Association of osteoprotegerin and lipid risk factors with severity of stenosis in coronary artery disease patients with diabetes mellitus

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ABSTRACT

Introduction and Aim: Type-2 diabetes mellitus (T2DM) is a major risk factor for coronary artery diease (CAD). CAD patients with severe stenosis undergo angiography as a part of diagnostic and interventional cardiac care. Gensini scoring system can quantify stenosis which is done during angiogam, an invasive procedure associated with substantial risk. Osteoprotegerin is a protein involved in calcification. Therefore, India being the diabetic capital of world, in the pressent study, we explored the link of osteoprotegerin (OPG) with the severity of stenosis in CAD patients with history of T2DM.

Materials and Methods: We assessed serum OPG, fasting blood sugar, calcium, lipid profile and lipid risk factors in CAD patients with history of T2DM (n=109) and gensisni score was assigned from angiogram. We analyzed the correlation of biochemical parameters with Gensisni score, the gold standard for the assessment of stenosis severity.

Results: Gensisni score was significantly correlated to the type of vessel disease ($p<0.000$) while osteoprotegerin was positively linked ($p<0.05$) in CAD patients with diabetes. Among the lipid risk factors, TG/HDL-C and atherogenic index of plasma (AIP) were positively linked to Gensisni score.

Conclusion: Serum level of osteoprotegerin and lipd risk factors correlate with severity of stenosis. Therefore, osteoprotegerin can be used as a novel screening tool in the Indian population, for CAD patients with a history of diabetes, as an indicator for undergoing angiogram.

Keywords: Coronary artery disease; type-2 diabetes mellitus; osteoprotegerin; stenosis.

INTRODUCTION

In people with type 2 diabetes mellitus, cardiovascular disease (CVD) is a major cause of mortality and morbidity globally (1). Coronary artery disease (CAD) is one among them and it therefore results in any one of the causes of the subsequent events such as cardiovascular death, myocardial infarction, unstable angina, stroke during the time period. Coronary angiogram remains the gold standard for diagnosis and evaluation of the degree of stenosis in coronary arteries that help the clinician to decide for life saving intervention procedures. Nevertheless, it is a risky invasive procedure, and hence performed in patients with specific indications for the procedure (2). Establishing the simple, accessible alternatives can add to the diagnosis where it is not commonly available. A stress echocardiogram is a noninvasive alternative that can assess structure and function of heart. However, this procedure also requires the expertise of a licensed medical professional, cardiologist, and a cardiac sonographer. Therefore, so far, there are no biochemical markers found that may complement these techniques like angiogram or echocardiography which require professionals to establish the diagnosis of stenosis of heart abnormality.

A potentially complementary approach to stratify risk and timing of intervention in patients with CAD, as well as to gain greater insight into causal factors for the disease, is the discovery of biomarkers. Biomarkers have been studied extensively in atherosclerotic cardiovascular disease, with some proving additive to risk scores in predicting future cardiovascular events, and some being recognized as causal factors in the disease. Research on biomarkers in risk assessment of CAD is much robust. However, biomarkers that correlate clinically with the ongoing process of atherosclerosis mirroring the clinical decision limit are not explored much. For the preliminary diagnosis of atherosclerosis indicating the extent of stenosis, it could be useful if such biochemical markers are identified. In addition, it would be very helpful as a screening tool, where specialist and provision for angiography are not accessible, and also as an add-on diagnosis as a prior test where this procedure is available.

The rise in the level of Osteoprotegerin (OPG; 3) is a marker of measure of existing calcification in vascular tissue (2, 4). In individuals with CAD with a history of diabetes mellitus elevated levels of OPG was noted (4). Similarly, hyperlipidemia is a strong risk factor that can promote atherosclerosis. Therefore, increasing level of OPG and dyslipidemia may be index of atherosclerosis. These markers

together have not been analyzed in Indian CAD population where the risk factors involved, and the incidence-prevalence pattern is much different compared to foreign population. Therefore, it would be interesting to study their blood levels in relation to established parameters of stenosis in the Indian population. To the best of our knowledge, no studies have been conducted yet to assess the link of OPG to CAD disease severity in Indian population, especially those with history of T2D. Accordingly, the present aim of our study was to determine whether OPG levels in serum could be a useful diagnostic tool to identify the extent of stenosis severity in CAD patients. Therefore, in the present study, we have assessed the link of OPG to gensini score, a well-established quantification system to assess degree of coronary artery stenosis in diabetic CAD patients.

MATERIALS AND METHODS

Study design

This was an interdepartmental cross-sectional observational study. It was conducted in the department of Biochemistry and Cardiology at (JIPMER), Puducherry. Ethics approval was attained from the Institute Ethics Committee before conducting the study. The study group consisted of CAD patients with history of T2D undergoing angiogram in cardiology OPD as a part of their routine health care. Patients with history of DM, aged between 20 and 75 years undergoing coronary angiogram in dept of cardiology with suspected CAD and subsequently advised to undergo coronary angiogram were recruited for the study. Suspected CAD patients with significant stenosis in at least in one major coronary artery were included. Patients with type 1 diabetes, infections, history of osteoporosis, renal diseases, malignancies, patients on glucocorticoid or immuno-suppressive therapy and post CABG CAD patients and those with implantable cardiac device were excluded.

Brief Procedure

The study protocol was explained to patients, and their relatives. After they agreed to take part in study, written informed consent was obtained. Their age, bodyweight, body mass index (BMI), systolic and diastolic blood pressure (SBP and DBP) was recorded. Following an overnight fast blood was collected on the day of angiogram before the procedure. The serum was separated and stored at −40°C for further biochemical analysis, after routine analysis of glucose and lipid profile in clinical biochemistry lab.

Biochemical parameters

Fasting serum glucose, lipid profile (triglycerides, total cholesterol, HDL- cholesterol, LDL- cholesterol & VLDL- cholesterol) were measured using commercial kits adapted to clinical chemistry autoanalyzer (AU 5800, Beckman Coulter, Orlando, FL, USA). Serum calcium was estimated by Arsenazo method in auto analyzer Beckman Coulter AU 5800. Coronary lipid risk factors of cardiovascular disease were calculated from lipid profile values as described before (5). Osteoprotegerin (OPG) was analyzed using ELISA kit (Wuhan fine Biotech Co., Ltd) following manufacturer's instructions.

Gensini score

Gensini score is calculated based on angiographic findings. Percentage of blockage in the arteries are calculated by visual assessment. It was carried out from the angiogram video recordings by the cardiologist and scores were assigned. A lesion was described as significant when there was ≥1% decrease in luminal diameter. The score of 1 for 1-25% block specifies severity of lesion. After the calculation, the percentage of block is entered the corresponding artery and the segment mentioned in the table given below. The sum of all the score for each artery, segment was calculated. Therefore, sum of all lesions score was the final gensini score as per previously described protocol (6, 7).

Statistical analysis of data

All statistical analyses were done using SPSS software package version 13 (Chicago, IL, USA). Data were analyzed by Kolmogrove-Smironoff test for the normality. All data were expressed as mean and SD. The strength of correlation among parameters was analyzed by Spearman's rank correlation analysis for nonparametric data.

RESULTS

All data is expressed as Mean ± SD and $P < 0.05$ was considered significant. The study group consist of 109 cases who underwent coronary angiogram. Their age ranges from 20 -75 years with the mean age 54.77 ± 9.05. Also, their mean BMI is 24.80 ± 2.15 Kg/m^2. The mean of systolic and diastolic blood pressure is given table 1.

Table 1: Characteristics of coronary artery disease (CAD) patients with DM (n= 109)

Characteristics	Study group
Age (years)	54.77 ± 9.05
BMI (Kg/m^2)	24.80 ± 2.15
Weight (Kg)	65.88 ± 6.66
SBP (mmHg)	125.88 ±20.35
DBP (mmHg)	78.53 ± 10.60

The values are expressed as Mean ± SD. BMI: Body mass index; SBP: Systolic blood pressure; DBP: Diastolic blood pressure

Analysis of biochemical and atherosclerotic markers in CAD patients is given in table 2. It includes the fasting blood glucose, lipid profile and lipid risk

factors. Calculation of atherogenic index of plasma was by using formula AI =$\log_{10}$ [TG/ HDL- C]. Markers of atherosclerosis includes the levels of OPG and the calcium (table 2).

Table 2: Biochemical profile and Gensini score of coronary artery disease (CAD) patients with DM (n= 109)

Routine parameters	
FBG (mg/dL)	130.28 ± 62.28
TC (mg/dL)	145.76 ± 92.09
HDL C (mg/dL)	32.92 ± 9.38
LDL C (mg/dL)	73.20 ± 39.39
TG (mg /dL)	130.25 ± 55.93
VLDL C (mg/dL)	26.05 ± 11.18
Lipid risk factors	
Non HDL-C	112.83 ± 86.08
TG/ HDL- C	4.08 ± 1.66
TC/ HDL-C	4.40 ± 1.52
LDL-C/ HDL-C	2.27 ± 1.18
AIP	0.57 ± 0.17
Markers related to atherosclerosis	
Gensini score	39.97 ± 33.99
Osteoprotegerin (pg/mL)	664.04 ± 714.57
Calcium (mg/dL)	8.94 ± 0.93

The values are expressed as Mean ± SD. AIP =$\log_{10}$ [TG/ HDL- C];

As gensini score data distribution was skewed (non-parametric) we used Spearman rank correlation analysis for correlation of gensini score with various lipid and atherosclerosis markers. Gensini score was significantly linked to OPG, TG/HDL-C and atherogenic index of plasma (AIP) and severity of disease (categorized as number of diseased vessels) as shown in table 3.

Table 3: Spearman rank correlation analysis of Gensini score with various parameters in coronary artery disease (CAD) patients with DM (n= 109)

Parameters	***r***	**P**
Severity of disease	0.502	0.000
OPG	0.211	0.027
NHDL-C	0.027	0.781
TG/HDL-C	0.211	0.030
TC/HDL-C	0.051	0.601
LDL-C/HDL-C	0.138	0.158
AIP	0.211	0.030

AIP: Atherogenic index of plasma; P < 0.05 was considered significant

DISCUSSION

Coronary artery disease is the most general cause of mortality globally. It involves the pathogenesis of atherosclerotic plaque, and during the course of time, it develops into stenosis which results in the narrowing of arteries. Atherosclerosis is a biochemical process of formation and growth of plaques inside arterial lumen which on expansion with time reduces the blood flow. Age is one of the foremost players in the course of clinically significant atherosclerosis. The endogenous repair mechanism responsible for slowing progression of atherosclerosis, hinders the ill effects of various risk factors of CVD. However, with aging, this capacity of to repair the damaged arteries becomes impaired (8) and calcification accompanies atherosclerosis. In our study group, the mean age of participants was 54 years, ranging from 44 to 64 years (Table 1). Calcification and advancement of atherosclerosis is expected in this age group. Among all risk factors involved, diabetes mellitus is the foremost risk involved in pathogenesis of CVD (9) such as coronary heart disease, peripheral vascular disease and stroke.

As our study participants had a history of DM and on treatment their blood glucose was higher than normal (100 mg/dL) but under control (below 200 mg/dl) as seen in Table 2. This was also reflected in their BMI and blood pressure status, which was normal, and age appropriate (Table 2). As most of the oral drugs for diabetes also take care of hyperlipidemia, the same was reflected in the lipid profile of the patients where there was no gross hyperlipidemia (Table 2).

Lipid risk factors calculated from fasting lipid profile are employed to assess the risk for atherosclerosis and have been implicated in the pathophysiology of CAD (10). The AIP is expressed as $\log_{10}$ (TG/HDL-C) where TG and HDL-C are expressed in mM/L. Both these lipid risk factors were significantly and positively associated with the gensini score (Table 3) suggesting that despite the appearance of a routine lipid profile, the rise in these lipids related risk for CVD is associated with the increasing degree of stenosis. The severity of atherosclerotic disease has been traditionally normed as single vessel, double vessel and triple vessel disease based on the number

of coronary arteries involved. In our study group 26% had single vessel disease, 37% had double vessel and 37% triple vessel disease. This assessment of disease severity was significantly associated with gensini scoring (r=0.502, p=0.000) suggesting agreement in both scoring system. Serum OPG level was positively linked to gensini scoring (r =0.211, p=0.027) suggesting that decreased OPG level is indicative of higher degree of stenosis in patients with indication for angiogram. In a previous report (11) in a group of patients with CAD, OPG was positively associated with carotid intima-media thickness, but not with the gensini score. Similarly, in another study in patients with SA and unstable angina pectoris/non-ST elevation myocardial infarction (12), it was also not related to the severity or the degree of coronary artery disease. On the other hand, in another study in suspected CAD patients undergoing diagnostic coronary angiography, OPG was positively associated with gensini score (13). Our findings corroborate with Maniatis *et al.*, and their reports have shown that elevated OPG level can independently predict a poor cardiac prognosis, especially in patients with intermediate coronary lesions (13,14). Therefore, in the present study population which were admitted to cardiology OPD and had to undergo percutaneous intervention which is performed when angiogram shows sever stenosis, the raised level of OPG and its association with genisini scoring is a substantial finding. The possible mechanism could be that OPG is involved most probably as a defense mechanism against vascular damage. Therefore, when there is increased stenosis and/or plaque erosion, it stimulates OPG expression (15).

CONCLUSION

Our data indicates that identification of biochemical markers from simple blood analysis can help in for early diagnosis of atherosclerosis indicating the degree of stenosis. OPG assessment can complement angiogram where facility is available while it may be useful in rural areas where experts and facility for angiographic assessment are not available as a preliminary screening test to select patients at a high risk of stenosis.

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CONFLICT OF INTEREST

The authors declare that they have no conflicting interest.

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