontin (OPN), fetuin-A, bone morphogenetic proteins (BMPs), and alkaline phosphatase (ALP), could be considered as predictors of CAC prevalence.[7-14] However, the association between bone turnover markers and the development of CAC has remained controversial, as shown in various studies.[14-16] As the prediction of artery calcification is related to the concentration of bone metabolism markers and leads to early diagnosis of individuals at high risk of developing CAC, this review discusses the potential role of bone metabolism markers in stratifying the risk of CAC. The molecular mechanisms of bone turn over markers in the etiology of CAC and bone-associated regulatory factors at the molecular level were investigated as novel therapeutic targets for coronary calcification.

1. Bone Metabolism Markers and Mechanistic Insights into Coronary Artery Calcification

In the majority of human tissues, including bone (osteoblasts) and the vasculature (endothelial and vascular smooth muscle cells, or VSMC), OPG, a soluble glycoprotein, is widely expressed.[17] tumor necrosis factor (TNF)-related apoptosis-inducing ligand, which is expressed by VSMC and T cells, is bound by OPG, a member of the TNF family, to counteract its pro-apoptotic effects.[18] RANK receptors, which are found on the surface of osteoclasts, monocytes, and dendritic cells, are coupled to RANKL, which is expressed in osteoblastic, stromal, and T-cells.[19] The interaction can initiate intracellular signaling cascades, including the NF-κB pathway, and initiate the activation of osteoclast differentiation.[20] OPG can compete with RANKL for binding to the receptor and prevents RANK/RANKL interaction, thereby inhibiting osteoclast differentiation and bone resorption is the outcome.[19] Since several similarities were observed between osteoporotic bone loss and arterial mineral accumulation, OPG and RANKL were suggested as key markers involved in CAC[21,22] (Figure 1). Fetuin-A is a glycoprotein that is synthesized in the liver and mainly found in bone tissue. It plays an important role in bone mineralization.[23,24] This protein is a carrier of lipids in the blood and can mediate inflammatory responses.[25,26] Fetuin-A binds to TGF-β and BMP, inhibits bone mineralization, and is considered a calcification inhibitor.[27,28] Because the binding ability of fetuin-A to calcium and phosphorus in the circulation could enhance their solubility and, in turn, inhibit coronary calcification, this protein was noticed as a candidate marker for assessing the risk of CAC.[29] The most prevalent non-collagenous peptide in the mineralized matrix of bone is OCN. OCN has been shown to be a marker of arterial calcification, and compared with healthy controls, patients with coronary atherosclerosis have higher levels of OCN on the surface of their endothelial progenitor cells (EPC).[30] Endothelial cells, fibroblasts, VSMCs, and chondrocytes express MGP, a 14-kDa γ-carboxylated protein that was first isolated from bone. This protein contains five glutamine and three serine residues. To become fully functional, MGP must be phosphorylated and undergo a glutamate carboxylation process that is dependent on vitamin K. MGP potentially acts in several ways to regulate calcium deposition.[31] The mechanism for the strong calcification inhibition activity of MGP was explained by Price et al.[32], who suggested that further calcium precipitation could be prevented by binding MGP to the calcium phosphate crystal nuclei. The other mechanism for the inhibitory effect of MGP on calcification is the binding of MGP to bone morphogenetic protein-2 (BMP-2) and its inactivation of this pro-mineralization factor.[33] Bone morphogenetic proteins (BMPs) are derived from the -β like growth factors family. BMPs are secreted by endothelial cells, smooth muscle cells, and foamy cells in atherosclerotic vascular areas.[34] Because they contribute to calcification, BMP-2, BMP4, and later BMP 5, 6, and 7 have the highest correlation with vascular disease among the proteins in this family. BMP-2 is a crucial molecule that controls vascular calcification and bone development.[35] OPN is an extracellular matrix protein that functions as a proinflammatory cytokine. In addition to binding to osteoblasts, OPN is also involved in most systemic inflammatory processes and tissue remodeling.[36] It has been indicated that OPN is a potent calcification inhibitor because it can potentially increase the speed of calcium and other mineral dissolution by accelerating the expression of monocyte's carbonic anhydrase II enzyme and acidifying the extracellular surrounding.[37] ALP is a ubiquitous enzyme found in most tissues. ALP increases the level of phosphate in the extracellular surroundings.[38] Binding calcium to these extra phosphates induces calcification via the formation of calcium crystals.[39,40]

2. Molecular Factors Involved in Bone Metabolism Regulation as Therapeutic Targets in CAC

Over the last few years, noncoding RNAs (ncRNAs) have been of interest as novel therapeutic targets because they contribute to cellular processes by regulating gene expression.[41,42] Although a number of blood markers have been linked to an elevated risk of cardiovascular endpoints, few have been demonstrated

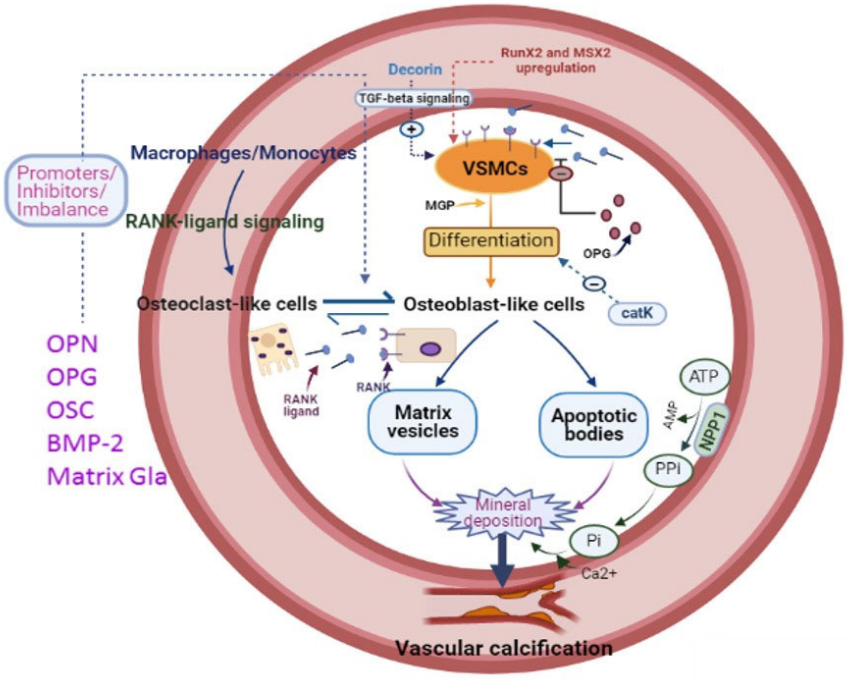

Figure 1: RANKL-RANK interactions initiate intracellular signaling cascades including NF-κB required for osteoclast differentiation and activity. OPG can compete with RANKL for binding to the receptor and inhibits RANK/RANKL, thereby preventing osteoclast differentiation and bone resorption. MGP binds to BMP-2 and inactivates this pro-mineralization factor. Among other effects, BMP-2, a member of the transforming growth factor-beta (TGF-beta) superfamily, promotes osteogenic conversion of VSMCs via the MSX2 transcription factor. BMP-2 5, 6, and 7 have the highest association with vascular disease due to their contributing role in calcification. Fetuin-A binds to TGF-β and BMP and prevents bone mineralization and calcification. High levels of OCN are located on the surface of endothelial progenitor cells (EPC) in coronary atherosclerosis. OCN can be undercarboxylated (ucOCN) due to low activity of the vitamin K-dependent carboxylase enzyme or vitamin K deficiency. ucOCN has less affinity to hydroxyapatite and is more readily released into circulation than OCN. It was indicated that OPN is a potent inhibitor of calcification as it can potentially increase the speed of calcium dissolution and other minerals by accelerating the expression of monocyte`s carbonic anhydrase II enzyme and acidifying the extracellular surrounding. ENPP1 could hydrolyze extracellular ATP and release PPi, which inhibit hydroxyapatite crystals by binding to their Pi sites, leading to prevent vascular mineralization. Decorin regulates transforming growth factor-β activity. During atherosclerosis and restenosis, extracellular decorin associates with SMC and collagen types I and III in the fibrous cap and with SMC and macrophages in the core region

RANKL: Receptor-activator of NF-kB ligand, OPG: Osteoprotegrin, MGP: Matrix gla protein, OCN: Osteocalcin, ucOCN: Undercarboxylated osteocalcin, OPN: Osteopontin, BMP-2: Bone morphogenetic protein-2

to have significant clinical implications or diagnostic value that would influence patient care.[6] As a result, novel biomarkers that can be utilized to evaluate the likelihood of atherosclerosis, the advancement of CAD, and the effectiveness of treatment are highly sought for.[7,8] Numerous studies have demonstrated the tight relationship between regulatory ncRNAs, such as microRNAs (miRNAs) (miRNA/miRs), short interfering RNAs (siRNAs), and long non-coding RNAs (lncRNAs), and the occurrence and progression of cardiovascular disorders. Recent studies on lncRNAs and miRNAs in cardiac disease have advanced rapidly.[9] MiRNA and lncRNA expression signatures in tissues and blood may play a role in disease diagnosis, prognosis, and treatment evaluation. In the cardiovascular system, ncRNAs are critical for the development of the heart and arteries as well as for the pathophysiology of cardiac disorders like CAD.[10,11] Because of their regulatory function in disease development, ncRNAs-a class of genetic, epigenetic, and translational regulators-ontain both short and long transcripts and offer fascinating potential as biomarkers.[13] Because ncRNAs, particularly miRNAs and lncRNAs, are persistent in bodily fluids like plasma, they may be used as disease biomarkers. Numerous studies have demonstrated the important roles of certain miRNAs and lncRNAs in the development of the heart and arteries as well as in the pathophysiology of the heart. To identify novel targets for the therapy of heart disease, we have compiled the most recent research findings here, with an emphasis on the molecular mechanism of miRNAs and lncRNAs in CAD. Small noncoding ribonucleic acid molecules called miRNAs, which have a length of 20-22 base pairs, are essential for controlling posttranscriptional levels of gene expression because they can

either prevent mRNA translation or cause mRNA destruction. In rare circumstances, miRNAs may improve a gene's transcription or translation of a gene, increasing the amount of the protein product.[14] A growing body of research indicates that miRNAs play a crucial role in controlling important signaling and lipid homeostasis pathways that change the ratio of atherosclerotic plaque advancement to reversal.[15] Crucially, miRNAs are involved in the control of endothelial cell inflammation and plaque development, in addition to lipoprotein metabolism.[16,17] Furthermore, leukocyte recruitment-one of the first harmful processes in atherosclerosis-is regulated by miRNAs.[17] In accordance with the ncRNA classification, lncRNAs are defined as ncRNAs longer than 200 base pairs. Despite the fact that the majority of lncRNAs have an unknown function, it is now evident that these molecules play crucial roles in many biological processes. lncRNAs can control gene expression programs by a number of methods, including alternative splicing, posttranscriptional gene regulation, epigenetic alterations of DNA, and mRNA stability and translation.[18,19] Considering their well-established functions in transcriptional control, lncRNAs are essential for many cellular processes, such as development, migration, apoptosis, and proliferation.[18] Because lncRNAs are now known to modulate the expression of genes that encode proteins, they can either favorably or adversely affect the expression of the genes they target.

Recently, the results of an animal study demonstrated that miR103a could reduce osteogenic trans-differentiation in VSMCs by inhibiting the expression of Runx2, an osteogenesis transcription factor that induces calcification. As it was indicated that Runx2 levels are significantly enhanced in calcified atherosclerotic plaques[43], inhibiting the expression of this transcription factor could be a research priority to identify promising combinations between related miRs and CAC incidence. Similar to the results of this study, another in vitro study showed that inhibiting miR32 could remarkably decrease the levels of Runx2 as well as the levels of BMP2, OPN, and MGP; therefore, vascular calcification was attenuated.[44] Interestingly, the result of an experimental study in 2021 showed that miR-223-3p could target the interleukin-6 (IL-6)/STAT3 signaling pathway and inhibit the osteogenic switch in VSMCs, thereby finding the associated miRs with STAT3 signaling pathway could potentially target atherosclerotic vascular calcification and can be considered a novel pharmacological intervention for CAC treatment.[45]

Lately, a study by Wicik et al.[46] applied correlation network analysis of 51 women that 21 of them had high CAC score and identified four bone metabolism genes, including PTGER3, FGFR1, ONECUT2, and SGCD, as the most contributed genes with other regulators of CAC. As demonstrated by the results of this study, the interaction of miRs and lncRNAs with these genes and signaling pathways could be considered novel therapeutic targets for reducing high CAC scores and improving cardiovascular disease (CVD) outcomes among patients.[46] PTGER3 contributes to the formation of prostaglandin receptors and the calcium signaling pathway, which can play an important role in cellular processes related to calcification in the arterial walls.[47,48] An emerging body of evidence from an *in vivo* study indicated that PTGER3 is less expressed during osteogenic activity and atherosclerosis, suggesting that manipulation of this gene expression could be a therapeutic target in altering the calcium signaling pathway during CAC.[47] Furthermore, FGFR1 plays a crucial role in bone calcification by acting as a receptor for FGF23, a bone-derived hormone that regulates the level of phosphate. The inhibition of FGFR1 expression leads to dysfunction in phosphate concentration and elevated calcification levels. Thus, targeting the expression of FGFR1 as a modulator factor could lay the foundation for novel therapeutic interventions against CAC.[49] The cumulative result of a study in an animal model demonstrated that SGCD contributes to the intracellular pathway related to calcification and myocardial dysfunction by regulating the signaling pathways in the cardiac muscle membrane.[50] Moreover, it was shown that ONECUT2 is a key driver in the molecular process of advanced CAD and aortic valve calcification, and interfering with the expression of FGFR1 and PTGER3 plays a role as a novel key regulator of CAC incidence.[46,51]

3. Prognostic Role of Osteogenic Activity Mediators in Determining the Risk of CAC

This review identified numerous observational studies investigating the association between OPG level and CAC risk in three different populations, including asymptomatic subjects, patients with type 2 diabetes, and individuals with a history of CVD.

3.1. Association between OPG and CAC in individuals with type 2 diabetes

According to a prospective cohort study involving 510 patients with type 2 diabetes, OPG was found to be an independent significant marker for predicting the risk of CAC [odds ratio (OR) = 2.84 (2.2-3.67), $P < 0.01$].[9] The cumulative results from the cross-sectional studies demonstrated the significant contribution of elevated OPG levels in identifying the enhanced risk of CAC incidence.[52-54] On the other hand, a clinical study with 168 participants reported that OPG was not correlated with CAC incidence.[55] However, the causality relationship between CAC and OPG could not be confirmed by these cross-sectional studies, and the strong association between OPG and CAC among patients with type 2 diabetes should be further evaluated in prospective cohort studies and clinical trials with larger sample sizes. The characteristics of the included studies are presented in the Table 1.

Table 1: Characteristics of the studies evaluating the association between bone turnover markers and CAC

Author (year)	Country	Age (year)	Population	Study design	Follow-up	Marker	Main finding	Reference number
Anand (2006)	UK	52.7	T2DM free of symptoms of cardiovascular disease	Prospective cohort, n=510	18±5 months	OPG	OPG levels retained a strong association with elevated CAC scores.	[9]
Berezin (2013)	Ukraine	58.34±9.60	Subjects with documented asymptomatic CAD	Prospective cohort, n=126	-	OPG OPN	OPG and OPN independently associated with coronary artery calcification.	[59]
Ishiyama (2009)	Japan	60.85	T2DM	Cross-sectional, n=168	-	OPG OPN	OPG was not a significant independent determinant of CAC. OPN was a significant independent determinant of CAC.	[55]
Jung (2009)	Korea	57.2±11.2	T2DM	Cross-sectional, n=110	-	OPG	CAC and OPG were significantly correlated with each other.	[52]
Maser (2015)	USA	62.5	T2DM	Cross-sectional, n=50	-	OPG OCN ucOCN	OPG is a useful serum biomarker for identifying those at increased risk of arterial calcification. OCN and ucOCN were not significant marker for CAC.	[53]
Pesaro (2018)	Brazil	57.95	Asymptomatic subjects	Cross-sectional, n= 130	-	OPG MGP RANKL Fetuin-A	MGP and RANKL were associated with CAC. No association was found between OPG or fetuin-A and CAC burden.	[58]
Diederichsen (2017)	Denmark	55.39±5.01	Asymptomatic subjects	Prospective cohort, n= 1006	5 years	OPG	OPG has no predictive value for CAC among asymptomatic subjects.	[57]
Mohammadpour (2012)	Iran	56.52±11.05	CAD	Cross-sectional, n=50	-	OPG RANKL	OPG level and RANKL/OPG ratio was correlated with CAC, but RANKL was not a significant marker.	[22]
Lieb (2010)	USA	61	Free of CVD	Prospective cohort (Framingham Study), n=3250	4.6 years	OPG RANKL	Prevalence of CAC increased non significantly with RANKL quartiles. OPG quartiles showed a weak association with CAC prevalence.	[54]

Table 1: Continued

Author (year)	Country	Age (year)	Population	Study design	Follow-up	Marker	Main finding	Reference number
Ix (2012)	USA	62±10	Free of CVD	Cohort [Multi-ethnic study of atherosclerosis (MESA)], n= 2457	3.2 years	Fetuin-A	Fetuin-A is inversely associated with CAC severity, while no associations were found between fetuin-A and CAC incidence or progression.	[29]
Ix (2011)	USA	70±11	Free of CVD	Cohort, n= 1375	4.6 years	Fetuin-A	Fetuin-A was associated with CAC severity.	[12]
Abedin (2007)	USA	44.75	Free of CVD	Prospective Cohort (Dallas study) , n= 3386	2 years	OPG	OPG is independently associated with CAC.	[56]
Esteghamat (2014)	Iran	56.8±11.2	CAD	Cross-sectional, n=155	-	OPG	Strong and independent association between CCS and OPG.	[60]
Nazemi (2018)	Iran	57.2±10.2	CAD	Cross-sectional, n= 83	-	BMP-2 BMP-7	BMP-2, BMP-7 were significantly associated with CAC.	[10]
Panh (2017)	France	60.9±10.8	Free of CVD	Cross-sectional, n=500	-	ALP OCN ucOCN	ALP was a significant marker for CAC but OCN and ucOCN were not associated with CAC.	[65]
Kiselova-Kaneva (2020)	Bulgaristan	62.12±12.00	CVD	Cross-sectional, n=99	-	ucMGP	CAC and ucMGP was not significantly associated with each other.	[68]
UZ (2009)	Turkey	49.5±10.9	Suspected CAD patients	Cross-sectional, n=64	-	OPN Fetuin-A	OPN was significantly correlated with CAC, but fetuin-A was not associated with CAC.	[14]
Mohammadpour (2018)	Iran	57.13±10.7	CAD	Cross-sectional, n=83	-	ENPP1	ENPP1 was significantly associated with CAC.	[71]
Kuipers (2015)	Trinidad and Tobago	62.9±8	Afro-Caribbean men	Case-control, n=191	-	Sclerostin	Sclerostin was a significant associated factor for CAC.	[72]
Nazemi (2018)	Iran	57.2±10.2	CAD	Cross-sectional, n=84	-	Decorin	No significant association between CAC and decorin was observed.	[70]

Table 1: Continued

Author (year)	Country	Age (year)	Population	Study design	Follow-up	Marker	Main finding	Reference number
Mori (2010)	Japan	62.4±10.4	Recently underwent coronary angiography	Cross-sectional, n=92	-	Fetuin-A	The correlation between CAC and Fetuin-A was indicated significant.	[61]
Okura (2010)	Japan	61	Essential hypertension	Cross-sectional, n=92	-	ucOCN	ucOCN and CAC were independently associated with each other.	[67]
Cho (2015)	Korea	51.8±8.2	Asymptomic patients	Cross-sectional, n=162	-	OCN ucOCN	ucOCN was associated with CAC in men.	[66]
Jono (2004)	Japan	64±11	Suspected CAD patients	Cross-sectional, n=115	-	MGP	MGP was an associated marker for CAC severity.	[7]
Krajnc (2019)	Slovenia	59±8	T2M	Cohort, n=45	18 months	Fetuin-A	CAC progression was independently associated with Fetuin-A.	[62]

T2DM: Type 2 diabetes, CAC: Coronary artery calcification, CVD: Cardiovascular disease, CCS: Coronary calcium score, CAD: Coronary artery disease, OPG: Osteoprotegrin, MGP: Matrix gla protein, ucMGP: Undercarboxylated MGP, OPN: Osteopontin, OCN: Osteocalcin, ucOCN: Undercarboxylated osteocalcin, BMP: Bone morphogenetic protein, ALP: Alkaline phosphatase, RANKL: Receptor-activator of NF-kB ligand

3.2. OPG and CAC risk in asymptomatic patients

The association between CAC and plasma OPG was evaluated in a Dallas heart study among 3,386 participants, and it was demonstrated that elevated OPG levels independently enhanced the risk of CAC prevalence by 39%.[56] In addition, multivariate analysis by a prospective cohort study in a population of 3250 asymptotic subjects indicated that OPG quartiles could weakly increase the prevalence of CAC and suggested serum OPG concentration as a predictive marker for CVD and mortality in the clinical health care system.[54] In contrast, the incidence odds ratio for coronary artery calcium score at baseline and follow-up was evaluated using the OPG level in 1006 individuals and revealed that OPG was an insignificant factor for CAC incidence.[57] Likewise, in a cross-sectional study with a population of 130 subjects, no significant association was found between OPG and CAC among asymptomatic individuals.[58] Overall, OPG might be a potential predictor of the risk of CAC, thereby identifying subclinical CVD burden.

3.3. Association between OPG and CAC risk in patients with CAD

As observed in studies on patients with CAD, a positive significant association was demonstrated between OPG and the incidence of CAC. The cohort study involving 126 patients with CAD indicated a positive correlation between OPG and CAC, in which elevated OPG levels could enhance the prevalence of CAC by 45%.[59] The study by Esteghamat et al.[60] recruited 155 subjects and demonstrated that OPG could be an independent detector of CVD mortality by predicting CAC prevalence. On the other hand, a study by Mohammadpour et al.[22] involving 50 patients with ischemic coronary disease reported a significant negative association between serum OPG and total CAC (P = 0.03, Correlation coefficient=−0.468), suggesting a protective effect of OPG on vascular calcification. They claimed that although OPG could not be a diagnostic marker for CAC incidence, the RANKL: OPG ratio might be a diagnostic marker for evaluating the risk of CAC in patients with a previous history of CAD.[22] Owing to limited and controversial findings, the ability of OPG to predict coronary artery calcium scores in CVD patients has yet to be established.

4. Association between RANKL and the Risk of CAC

Several studies have investigated the correlation between RANKL and CAC and have indicated that RANKL cannot be recognized as a reliable bone turnover marker for identifying the risk of CAC. As observed in a study by Lieb et al.[54] with a population of 3250 individuals having no symptoms of CVD at baseline, no significant correlation was confirmed between the prevalence of CAC and the RANKL quartiles after 4.6 years of follow-up. Furthermore, a cross-sectional study in 2012 found no significant association between CAC and RANKL in patients with ischemic heart disease.[22] In contrast, in a clinical study by Pesaro et al.[13], investigating osteogenic proteins among 170 participants without known CAD, RANKL was recognized as a positive significant factor for the detection of CAC after

fully adjusting for potential CVD confounders. Thus, a high level of RANKL increased the risk of CAC [OR=1.75 (1.04; 2.94), P = 0.03] and suggested RANKL as a novel marker for identifying subclinical CVD burden among individuals. An additional analysis conducted on 70 participants (40 acute MI patients and 30 controls) revealed that RANKL concentration was not significantly correlated with MI or 1-2 months post-MI.[13] Overall, the results of the studies identifying the association of RANKL or RANKL: OPG ratio with CAC were inconsistent and inclusive. Hence, conducting additional clinical investigations can benefit the existing gap and provide robust evidence.

5. Fetuin A Level and Elevated Incidence of CAC

The hypothesized relationship between fetuin-A and the severity of CAC and CVD burden has been widely investigated in clinical settings. Accordingly, a prospective cohort study was conducted on 1375 participants without known prevalence of clinical CVD, and the protective effect of fetuin-A on CAC was determined. Based on our results, 31% of the risk of CAC severity was reduced by high levels of circulating fetuin-A.[12] Furthermore, a study by Mori et al.[61] confirmed the protective effect of fetuin-A on CAC by reducing the incidence of CAC by up to 46% in patients who recently underwent coronary angiography. In addition, a significant negative association between fetuin-A and relative CAC progression was also verified in type 2 diabetic patients comparing baseline and 18-month follow-up (coefficient for the relative change in calcium score=-0.345, P = 0.02).[62] As demonstrated in a cross-sectional study of 88 non-dialyzed individuals with diabetic nephropathy, enhanced prevalence and severity of CAC were observed among individuals with diabetic nephropathy compared with diabetic controls. Hence, there was a direct, potent association between fetuin-A levels and the CAC score (r=0.22, P = 0.038) which is dependent on the status of nephropathy.[63] In line with these studies, inversely independent associations were identified between plasma fetal fetuin-A concentrations and CAC severity. For each SD higher fetuin-A level, there was a 12% decline in CAC severity in fully adjusted models for conventional risk factors of CVD in addition to kidney function and lifestyle variables. However, no association was found between fetuin-A and the incidence or progression of CAC.[29] Despite the observed significant associations between fetuin-A and CAC in previously described studies, a clinical study with 64 patients with suspected CVD indicated no significant correlation between fetuin-A and CAC incidence (r=0.17, P = 0.22).[14] The result of comparing the baseline data and a 7-year follow-up indicated that serum fetuin-A concentrations were not associated with the risk of cardiovascular events among 2647 individuals recruited from the multi-ethnic study of atherosclerosis cohort [HR=1.01 (0.84; 1.23)].[64] Additionally, in a study by Pesaro et al.[58], an association between fetuin-A and abnormal coronary artery calcium score was not found in both univariate and multivariate analyses.

6. OCN Connection to CAC Development

The possible association between carboxylated OCN (ucOCN) and the incidence of CAC remains controversial. However, total OCN did not significantly predict CAC development among the evaluated studies. As observed in a cross-sectional study recruiting 50 patients with type 2 diabetes, no significant correlation was reported between CAC and OCN or ucOCN by logistic regression analysis.[53] Moreover, the study by Panh et al.[65] among 500 asymptomatic participants indicated the same results: neither OCN nor ucOCN was not associated with abnormal coronary artery calcium score. However, ucOCN was a significant indicator of CAC prevalence in two cross-sectional studies. By investigating the relationship between CAC incidence and ucOCN levels among 162 asymptomatic healthy men, 29% of CAC prevalence was associated with ucOCN.[66] Likewise, in a population with essential hypertension, ucOCN was responsible for 18% of CAC incidence, demonstrating that ucOCN is a potential marker for predicting CVD outcomes.[67]

7. MGP and Risk of CAC Incidence

Few studies have investigated the association between MGP and carboxylated MGP and CAC development among patients diagnosed with CVD and those without known CVD. Accordingly, the multivariate analysis for MGP in 170 patients indicated that MGP could increase the risk of CAC by more than triple times (OR= 3.12 (1.20-8.11), P = 0.02).[13] In parallel, the presence of MGP among 115 patients with suspected coronary artery disease decreased the severity of CAC ($P < 0.001$), suggesting a contributing role of MGP in vascular calcification and reflecting subclinical CVD diagnosis in clinical settings.[7] However, an increasing trend of ucMGP was observed with a high score of coronary artery calcium among 99 patients with CVD, which was not statistically significant in this cross-sectional study.[68] The causality relationship between MGP and CAC could not be addressed by these cross-sectional studies and should be investigated by high-quality prospective cohorts and clinical trials.

8. The Relationship between OPN and CAC Development

OPN reflected a possible association with identifying CAC burden and providing an opportunity for early diagnosis of CVD incidence among patients. By comparing the data between baseline and follow-up using Cox regression analysis, we found that an elevated level of OPN can increase the risk of CAC by 14% among 126 individuals with a history of CAD.[69] Likewise, a cross-sectional study that recruited 64 patients with CAD supported the positive association of OPN with CAC incidence.[14] Moreover, using linear regression analyses, the relationship

between OPN and CAC was evaluated in patients with type 2 diabetes, indicating that OPN was a significant determinant of coronary artery calcium score and CVD burden ($P < 0.0001$).[55]

9. Association between BMP Levels and Abnormal Coronary Artery Calcium Score

Because limited studies have evaluated the association between BMPs and CAC, we obtained relevant cross-sectional literature. This pilot study included 83 patients with CAD aged >40 years. The plasma concentrations of BMP-2, BMP-7, and the Agatston score were measured, and linear regression analysis demonstrated a positive correlation of BMP-2 and BMP-7 with total CAC ($P < 0.001$).[10] However, this association should be verified by additional cohort studies that recruit a large number of individuals.

10. ALP and CAC risk

As observed in a cross-sectional study that evaluated the tertiles of ALP and calcium scores among 500 individuals without any signs of CAD, high ALP levels could be considered a potential risk factor for coronary calcification [OR=3.84 (2.01-7.54), = 0.001]. In order to identify the risk of high coronary artery calcium scores among patients under statin therapy and without a statin, logistic regression analysis showed that ALP was significantly associated with abnormal coronary artery calcium scores among patients not administering lipid-lowering therapy, whereas the association was not significant in the statin treatment group.[65] Due to limited data identifying the role of ALP in determining the patients exposed to high risk of CAC, further studies are needed to confirm the association.

11. Association between Other Bone Markers and CAC Risk

The prognostic role of novel bone turnover markers, including decorin, ENPP1, sclerostin, and Gremlin-1 were also investigated in determining the risk of CAC. Circulating decorin concentrations were investigated in our laboratory after recruiting 84 patients with CAD. The results of the study showed no significant correlation between serum decorin levels and the Agatston score (r=-0.121, $P = 0.28$).[70] Recently, in our previous study, the association between ENPP1 and CAC was assessed among 83 patients diagnosed with CAD (80.7% non-diabetic patients), and a negative correlation was found between ENPP1 and the prevalence of total CAC. Our findings indicated that serum ENPP1 concentrations could be useful markers for identifying subclinical CVD burden in patients without diabetes.[71] Multivariate analysis showed that elevated serum sclerostin levels could increase the risk of CAC by 76% in 191 Afro-Caribbean men.[72] Moreover, the contribution role of Gremlin-1 as an extracellular antagonist of BMPs was demonstrated by our previously described study. The high concentrations of serum Gremlin-1 may consider a predictor of decreased risk of CAC. Further studies with larger populations are necessary to verify this association.[73]

CONCLUSION

Because CAC measurement is rather expensive and patients are exposed to radiation, applying the prognostic role of bone turnover markers in predicting elevated coronary artery calcium score could improve treatment adherence and reduce the rate of CVD mortality. Accumulating evidence supports the role of bone marker-mediated pathways in the progression of CAC, which facilitates early diagnosis of CVD complications and the establishment of innovative targets for pharmacological therapy. Indeed, miRNAs and lncRNAs, as novel therapeutic interventions, can be a research priority in regulating bone metabolism at the gene expression level to attenuate high CAC and improve CVD outcomes.

Ethics

Authorship Contributions

Concept: A.H.M., A.H.M., N.O., Design: D.W., A.I.M., N.O., Data Collection or Processing: S.S., F.V., N.S., D.W., A.I.M., Analysis or Interpretation: S.S., F.V., N.S., D.W., A.I.M., A.H.M., N.O., Literature Search: S.S., F.V., N.S., Writing: S.S., F.V., A.H.M., N.O.

Footnotes

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

REFERENCES

1. Tajani A, Sadeghi M, Omidkhoda N, Mohammadpour AH, Samadi S, Jomehzadeh V. The association between C-reactive protein and coronary artery calcification: a systematic review and meta-analysis. BMC Cardiovasc Disord. 2024;24:204.
2. Diederichsen SZ, Grønhøj MH, Mickley H, Gerke O, Steffensen FH, Lambrechtsen J, et al. CT-Detected Growth of Coronary Artery Calcification in Asymptomatic Middle-Aged Subjects and Association With 15 Biomarkers. JACC Cardiovasc Imaging. 2017;10:858-66. Erratum in: JACC Cardiovasc Imaging. 2017;10:1088-9.
3. Abedi F, Sadeghi M, Omidkhoda N, Kelesidis T, Ramezani J, Samadi S, et al. HDL-cholesterol concentration and its association with coronary artery calcification: a systematic review and meta-analysis. Lipids Health Dis. 2023;22:60.
4. Mehta A, Pandey A, Ayers CR, Khera A, Sperling LS, Szklo MS, et al. Predictive Value of Coronary Artery Calcium Score Categories for Coronary Events Versus Strokes: Impact of Sex and Race: MESA and DHS. Circ Cardiovasc Imaging. 2020;13:e010153. Erratum in: Circ Cardiovasc Imaging. 2021;14:e000072.
5. Budoff MJ, Young R, Burke G, Jeffrey Carr J, Detrano RC, Folsom AR, et al. Ten-year association of coronary artery calcium with atherosclerotic cardiovascular disease (ASCVD) events: the multi-ethnic study of atherosclerosis (MESA). Eur Heart J. 2018;39:2401-8.

6. Vazirian F, Sadeghi M, Wang D, Javidi Dashtbayaz R, Gholoobi A, Samadi S, et al. Correlation between osteoprotegerin and coronary artery calcification in diabetic subjects: a systematic review of observational studies. BMC Cardiovasc Disord. 2023;23:96.
7. Jono S, Ikari Y, Vermeer C, Dissel P, Hasegawa K, Shioi A, et al. Matrix Gla protein is associated with coronary artery calcification as assessed by electron-beam computed tomography. Thromb Haemost. 2004;91:790-4.
8. Salari P, Keshtkar A, Shirani S, Mounesan L. Coronary Artery Calcium Score and Bone Metabolism: A Pilot Study in Postmenopausal Women. J Bone Metab. 2017;24:15-21.
9. Anand DV, Lahiri A, Lim E, Hopkins D, Corder R. The relationship between plasma osteoprotegerin levels and coronary artery calcification in uncomplicated type 2 diabetic subjects. J Am Coll Cardiol. 2006;47:1850-7.
10. Nazemi S, Rezapour S, Moallem SMH, Afshar M, Elyasi S, Hashemi E, et al. BMP-2 and BMP-7 be biomarkers of coronary artery disease? A pilot clinical study. Artery Research. 2018;23:14-9.
11. Panh L, Ruidavets JB, Rousseau H, Petermann A, Bongard V, Bérard E, et al. Association between serum alkaline phosphatase and coronary artery calcification in a sample of primary cardiovascular prevention patients. Atherosclerosis. 2017;260:81-6.
12. Ix JH, Barrett-Connor E, Wassel CL, Cummins K, Bergstrom J, Daniels LB, et al. The associations of fetuin-A with subclinical cardiovascular disease in community-dwelling persons: the Rancho Bernardo Study. J Am Coll Cardiol. 2011;58:2372-9.
13. Pesaro AE, Katz M, Liberman M, Pereira C, Mangueira CLP, de Carvalho AEZ, et al. Circulating osteogenic proteins are associated with coronary artery calcification and increase after myocardial infarction. PLoS One. 2018;13:e0202738.
14. Uz O, Kardeşoğlu E, Yiğiner O, Baş S, Ipçioğlu OM, Ozmen N, et al. The relationship between coronary calcification and the metabolic markers of osteopontin, fetuin-A, and visfatin. Turk Kardiyol Dern Ars. 2009;37:397-402.
15. Zhelyazkova-Savova MD, Yotov YT, Nikolova MN, Nazifova-Tasinova NF, Vankova DG, Atanasov AA, et al. Statins, vascular calcification, and vitamin K-dependent proteins: Is there a relation? Kaohsiung J Med Sci. 2021;37:624-31.
16. Bakhireva LN, Laughlin GA, Bettencourt R, Barrett-Connor E. Does osteoprotegerin or receptor activator of nuclear factor-kappaB ligand mediate the association between bone and coronary artery calcification? J Clin Endocrinol Metab. 2008;93:2009-12.
17. Kiechl S, Werner P, Knoflach M, Furtner M, Willeit J, Schett G. The osteoprotegerin/RANK/RANKL system: a bone key to vascular disease. Expert Rev Cardiovasc Ther. 2006;4:801-11.
18. Sandra F, Hendarmin L, Nakamura S. Osteoprotegerin (OPG) binds with tumor necrosis factor-related apoptosis-inducing ligand (TRAIL): suppression of TRAIL-induced apoptosis in ameloblastomas. Oral Oncol. 2006;42:415-20.
19. Lacey DL, Timms E, Tan HL, Kelley MJ, Dunstan CR, Burgess T, et al. Osteoprotegerin ligand is a cytokine that regulates osteoclast differentiation and activation. Cell. 1998;93:165-76.
20. Soysa NS, Alles N. NF-κB functions in osteoclasts. Biochemical and Biophysical Research Communications, 2009;378:1-5.
21. Bucay N, Sarosi I, Dunstan CR, Morony S, Tarpley J, Capparelli C, et al. osteoprotegerin-deficient mice develop early onset osteoporosis and arterial calcification. Genes Dev. 1998;12:1260-8.
22. Mohammadpour AH, Shamsara J, Nazemi S, Ghadirzadeh S, Shahsavand S, Ramezani M. Evaluation of RANKL/OPG Serum Concentration Ratio as a New Biomarker for Coronary Artery Calcification: A Pilot Study. Thrombosis. 2012;2012:306263.
23. Termine JD. Non-collagen proteins in bone. Ciba Found Symp. 1988;136:178-202.
24. Brylka L, Jahnen-Dechent W. The role of fetuin-A in physiological and pathological mineralization. Calcif Tissue Int. 2013;93:355-64.
25. Li W, Zhu S, Li J, Huang Y, Zhou R, Fan X, et al. A hepatic protein, fetuin-A, occupies a protective role in lethal systemic inflammation. PLoS One. 2011;6:e16945.
26. Kumbla L, Cayatte AJ, Subbiah MT. Association of a lipoprotein-like particle with bovine fetuin. FASEB J. 1989;3:2075-80.
27. Demetriou M, Binkert C, Sukhu B, Tenenbaum HC, Dennis JW. Fetuin/alpha2-HS glycoprotein is a transforming growth factor-beta type II receptor mimic and cytokine antagonist. J Biol Chem. 1996;271:12755-61.
28. Ketteler M, Bongartz P, Westenfeld R, Wildberger JE, Mahnken AH, Böhm R, et al. Association of low fetuin-A (AHSG) concentrations in serum with cardiovascular mortality in patients on dialysis: a cross-sectional study. Lancet. 2003;361:827-33.
29. Ix JH, Katz R, de Boer IH, Kestenbaum BR, Peralta CA, Jenny NS, et al. Fetuin-A is inversely associated with coronary artery calcification in community-living persons: the Multi-Ethnic Study of Atherosclerosis. Clin Chem. 2012;58:887-95.
30. Zhang H, Wang LJ, Si DL, Wang C, Yang JC, Jiang P, et al. Correlation between osteocalcin-positive endothelial progenitor cells and spotty calcification in patients with coronary artery disease. Clin Exp Pharmacol Physiol. 2015;42:734-9.
31. Speer MY, Yang HY, Brabb T, Leaf E, Look A, Lin WL, et al. Smooth muscle cells give rise to osteochondrogenic precursors and chondrocytes in calcifying arteries. Circ Res. 2009;104:733-41.
32. Price PA, Faus SA, Williamson MK. Warfarin causes rapid calcification of the elastic lamellae in rat arteries and heart valves. Arterioscler Thromb Vasc Biol. 1998;18:1400-7.
33. Sweatt A, Sane DC, Hutson SM, Wallin R. Matrix Gla protein (MGP) and bone morphogenetic protein-2 in aortic calcified lesions of aging rats. J Thromb Haemost. 2003;1:178-85.
34. Rahman MS, Akhtar N, Jamil HM, Banik RS, Asaduzzaman SM. TGF-β/BMP signaling and other molecular events: regulation of osteoblastogenesis and bone formation. Bone Res. 2015;3:15005.
35. Li X, Yang HY, Giachelli CM. BMP-2 promotes phosphate uptake, phenotypic modulation, and calcification of human vascular smooth muscle cells. Atherosclerosis. 2008;199:271-7.
36. Denhardt DT, Noda M. Osteopontin expression and function: role in bone remodeling. J Cell Biochem Suppl. 1998;30-31:92-102.
37. Steitz SA, Speer MY, McKee MD, Liaw L, Almeida M, Yang H, *et al.* Osteopontin inhibits mineral deposition and promotes regression of ectopic calcification. Am J Pathol. 2002;161:2035-46.
38. Millán JL. Alkaline Phosphatases: Structure, substrate specificity and functional relatedness to other members of a large superfamily of enzymes. Purinergic Signal. 2006;2:335-41.
39. Villa-Bellosta R. Synthesis of extracellular pyrophosphate increases in vascular smooth muscle cells during phosphate-induced calcification. Arterioscler Thromb Vasc Biol. 2018;38:2137-47.
40. Azpiazu D, Gonzalo S, Villa-Bellosta R. Tissue non-specific alkaline phosphatase and vascular calcification: a potential therapeutic target. Curr Cardiol Rev. 2019;15:91-95.
41. Klimczak D, Pączek L, Jażdżewski K, Kuch M. MicroRNAs: powerful regulators and potential diagnostic tools in cardiovascular disease. Kardiol Pol. 2015;73:1-6.
42. Lim YH, Ryu J, Kook H, Kim YK. Identification of long noncoding rnas involved in differentiation and survival of vascular smooth muscle cells. Mol Ther Nucleic Acids. 2020;22:209-21.

43. He L, Xu J, Bai Y, Zhang H, Zhou W, Cheng M, *et al.* MicroRNA-103a regulates the calcification of vascular smooth muscle cells by targeting runt-related transcription factor 2 in high phosphorus conditions. Exp Ther Med. 2021;22:1036.

44. Liu J, Xiao X, Shen Y, Chen L, Xu C, Zhao H, *et al.* MicroRNA-32 promotes calcification in vascular smooth muscle cells: Implications as a novel marker for coronary artery calcification. PLoS One. 2017;20;12:e0174138.

45. Han Y, Zhang J, Huang S, Cheng N, Zhang C, Li Y, *et al.* MicroRNA-223-3p inhibits vascular calcification and the osteogenic switch of vascular smooth muscle cells. J Biol Chem. 2021;296:100483.

46. Wicik Z, Jales Neto LH, Guzman LEF, Pavão R, Takayama L, Caparbo VF, *et al*. The crosstalk between bone metabolism, lncRNAs, microRNAs and mRNAs in coronary artery calcification. Genomics. 2021;113:503-13.

47. Mak S, Sun H, Acevedo F, Shimmin LC, Zhao L, Teng BB, *et al*. Differential expression of genes in the calcium-signaling pathway underlies lesion development in the LDb mouse model of atherosclerosis. Atherosclerosis. 2010;213:40-51.

48. Trump BF, Berezesky IK. Calcium-mediated cell injury and cell death. FASEB J. 1995;9:219-28.

49. Gattineni J, Alphonse P, Zhang Q, Mathews N, Bates CM, Baum M. Regulation of renal phosphate transport by FGF23 is mediated by FGFR1 and FGFR4. Am J Physiol Renal Physiol. 2014;306:F351-8.

50. Elsherif L, Huang MS, Shai SY, Yang Y, Li RY, Chun J, *et al.* Combined deficiency of dystrophin and beta1 integrin in the cardiac myocyte causes myocardial dysfunction, fibrosis and calcification. Circ Res. 2008;102:1109-17.

51. Talukdar HA, Foroughi Asl H, Jain RK, Ermel R, Ruusalepp A, Franzén O, *et al*. Cross-Tissue Regulatory Gene Networks in Coronary Artery Disease. Cell Syst. 2016;23;2:196-208.

52. Jung CH, Lee WY, Kim SY, Jung JH, Rhee EJ, Park CY, *et al.* The relationship between coronary artery calcification score, plasma osteoprotegerin level and arterial stiffness in asymptomatic type 2 DM. Acta Diabetol. 2010;47 Suppl 1:145-52.

53. Maser RE, Lenhard MJ, Sneider MB, Pohlig RT. Osteoprotegerin is a better serum biomarker of coronary artery calcification than osteocalcin in type 2 diabetes. Endocr Pract. 2015;21:14-22.

54. Lieb W, Gona P, Larson MG, Massaro JM, Lipinska I, Keaney JF Jr, *et al.* Biomarkers of the osteoprotegerin pathway: clinical correlates, subclinical disease, incident cardiovascular disease, and mortality. Arterioscler Thromb Vasc Biol. 2010;30:1849-54.

55. Ishiyama M, Suzuki E, Katsuda J, Murase H, Tajima Y, Horikawa Y, *et al*. Associations of coronary artery calcification and carotid intima-media thickness with plasma concentrations of vascular calcification inhibitors in type 2 diabetic patients. Diabetes Res Clin Pract. 2009 Aug;85(2):189-96.

56. Abedin M, Omland T, Ueland T, Khera A, Aukrust P, Murphy SA, *et al.* Relation of osteoprotegerin to coronary calcium and aortic plaque (from the Dallas Heart Study). Am J Cardiol. 2007;15;99:513-8.

57. Diederichsen SZ, Grønhøj MH, Mickley H, Gerke O, Steffensen FH, Lambrechtsen J, *et al.* CT-detected growth of coronary artery calcification in asymptomatic middle-aged subjects and association with 15 biomarkers. JACC Cardiovasc Imaging. 2017;10:858-866.

58. Nakahara T, Dweck M, Narula N, *et al*. Coronary artery calcification: from mechanism to molecular imaging. J Am Coll Cardiol Img. 2017;10:582-93.

59. Bjerre M. Osteoprotegerin (OPG) as a biomarker for diabetic cardiovascular complications. SpringerPlus. 2013;2:658.

60. Esteghamati A, Sheikhbahaei S, Hafezi-Nejad N, Mousavizadeh M, Noshad S, Gilani Larimi N, *et al*. Serum osteoprotegerin in relation to metabolic status, severity, and estimated risk of subsequent coronary heart disease. Arch Iran Med. 2014;17:596-601.

61. Mori K, Ikari Y, Jono S, Emoto M, Shioi A, Koyama H, *et al.* Fetuin-A is associated with calcified coronary artery disease. Coron Artery Dis. 2010;21:281-5.

62. Krajnc M, Pečovnik Balon B, Krajnc I. Non-traditional risk factors for coronary calcification and its progression in patients with type 2 diabetes: The impact of postprandial glycemia and fetuin-A. J Int Med Res. 2019;47:846-58.

63. Mehrotra R, Westenfeld R, Christenson P, Budoff M, Ipp E, Takasu J, *et al.* Serum fetuin-A in nondialyzed patients with diabetic nephropathy: relationship with coronary artery calcification. Kidney Int. 2005;67:1070-7.

64. Aroner SA, St-Jules DE, Mukamal KJ, Katz R, Shlipak MG, Criqui MH, *et al.* Fetuin-A, glycemic status, and risk of cardiovascular disease: The Multi-Ethnic Study of Atherosclerosis. Atherosclerosis. 2016;248:224-9.

65. Panh L, Ruidavets JB, Rousseau H, Petermann A, Bongard V, Bérard E, *et al.* Association between serum alkaline phosphatase and coronary artery calcification in a sample of primary cardiovascular prevention patients. Atherosclerosis. 2017;260:81-86.

66. Cho E. Association between osteocalcin and coronary calcium score in Korean adults. Osteoporosis International. 2016;27:S710-1.

67. Okura T, Kurata M, Enomoto D, Jotoku M, Nagao T, Desilva VR, *et al.* Undercarboxylated osteocalcin is a biomarker of carotid calcification in patients with essential hypertension. Kidney Blood Press Res. 2010;33:66-71.

68. Kiselova-Kaneva DY, Nazifova-Tasinova N, Vankova D, Nikolova M, Pasheva M, *et al.* Matrix Gla-protein expression in peripheral blood mononuclear cells is related to risk factors in cardiovascular diseased patients. Turkish Journal of Biochemistry. 2022;47:247-55.

69. Berezin A. and Kremzer A. Predict value of circulating bone-related glycopeptide osteoprotegerin in asymptomatic coronary artery disease patients with second type diabetes mellitus. European Heart Journal. 2013;34:P5515.

70. Nazemi S, Rezapour A, Moallem SMH, Afshar M, Elyasi S, Mashreghi Moghadam HR, *et al.* Could decorin be a biomarker of coronary artery disease? A pilot study in human beings. Acta Biomed. 2018;8:89:365-69.

71. Mohammadpour AH, Nazemi S, Mashhadi F, Rezapour A, Afshar M, Afzalnia S, *et al*. Evaluation of NPP1 as a Novel Biomarker of Coronary Artery Disease: A Pilot Study in Human Beings. Adv Pharm Bull. 2018;8:489-93.

72. Kuipers AL, Miljkovic I, Carr JJ, Terry JG, Nestlerode CS, Ge Y, *et al.* Association of circulating sclerostin with vascular calcification in Afro-Caribbean men. Atherosclerosis. 2015;239:218-23.

73. Rezapour A, Nazemi S, Mashhadi F, Moallem SMH, Afshar M, Elyasi S, *et al.* Evaluation of Coronary Artery Calcification and Gremlin-1 Serum Level Correlation in Patients with Chronic Ischemic Heart Disease. J Pharm Care. 2021;9:82-87.