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Original article

Could decorin be a biomarker of coronary artery disease? A pilot study in human beings

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Summary. *Background and aim:* Nowadays there is a strong necessity in identifying patients who may be exposed to the risk for future cardiovascular events like progressive atherosclerotic disease. Biomarkers are valuable tools for this purpose. Coronary artery calcification (CAC) is utilized as an important tool for the global risk assessment of cardiovascular events in individuals with intermediate risk. Decorin (DCN) is a small leucine-rich proteoglycan that induces calcification of arterial smooth muscle cell and localizes to mineral deposition in human atherosclerotic plaque. The main purpose of this clinical study was to find out the correlation between Decorin serum concentration and CAC in human for the first time. *Methods:* In this study 84 patients with coronary artery disease who fulfilled inclusion and exclusion criteria, entered the study. For all patients a questionnaire consisting demographic data and traditional cardiovascular risk factors were completed. CT-Angiography was carried out to determine coronary artery calcium score and ELISA method was used for measuring DCN serum concentrations. *Results:* No significant correlation between DCN serum concentration and total CAC score and also CAC of left anterior descending, right coronary artery, left main coronary artery and circumflex was found in the study population (P>0.05). *Conclusions:* On the basis of our results DCN serum concentration is not a suitable biomarker of coronary artery disease. However, more studies with higher sample size are necessary for its confirmation. (www.actabiomedica.it)

Key words: coronary artery calcification, decorin- tumor necrosis factor β, glycosaminoglycan, proteoglycans, biomarker

Introduction

Vascular calcification is a threatening the survival complication of cardiovascular disease and is an independent risk factor for high morbidity and mortality (1).Vascular calcification is an important feature of atherosclerosis and cardiovascular diseases, and it is an inevitable process particularly in the advanced stages of atherosclerosis which can create break in the vessels and cause the plaque rupture. Coronary artery calcification (CAC) is a surrogate marker for subclinical atherosclerosis and is known to reflect atherosclerotic burden. Increased coronary artery calcium score (CACS) correlate with the risk of cardiovascular dis-

ease (2). CAC determined by electron beam-computed tomography (EBCT). EBCT was recently determined a strong predictor that comforts the prediction of future cardiovascular events particularly in intermediate risk subjects while in the past CAC has been a poor prognosis for vascular disease (3).

Recent studies have provided impetus to shift from cellular interaction based calcification models to models emphasizing on the important role of extracellular matrix (ECM) in calcification. The ECM contains number of non-collagenous matrix molecules such as proteoglycans, which is important regulator of bone mineralization, because this regulates collagen fibril formation and tendency and directly controls hydroxyapatite crystal growth (4). Proteoglycans, especially those belonging to the small leucine-rich proteoglycan family which DCN is an exponent example, have a significant role in calcification. The study of in vitro and in vivo animal models suggest an important role of DCN in arterial calcification, however this role is not very clear. The proteoglycans are the ingredient of superfamily leucine-rich repeat (LRR) (>300 members) (5). Proteoglycans consist of one or more glycosaminoglycan (GAG) side chains bound to a core protein. According to the type of the GAG, the proteoglycans classified into, dermatan sulfate, heparan sulfate, keratan sulfate and chondroitin sulfate proteoglycans (6,7). DCN is composed of a 38 Kdalton core protein with 12LRRs containing one dermatan sulfate or chondroitin sulfate chain, and is expressed in skeletal tissues, the adventitia of blood vessels and the skin (8,9). DCN overexpression increases calcification arterial smooth muscle cells (SMCs) and aggregates to areas of atherosclerotic plaques in arteries involved in calcification (10).The DCN core protein binds to TGF-β isoforms by means of GAG chain which control TGF-β/ECM interactions. Decorin GAG chain has a very important function as an inducer of VSMC bio mineralization which actives TGF-β signaling and Ox-LDL-induced SMC mineralization. Overexpression of decorin increases TGF-β activation and regulates TGF-B bio activity considerably; In addition to, decorin-induced TGF-β signaling expedites osteogenic differentiation of VSMCs. TGF-β and DCN alike were implicated in promoting vascular calcification (10-12).The TGF-B is one of the influential factors which acts via expressing the properties of osteoblastic on the arteries calcification in atherosclerotic plaques. In addition, TGF-B with high concentration can be fined in atherosclerotic plaques and increases the formation speed of mineralized nodules (13). From the other point of view, overexpression of decorin induces collagen gel stiffness and accumulation and promotes collagen synthesis, enlarges fibronectin fibrillogenesis, and results in the formation of a dense collagenous matrix in the intima of injured arteries in vivo. Also, DCN directly binds to hydroxyapatite that might be involved in the effect of decorin on calcification (14, 15). Having examined the previous studies in this field, all of investigations were done based on the in vitro and yet, any studies have not been done on humans.

According to this, we evaluated the DCN as a diagnostic biomarker in human to determine the extent of vascular calcification and subsequent coronary disorders such as CAC.

Methods

Patients

Eighty-four patients with diagnosis of coronary artery disease are enrolled in this study between November 2015 and March 2016. Patients were recruited from Cardiology ward of Razavi Hospital, Mashhad, Iran. This study was accepted by ethics committee of Mashhad University of Medical Sciences (code: 931459). Patients with calcium and Phosphor metabolic disorder, parathyroid disease, renal dysfunction, history of osteoarticular disorders, zero calcium score, and were excluded from the study. A questionnaire containing demographic data, laboratory data, drug history, medical history, familial history of cardiovascular risk factors was completed for all patients. All patients signed the consent form prior to entry in the study.

Determination of decorin serum concentration and CAC

Whole blood was collected from patients and centrifuged at 2500 rpm for 10 min. The plasma frac-

tion was isolated and stored at -70°C until required for analysis. Routine biochemical measurements such as plasma glucose, total cholesterol (TC), triglycerides, low density lipoprotein Cholesterol (LDL-c), high-density lipoprotein cholesterol (HDL-c), and serum calcium and phosphorus level were carried out by routine laboratory methods. Serum level of soluble DCN was measured with an enzyme-linked Immunosorbant assay (ELISA) -kit (Zellbio, Germany); each assay was calibrated using BGN standard curve following the manufacturer's instructions. Coronary Artery Calcification was determined by CT-Angiography.

Statistical analysis

Statistical analysis was carried out by SPSS16, All measured values are presented as mean ± SD.

Correlation between Serum Concentration of DCN with CAC was analyzed using spearman correlation test. To compare serum concentration of DCN between different groups, Independent-sample T Test was used. Results were considered significant at $p<0.05$.

Results

Characteristics of the study population

The study population consists of 84 patients, male (77%) and female (23%). The mean age of population was 56.80±10.73 years. Patients' characteristics, laboratory tests including biochemical parameters, traditional cardiovascular risk factors and mean decorin serum level are summarized in Table 1.

Table 1. Patients' characteristic, laboratory data, traditional cardiovascular risk factors and mean decorin serum level of patients

Patients' characteristics	Mean±SD
Age (year)	57.13±10.7
BMI (kg/m^2)	28.36±4.78
Female/male ratio	0.29
Laboratory tests	**Mean±SD**
HDL-C (mg/dl)	41.92±9.97
LDL-C (mg/dl)	90.81±29.14
Total cholesterol (mg/dl)	163.30±33.32
FBS (mg/dl)	104.56±24.00
Traditional risk factors	**Frequency (%)**
Hypertension (%)	45.88
Dyslipidemia (%)	63.52
Positive family history (%)	51.76
Diabetes (%)	20.58
Current Smoking (%)	35.29
Concentration of DCN (pg/mL, Mean±SD)	34.45±19.78

BMI: Body Mass Index, HDL-C: High Density Lipoprotein-Cholesterol, LDL-C: Low Density Lipoprotein-Cholesterol, FBS: Fast Blood Sugar

Correlation between DCN serum level and coronary artery calcification agatson score

There was no significant correlation between DCN serum level and total coronary artery calcification score and also CAC score of LAD, LMCA, RCA and CX ($P>0.05$) (Table 2).

Table 2. Correlation between DCN serum concentration with LAD, RCA, LMCA, and CX coronary artery calcification score

Coronary artery Calcium score	Mean ±SD	P value Spearman Correlation Test	r s Correlation coefficient
Total calcification of coronary vessels (agatson score)	357.29±590.81	0.28	-0.121
Calcification in coronary LAD (agatson score)	184.60±304.46	0.763	0.044
Calcification in coronary RCA (agatson score)	63.37±101.86	0.125	-0.22
Calcification in coronary CX (agatson score)	44.86±99.00	0.064	-0.264
Calcification in coronary LMCA (agatson score)	34.11±116.00	0.460	-0.107

LAD: Left Anterior Descending, RCA: Right Coronary Artery, CX: Circumflex, LMCA: Left Main Coronary Artery, DCN: Decorin

Discussion

In this study, the correlation of the DCN serum level with CAC was evaluated in patients with coronary artery disease. As can be found from aforementioned results, there was no significant correlation between DCN serum level and total CAC and CAC of RCA, LAD, LM and CX (P>0.05). Until now, the relationship between the DCN serum concentration and CAC has been investigated in vitro or only in animal models. In accordance with the obtained results from the previous studies, it is entirely apparent that the calcification is stimulated by Decorin by the means of TFG-β.

In two studies conducted in vitro, it was shown that decorin induces the osteogenic differentiation of the smooth muscle cells. Decorin affects this differentiation via TGF-β signaling pathways. The decorin central protein connects to TGF-β isoforms via side chain. The oxidized LDL adjusts the synthesis of decorin side chain. The difference in the composition of the glycoseaminoglycon chain may occur in various cases such as the spread of atherosclerosis and progressive calcification and vascular rearrangement. High sulfate level of chondrotin sulfate stimulates osteoblastic mineralization and increases the XT-I level which is the enzyme responsible for the synthesis of the chain. The side chain acts as the inductor of bio mineralization of smooth muscle cells of vessels and activator of the signaling pathways TGF-β and adjusting MSX-2 with support of mineralization induced by oxidized LDL. Therefore, the increase in decorin expression significantly increases the TGF-β activity and TGF-β induces the calcification of smooth muscle cells of the vessels. Also, the signaling induced by decorin accelerates osteogenic differentiation in the smooth muscle cells of the vessels (16, 17).

In an in vitro study, the increase of the expression of decorin in retroviral induces the collagen gel contractions and stimulates collagen synthesis. In an invivo study, the increased synthesis of fibrin fibronectin leads to the formation of dense collagenous matrix within the damaged intima of the artery. So, it is possible that the decorin connects the matrix components such as collagen and fibronectin as the onset or the nucleus for the growth of hydroxyapatite crystals. In another study, it has been shown that decorin increases the hydroxyapatite formation for mineralization of collagen gel (16, 17).

Moreover, an in vitro study revealed that DCN prevents of matrix mineralization with unknown mechanism. DCN binds via leucin-rich chain to collagen and thereafter, the GAG component is exposed at the surface of collagen fibrils. This enlarged GAG, possibly with partially exposed protein core, may inhibit hydroxyapatite development in sides of collagen fibrils. Followed by, regulating the assembly and stableness of collagen fibrils may lead to the inhibition of matrix mineralization. Further studies are necessary to clearly elucidate the inhibitory mechanisms (18). According to the results mentioned above, there was no significant relationship between the coronary artery calcification and decorin serum concentration.

It is possible that by increasing the studied population size, -as there is enough data about calcium score in different sub-groups- we can understand the relationship between this biomarker and coronary artery calcification much better. On the other hand, the coronary artery calcium score of the studied patients in the sub-groups was not distributed uniformly. Perhaps if the calcium score distribution was balanced, a significant relationship could be found.

Conclusion

In this study, the correlation of the DCN serum level with CAC was clinically evaluated for the first time that there was no significant correlation between DCN serum level and total CAC, and CAC of RCA, LAD, LM and CX (P>0.05).

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