ELSEVIER

Archives of Medical Research ■ (2015) ■

Archives of Medical Research

ORIGINAL ARTICLE

Serum Osteopontin Concentrations in Relation to Coronary Artery Disease

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Received for publication November 30, 2014; accepted February 18, 2015 (ARCMED-D-14-00704).

Background and Aims. Coronary artery disease (CAD) is a common form of vascular disease and is associated with high mortality and morbidity globally. It has been suggested that serum osteopontin (OPN) may be a useful biomarker of atherosclerosis and vascular calcification. The aim of this study was to assess the association between serum OPN levels and severity of CAD.

Methods. Three hundred and four subjects were studied, 111 with clinically significant angiographically defined CAD (CAD+) (>50% stenosis), 96 with negative angiography (CAD-) (<50% stenosis) and 97 healthy controls. Fasting blood samples were collected from all patients before coronary angiography and serum OPN levels were determined using ELISA.

Results. Serum concentrations of OPN were significantly higher in both CAD+ (72.99 [51.05−103.64]) and CAD- (11.11 [8.11−18.23]) ($p = 0.001$) groups compared with the control group (5.99 [4.26−7.91]) ($p = 0.001$). CAD+ subjects also had higher serum OPN levels compared with CAD-subjects ($p = 0.001$). However, OPN levels were comparable between subgroups of CAD+ subjects stratified according to the number of narrowed vessels in angiography.

Conclusions. The present results suggest a positive association between circulating OPN concentrations and the presence but not the extent of CAD. © 2015 IMSS. Published by Elsevier Inc.

Key Words: Coronary artery disease, Atherosclerosis, Osteopontin.

Introduction

According to updated statistics, cardiovascular disease is the first cause of death both in the U.S. (source: American Heart Association, 2013) and worldwide (data from the World Health Organization, 2013). Cardiovascular disorders represent the foremost cause of preventable death worldwide (1). Coronary artery disease (CAD) is the most prevalent form of cardiovascular disease with high mortality and morbidity rates. Calcification of the arterial wall has been shown to be associated with an elevated risk of cardiovascular events, although the causality of this association remains elusive (2,3). Osteopontin (OPN) is a glycoprotein secreted by macrophages, vascular smooth muscle cells, and endothelial cells and has been demonstrated to promote macrophage chemotaxis (4,5). Expression of OPN has been shown in the neointima of injured vessels, calcified atheromatous plaque (6,7), and macrophages at the site of inflammation where it is thought to mediate monocyte adhesion (8), migration (9) differentiation (10), and phagocytosis (11). Serum OPN has also been reported to be increased in patients with atherosclerosis, valvular stenosis and myocardial infarction (12−14). Circulating OPN levels are associated with increased aortic pulse

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0188-4409/$ - see front matter.
http://dx.doi.org/10.1016/j.arcmed.2015.02.005

wave velocity in patients with rheumatoid arthritis (15) and with increased intima-media thickness and mean systolic and diastolic flow velocities in patients with essential hypertension (16). It is known that oxidative stress plays an important role in the pathogenesis of atherosclerosis and that an association exists between OPN and atherosclerosis. Previous studies have shown an association between OPN and malondialdehyde (MDA) levels in patients with CAD, suggesting potential involvement of oxidative stress in the regulation of OPN expression (17). In light of the aforementioned observations, the present study aimed to assess the association between serum OPN concentrations and severity of CAD in a group of patients undergoing coronary angiography.

Methods

Study Population

The study was performed on a total of 304 patients. Of these (111 + 96), 207 underwent angiography. Of the 207 patients, 111 were CAD+ and 96 were CAD-. The remaining enrolled persons (n = 97) were healthy controls who did not undergo coronary angiography. The study subjects were selected from those subjects who underwent coronary angiography in the Ghaem Hospital (Mashhad, Iran). Written informed consent was obtained from all participants using protocols approved by the Ethics Committee of the Mashhad University of Medical Science and a standardized questionnaire was used to collect demographic information.

Coronary Angiography

Angiography was indicated principally for stable angina in patients who were positive for at least one objective test of myocardial ischemia including exercise stress test, dobutamine stress echocardiography, and thallium SPECT (single photon emission computed tomography). Exclusion criteria were as follows: oral contraceptives or hormone replacement therapy, pregnancy, prior history of coronary angioplasty or coronary artery bypass graft, and having overt clinical features of infection or chronic inflammatory disease. All subjects were negative for viral markers of hepatitis and anti-HIV antibody. Moreover, patients with myocardial infarction within the previous 3 months or with renal, hepatic or malignant diseases were excluded. Subjects who were candidates for emergency percutaneous coronary intervention were also excluded from the study. Coronary angiograms were performed using routine procedures. Analysis of the angiograms was performed offline by a specialist cardiologist. The presence of one or more stenoses ≥50% in diameter of at least one major coronary artery (left main, right coronary artery, left anterior descending, circumflex) was considered evidence of significant CAD (18). Patients with significant CAD were further stratified according to the number of narrowed vessels into those with one (SVD), two (2VD), or three-vessel disease (3VD) depending on the number of coronary arteries involved. A ≥50% narrowing of the left main coronary artery was considered as two-vessel disease. Eighty-three age- and sex-matched healthy volunteers were also recruited as a normal control group. These individuals had no personal or family history of cardiovascular disease or diabetes. Information on smoking, drug use and family history of CAD was obtained via a questionnaire. The study protocol was approved by the ethics committee of Mashhad University of Medical Sciences and written informed consent was obtained from each participant.

Laboratory Evaluation

Blood samples were collected from all patients before coronary angiography and after an overnight fast. Serum OPN levels were determined using a commercially available ELISA kit (D0ST00; R&D Systems, Italy) according to the manufacturer's instructions. Sensitivity of the assay method was 3.33 ng/mL with an intra- and inter-assay CV of <5 and <10%, respectively. OPN was measured with a sandwich enzyme-linked immunosorbent assay using a commercially available kit (D0ST00; R&D Systems). In brief, 1:2 diluted testing samples were incubated in the N-terminal OPN antibody pre-coated wells at 37°C for 1 h. Following washing, 100 μL of labeled OPN antibody solution was added to each well and incubated for 30 min at 4°C. After washing, tetramethylbenzidine was added and the absorbance at 450 nm was measured with an automatic ELISA reader (Bio-Rad, Segrate, Italy).

Statistical Analysis

Statistical analyses were performed using the SPSS software v.16.0 (Chicago, IL). Data were expressed as mean ± SD (for normally distributed variables) or median (interquartile range) (for non-normally distributed variables). Comparison of serum OPN levels among study groups was made using one-way ANOVA (for normally distributed data) or Kruskal-Wallis (for non-normally distributed data) tests. Pearson or Spearman correlation coefficients were used to determine the association between OPN levels and clinical and biochemical factors. The impact of confounding parameters including age, gender, smoking status, diabetes mellitus and BMI on the association between serum OPN levels and CAD was assessed using binary logistic regression. In all analyses, a two-sided p value of ≤0.05 was considered as statistically significant.

Results

There were a total of 304 participants, of which 111 were CAD+, 96 CAD-, and 97 apparently healthy control

subjects. CAD+ subjects were stratified according to the number of narrowed vessels into SVD ($n = 43$, 36.44%), 2VD ($n = 29$, 26.13%) and 3VD ($n = 46$, 41.44%).

Demographic Characteristics

BMI, waist circumference, and hip circumference in the control group were higher than for the CAD+ and CAD- groups ($p > 0.05$). Mean systolic blood pressure and fasting blood glucose (FBG) were higher in the CAD+ and CAD- groups compared with the control group ($p < 0.05$). The control group had higher mean diastolic blood pressure compared to the CAD− group ($p > 0.05$) and CAD+ group ($p > 0.05$). No significant difference in lipid profile (HDL-C, LDL-C, and triglycerides) was observed among the three groups ($p > 0.05$, Table 1), which may be partially because of statin treatment in both CAD+ and CAD- groups. In regard to the subgroups of CAD+ patients with different numbers of stenosed vessels (single-vessel disease [SVD], double-vessel disease [2VD], and triple-vessel disease [3VD]), no significant difference in demographic parameters was observed between different subgroups ($p > 0.05$, Table 2). Demographic characteristics of study subjects are summarized in Tables 1 and 2.

OPN Values Among Different Groups

Mean OPN levels were significantly higher in both CAD+ (72.99 [51.05−103.64]) and CAD- (11.11 [8.11−18.23]) groups compared with control group (5.99 [4.26−7.91]) ($p < 0.001$) (Figure 1). This association remained significant after adjustment for age, gender, BMI, smoking status and diabetes in the regression model ($p < 0.001$). Comparison of OPN levels between CAD+ and CAD- groups revealed a significant elevation in the former group ($p < 0.001$). However, serum OPN levels were comparable among SVD (72.99 [23.86−108.41]), 2VD (68.75 [55.35−102.10]) and 3VD (71.11 [43.75−87.90]) subgroups of CAD+ subjects ($p > 0.05$) (Figure 2). Binary logistic regression analysis did not indicate any association between serum OPN concentrations and severe CAD (defined as 3VD) ($p = 0.458$).

Associations Between Plasma OPN Levels and Coronary Risk Factors

In the CAD-group, serum OPN levels were higher in the hypertensive vs. non-hypertensive group ($p < 0.05$). In CAD+ group: serum OPN levels were higher in current smokers in comparison to non-smokers ($p < 0.05$). Plasma OPN levels were not associated with other risk factors including gender, dyslipidemia and anthropometric parameters ($p > 0.05$) (Table 3).

Associations Between Plasma OPN and Hs-CRP Concentrations

Bivariate association between plasma levels of OPN and hs-CRP was evaluated using Spearman's correlation coefficient. A significant correlation between these parameters

Table 1. Demographic and clinical characteristics of the study groups

	CAD+	CAD-	CAD-controls
Number	96	111	97
Gender (F/M)	45/50	67/46	23/74
Smoking or addiction (%)	36.4[a*]	38.3	19.2
Hypertension (%)	51.2	45.7	30.0
Hyperlipidemia (%)	43.1	26.8	22.2
TG (mg/dL)	146 (105−172.50)	119 (89−157.50)	121 (86.25−156)
Age (year)	54.93 ± 9.55	53.19 ± 11.52	54.03 ± 6.42
FBG (mg/dL)	106.68 ± 39.23[a*]	99.08 ± 36.61[a*]	88.24 ± 25.34
BMI (kg/m^2)	27.54 ± 6.63	25.84 ± 4.71	28.65 ± 4.53[a*]
WHR	0.93 ± 0.11	0.94 ± 0.13	0.93 ± 0.069
WC (cm)	91.81 ± 14.55	90.25 ± 13.38	96.96 ± 11.45[a*]
HC (cm)	98.27 ± 12.87	95.88 ± 10.85	103.48 ± 8.65[a*]
LDL-C (mg/dL)	94.72 ± 43.70	102.09 ± 81.11	119.99 ± 40.03
HDL-C (mg/dL)	43.31 ± 11.66	42.90 ± 10.92	50.66 ± 50.34
SBP (mm Hg)	144.37 ± 30.59[a*]	149.06 ± 26.09[a*]	123.27 ± 11.24
DBP (mm Hg)	77.03 ± 14.04[a*]	75.75 ± 15.50[a*]	78.27 ± 9.12
TC (mg/dL)	165.59 ± 61.14	164.96 ± 46.74	197.84 ± 39.54
WHR category (%)	93.87	94.81	97
hsCRP (mg/L)	3.99 (13.25−1.34)[a***]	2.86 (9.30−0.99)	2.07 (4.83−1.25)

FBG, fasting blood glucose; BMI, body mass index; WHR, waist-hip ratio; WC, waist circumference; HC, hip circumference; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides; SBP, systolic blood pressure; DBP, diastolic blood pressure; Cad+, CAD-, compared with the control group.
Values are presented as mean ± SD.
[a*]$p < 0.05$, [a**]$p < 0.01$, [a***]$p < 0.001$, CAD+ compared with the CAD-group.
[b*]$p < 0.05$, [b**]$p < 0.01$, [b***]$p < 0.001$.

Table 2. Demographic and clinical characteristics of different subgroups of CAD patients according to the number of stenosed vessels

SVD		2VD	3VD
Number	43	29	46
Gender (F/M)	22/21	18/11	18/28
Smoking (%)	38.10[a*]	37.94	34.78
Hypertension (%)	46.84	44.82	46.66
Hyperlipidemia (%)	28.20	35.71	28.88
Age (years)	72.32 ± 44.95	56.52 ± 9.39	56.24 ± 9.80
TG (mg/dL)	114 (83−175.5)	129 (88−167)	129 (103−187)
Waist/hip category (%)	89.47	100	95
Age risk (%)	53.48	79.31	69.56
FBG (mg/dL)	102.76 ± 31.04	97.87 ± 34.82	98.18 ± 29
BMI (kg/m^2)	28.43 ± 8.36	27.37 ± 5.53	27.23 ± 5.35
WHR	0.92 ± 0.14	0.92 ± 0.07	0.963 ± 0.96
WC (cm)	92 ± 17.32	89.20 ± 13.09	93 ± 13.22
HC (cm)	99.57 ± 10.62	96.40 ± 13.74	97.40 ± 13.96
LDL-C (mg/dL)	97.01 ± 33.99	100.77 ± 41.72	104.56 ± 42.10
HDL-C (mg/dL)	46.35 ± 12.27	42.26 ± 11.20	41.63 ± 11.15
SBP (mm Hg)	123.90 ± 41.40[a*]	143.04 ± 23.93[a*]	135.28 ± 22.96
DBP (mm Hg)	73.57 ± 13[a*]	80.89 ± 12.02[a*]	79.48 ± 11.04
hsCRP (mg/L)	3.99 (13.25−1.34)[b***]	2.86 (9.30−0.99)[b***]	2.07 (4.83−1.25)[a***]

FBS, fasting blood glucose; BMI body mass index; WHR, waist-hip ratio; WC, waist circumference; HC, hip circumference; LDL-C low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides; SBP, systolic blood pressure; DBP, diastolic blood pressure; CAD+, CAD-, compared with the control group.
Values are presented as mean ± SD.
[a*]$p < 0.05$, [a**]$p < 0.01$, [a***]$p < 0.001$, CAD+ compared with the CAD-group.
[b*]$p < 0.05$, [b**]$p < 0.01$, [b***]$p < 0.001$.

was found only in the CAD+ group ($r = 0.99$, $p = 0.001$) (Table 3).

Discussion

In this case-control study, mean plasma OPN levels were found to be significantly higher in subjects with angiographically defined CAD. Elevation of OPN levels in CAD-subjects implies an association between this protein and cardiovascular risk, because CAD-group consisted of subjects with degrees of coronary atherosclerosis who were indicated for angiography due to suspicious CAD. Our results did not show any difference in OPN levels among CAD+ subgroups with different number of narrowed vessels. This latter finding does not violate our main results on the association between OPN levels and coronary risk because number of narrowed vessels does not necessarily reflect the severity of mycocardial ischemia or extent of coronary atherosclerosis. Several reports illustrated that OPN was increased after vascular injury such as atherosclerosis and restenosis following angioplasty. The increase in

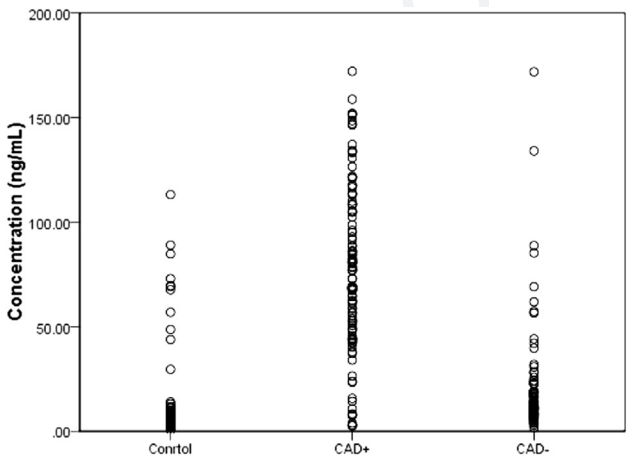

Figure 1. Comparison of osteopontin (OPN) levels among the study groups.

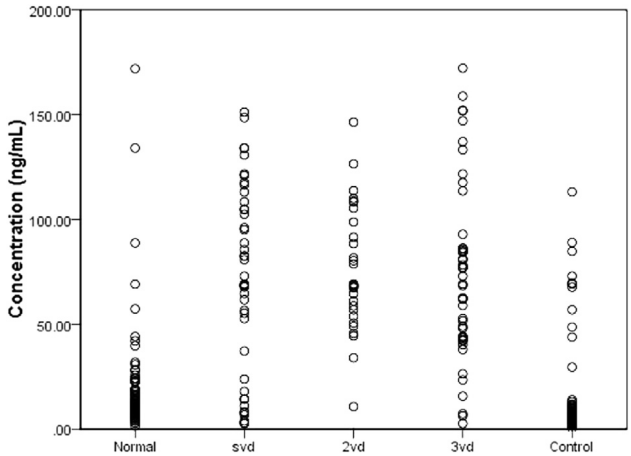

Figure 2. Comparison of OPN levels between subgroups of CAD+ patients with different number of stenosed vessels (single-vessel disease [SVD], double-vessel disease [2VD], and triple-vessel disease [3VD]).

Table 3. Evaluation of plasma OPN levels in relation to coronary risk factors

	CAD+	CAD-	Control
Gender			
Male	80.68 ± 39.01	15.80 ± 20.98	14.13 ± 22.90
Female	72.97 ± 39.74	23.00 ± 28.05	8.23 ± 13.84
p	0.299	0.097	0.221
Dyslipid			
Yes	81.39 ± 34.42	24.48 ± 25.07	22.69 ± 31.43
No	74.68 ± 42.48	19.07 ± 26.88	10.84 ± 18.28
p	0.494	0.455	0.358
Hypertension			
Yes	73.46 ± 38.45	22.32 ± 20.45	17.86 ± 26.59
No	80.04 ± 41.04	19.46 ± 29.34	12.14 ± 20.59
p	0.412	0.036	0.55
Smoking			
Yes	74.70 ± 42.34	22.32 ± 20.45	22.27 ± 28.15
No	77.92 ± 37.98	19.46 ± 29.34	9.63 ± 18.48
p	0.772	0.047	0.05
BMI			
<25	74.45 ± 43.10	20.30 ± 26.94	8.30 ± 12.75
25 < 25 <30	86.93 ± 35.02	21.78 ± 28.73	18.15 ± 28.16
>30	70.14 ± 38.88	18.18 ± 17.98	9.30 ± 13.92
p	1	1	1

OPN levels after percutaneous coronary interventions (PCI) suggests that vascular injury due to PCI is responsible for this phenomenon. Mazzone et al. reported an association between inflammatory status and accelerated atherosclerosis in patients with CAD undergoing PCI. The baseline and persistent rise of OPN is an expression of its contribution to the accelerated plaque advancement; hence, OPN may be a useful prognostic biomarker. OPN is an integrin-binding ligand, N-linked glycoprotein, which was identified as an important factor in the atherosclerotic inflammatory environment (19). Several reports have recognized OPN as a single peptide, and their results are mostly attributed to the full-length protein. As well, current evidence suggests that this view might be incomplete, ignoring the full extent of the bio-effects of the OPN family of proteins. Thrombin cleavage of OPN results in formation of OPN N-terminal fragment with a higher pro-inflammatory potential than the full length protein or the OPN C-terminal fragment (6,20,21). Higher plasma thrombin cleaved OPN was illustrated to associate with evidence of symptomatic cerebrovascular disease (22,23). Therefore, the OPN N-terminal fragment was measured, owing to its stronger association with increased carotid plaque inflammation (22). Chen et al. reported that plasma OPN levels are correlated with the severity of coronary artery lesions (24). In another study, Yan et al. reported that there is an independent association between plasma levels of OPN, but not thrombin-cleaved (N-half) OPN, and the presence and severity of nephropathy and CAD in diabetic subjects (25). Increased OPN levels have been previously reported in atherosclerotic vessels but not in normal vessels (26). These elevated concentrations of OPN can facilitate macrophage adhesion to endothelium via integrin receptors followed by subsequent penetration into subendothelial space and formation of foam cells. Also, there is evidence regarding induction of leukocyte chemotaxis, vascular smooth muscle cell proliferation and migration (27,28), whereas repression of nitric oxide production in macrophages and endothelial cells is shown by OPN (29). These findings suggest possible involvement of OPN in the pathophysiology of atherosclerosis. On the other hand, coronary calcification occurs in the majority of patients with CAD, and its presence is related to lesion vulnerability and the overall burden of atherosclerotic disease (30). Plasma OPN has been found to be positively correlated with coronary artery calcification in patients with stable angina (31) and diabetes (32). In the present study, the impact of traditional cardiovascular risk factors on serum levels of OPN was determined. It was found that in CAD-group, serum OPN levels were higher in the hypertensive vs. non-hypertensive group. Furthermore, serum OPN levels were higher in current smokers in comparison with non-smokers in the CAD+ group. Plasma OPN levels were not significantly associated with other risk factors in any of the study groups. Arnlov et al. investigated the relationship between cardiovascular risk factors and plasma OPN level. OPN level was not associated with blood pressure, the ratio of total to high-density lipoprotein cholesterol, smoking, diabetes, body mass index, and heart rate (33). Nevertheless, contradictory findings also exist where mean plasma OPN levels were found to be positively associated with diabetes, age and hypertension (17). In another study, diabetes, age, female gender, smoking and CAD were reported as significant determinants of plasma OPN levels (34). In another clinical study in patients with coronary artery disease, plasma OPN levels showed a positive relationship with plasma hs-CRP and lipid profile indices (35). Taken together, in addition to its role in coronary artery calcification, the majority of clinical findings indicate that OPN may be a significant contributor to several cardiometabolic comorbidities that accompany CAD as this protein has been shown to serve as a link among obesity, adipose tissue inflammation and insulin resistance (36).

Limitation

There were some limitations in this study. This study was conducted on a pilot scale and the results need to be confirmed in larger populations, although the significant difference observed in this study may itself reflect sufficient statistical power. As another limitation, in this study we had a single measurement of serum OPN, whereas repeated measurements are recommended to improve the reliability of findings. Finally, future studies are warranted to assess the association between serum OPN levels and Framingham risk score as well as cardiovascular events before

further consideration of OPN as a cardiovascular biomarker or risk factor.

In conclusion, this study showed that serum OPN levels are increased in patients with coronary artery stenosis >50% compared with those having <50% stenosis or healthy subjects. Therefore, serum OPN concentrations might serve as a risk marker for CAD. However, future research is required to assess the causality of association between CAD and raised serum OPN concentrations and also the association of serum OPN levels with other surrogate markers of atherosclerosis, i.e., carotid intima-media thickness, pulse-wave velocity and flow-mediated dilation.

References

1. Santulli G. Epidemiology of cardiovascular disease in the 21st century: updated numbers and updated facts. JCVD 2013;1:1–2.
2. Abdel-Azeez HA, Al-Zaky M. Plasma osteopontin as a predictor of coronary artery disease: association with echocardiographic characteristics of atherosclerosis. J Clin Lab Anal 2010;24:201–206.
3. Safder O, Al-Sharif S, Kari JA. Pediatric CKD and cardiovascular disease. Cardiovasc Hematol Disord Drug Targets 2014;14:177–184.
4. Gravallese EM. Osteopontin: a bridge between bone and the immune system. J Clin Invest 2003;112:147–149.
5. Sodek J, Ganss B, McKee M. Osteopontin. Crit Rev Oral Biol Med 2000;11:279–303.
6. Scatena M, Liaw L, Giachelli CM. Osteopontin: a multifunctional molecule regulating chronic inflammation and vascular disease. Arterioscler Thromb Vasc Biol 2007;27:2302–2309.
7. Takemoto M, Yokote K, Nishimura M, et al. Enhanced expression of osteopontin in human diabetic artery and analysis of its functional role in accelerated atherogenesis. Arterioscler Thromb Vasc Biol 2000;20: 624–628.
8. Reinholt FP, Hultenby K, Oldberg A, et al. Osteopontin–a possible anchor of osteoclasts to bone. Proc Natl Acad Sci 1990;87:4473–4475.
9. Giachelli CM, Lombardi D, Johnson RJ, et al. Evidence for a role of osteopontin in macrophage infiltration in response to pathological stimuli *in vivo*. Am J Pathol 1998;152:353–358.
10. Nyström T, Dunér P, Hultgårdh-Nilsson A. A constitutive endogenous osteopontin production is important for macrophage function and differentiation. Exp Cell Res 2007;313:1149–1160.
11. Murry CE, Giachelli CM, Schwartz SM, et al. Macrophages express osteopontin during repair of myocardial necrosis. Am J Pathol 1994; 145:1450–1462.
12. O'Brien KD, Kuusisto J, Reichenbach DD, et al. Osteopontin is expressed in human aortic valvular lesions. Circulation 1995;92: 2163–2168.
13. Fitzpatrick L, Severson A, Edwards W, et al. Diffuse calcification in human coronary arteries. Association of osteopontin with atherosclerosis. J Clin Invest 1994;94:1597–1604.
14. Tamura A, Shingai M, Aso N, et al. Osteopontin is released from the heart into the coronary circulation in patients with a previous anterior wall myocardial infarction. Circ J 2003;67:742–744.
15. Bazzichi L, Ghiadoni L, Rossi A, et al. Osteopontin is associated with increased arterial stiffness in rheumatoid arthritis. Mol Med 2009;15: 402–406.
16. Kurata M, Okura T, Watanabe S, et al. Osteopontin and carotid atherosclerosis in patients with essential hypertension. Clin Sci 2006;111: 319–324.
17. Georgiadou P, Iliodromitis EK, Varounis C, et al. Relationship between plasma osteopontin and oxidative stress in patients with coronary artery disease. Expert Opin Ther Targets 2008;12:917–920.
18. Marroquin OC, Kip KE, Kelley DE, et al. Metabolic syndrome modifies the cardiovascular risk associated with angiographic coronary artery disease in women a report from the Women's Ischemia Syndrome Evaluation. Circulation 2004;109:714–721.
19. Wolak T. Osteopontin—a multi-modal marker and mediator in atherosclerotic vascular disease. Atherosclerosis 2014;236:327–337.
20. Denhardt DT, Noda M, O'Regan AW, et al. Osteopontin as a means to cope with environmental insults: regulation of inflammation, tissue remodeling, and cell survival. J Clin Invest 2001;107:1055–1061.
21. Lai CF, Seshadri V, Huang K, et al. An osteopontin-NADPH oxidase signaling cascade promotes pro-matrix metalloproteinase 9 activation in aortic mesenchymal cells. Circ Res 2006;98:1479–1489.
22. Wolak T, Sion-Vardi N, Novack V, et al. N-terminal rather than full-length osteopontin or its C-terminal fragment is associated with carotid-plaque inflammation in hypertensive patients. Am J Hypertens 2013;26:326–333.
23. Kurata M, Okura T, Kumon Y, et al. Plasma thrombin-cleaved osteopontin elevation after carotid artery stenting in symptomatic ischemic stroke patients. Hypertens Res 2012;35:207–212.
24. Chen J, Lu Y, Huang D, et al. Relationship of osteopontin and renal function with severity of coronary artery lesions. Int J Clin Exp Med 2014;7:1122–1127.
25. Yan X, Sano M, Lu L, et al. Plasma concentrations of osteopontin, but not thrombin-cleaved osteopontin, are associated with the presence and severity of nephropathy and coronary artery disease in patients with type 2 diabetes mellitus. Cardiovasc Diabetol 2010;9:70–78.
26. Cho HJ, Cho HJ, Kim HS. Osteopontin: a multifunctional protein at the crossroads of inflammation, atherosclerosis, and vascular calcification. Curr Atheroscler Rep 2009;11:206–213.
27. Mazzali M, Kipari T, Ophascharoensuk V, et al. Osteopontin—a molecule for all seasons. QJM 2002;95:3–13.
28. Gadeau A-P, Campan M, Millet D, et al. Osteopontin overexpression is associated with arterial smooth muscle cell proliferation in vitro. Arterioscler Thromb Vasc Biol 1993;13:120–125.
29. Rollo E, Laskin D, Denhardt D. Osteopontin inhibits nitric oxide production and cytotoxicity by activated RAW264.7 macrophages. J Leukoc Biol 1996;60:397–404.
30. Schmermund A, Erbel R. Unstable coronary plaque and its relation to coronary calcium. Circulation 2001;104:1682–1687.
31. Ohmori R, Momiyama Y, Taniguchi H, et al. Plasma osteopontin levels are associated with the presence and extent of coronary artery disease. Atherosclerosis 2003;170:333–337.
32. Ishiyama M, Suzuki E, Katsuda J, 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;85:189–196.
33. Ärnlöv J, Evans J, Benjamin E, et al. Clinical and echocardiographic correlates of plasma osteopontin in the community: the Framingham Heart Study. Heart 2006;92:1514–1515.
34. Berezin A, Kremzer A. Circulating osteopontin as a marker of early coronary vascular calcification in type two diabetes mellitus patients with known asymptomatic coronary artery disease. Atherosclerosis 2013;229:475–481.
35. Kato R, Momiyama Y, Ohmori R, et al. Prognostic significance of plasma osteopontin levels in patients undergoing percutaneous coronary intervention. Circ J 2009;73:152–157.
36. Nomiyama T, Perez-Tilve D, Ogawa D, et al. Osteopontin mediates obesity-induced adipose tissue macrophage infiltration and insulin resistance in mice. J Clin Invest 2007;117:2877–2888.