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A study of correlation of PCSK9 enzyme levels in patients with coronary artery disease and diabetes mellitus

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Abstract

Background: *Proprotein convertase subtilisin/kexin type 9 (PCSK9) has a vital role in lipid metabolism and pathophysiology of atherosclerosis. It is also reported to be associated with diabetes mellitus.*

Objective: *To determine PCSK9 enzyme levels in patients having coronary artery disease (CAD) with diabetes mellitus and to correlate the enzyme levels with severity of CAD based on angiogram reports.*

Material and Methods: *The present study was undertaken as a hospital based cross sectional study among 40 patients of either sex in the age group of 30-65 years who were already diagnosed to have CAD with diabetes mellitus. The study was carried out during the period starting from May 2018 to November 2018. Difference in means between two independent groups having non parametric distribution was tested using Mann Whitney U test. A p value <0.05 was considered statistically significant.*

Results: *Mean PCSK9 levels were found to be considerably high in patients with triple vessel disease as compared to those patients with one or two vessel involvements. For a PCSK9 cut off value of 51.5 ng/dl, the sensitivity and specificity for triple vessel disease was calculated to be 88.24% (65.7% -96.7%) and 95.65% (79%-99.2%), respectively*

Conclusion: *PCSK9 levels correlated significantly with number of vessels involved in CAD, as evaluated by angiography, among patients with CAD and DM. A PCSK9 cut off level of 51.5 ng/dl had remarkable sensitivity and specificity for triple vessel disease.*

Keywords: *Diabetes Mellitus, Coronary Artery Disease, Proprotein convertase subtilisin/kexin type 9 (PCSK9), Triple vessel disease.*

Introduction

Almost 60% of all deaths in India are attributed to non-communicable diseases, with Coronary Artery Disease (CAD) being one of the major cause.[1,2] Nearly 15.5% of the deaths are attributed to CAD, globally and has risen to even higher proportions in India.[1] In the years 2015, CAD was the cause of nearly 26.9% of the medically certified deaths in India, the actual numbers in 2012 was 1,200,000 deaths.[3] With increasing prevalence of CAD and its risk factors,[4] CAD is now an important public health problem in India, which also leads to considerable economic burden.[5]

Proprotein convertase subtilisin/kexin type 9 (PCSK9) is a hepatic protease enzyme determined by the PCSK9 gene of chromosome 1.[6] The 1p32.3 locus of PCSK9 gene was found to be associated with CAD.[7]PCSK9 was first identified in 2003, as a vital regulator of low density lipoprotein (LDL) receptor.[8] Subsequently, during the next few years, there were considerable research on the role of PCKS9 in LDL physiology.PCSK9 is largely secreted by the liver, kidney, and small intestine, though evidence from the researches of the recent past portrayed that vascular smooth muscle cells, endothelial cells, and macrophages when exposed to inflammation does express PCSK9 and is considered an inflammatory molecule.[9,10,11,12] The chief transcription factors of PCSK9 are sterol response element binding protein 2 (SREBP-2) and hepatocyte nuclear factor 1 a (HNF1a), which also drives the upregulation of PCSK9.[13] Existing evidence suggest that the PCSK9 levels are linked with risk of adverse cardiovascular events in the furture and its inhibition to reduce LDL levels in turn is found to decrease the risk of CAD.[14,15,16] Also researchers have documented that PCSK9 levels may successfully predict, adverse cardiovascular events, even in patients having controlled levels of LDL.[17] These suggest that there is consistently developing evidence over a period, that PCSK9 has a vital role in pathophysiology of atherosclerosis and CAD.[18] Additionally, a meta-analysis also has documented that those physical activities of moderate-to-vigorous intensity, were found to increase PCSK9 levels in plasma, especially among those were already being administered with statins.[19] Furthermore, in an intervention trial, it was observed that replacing saturated fats with unsaturated fats in diet, lowered the level of LDL cholesterol and the risk of CAD, but did not affect the PCSK9 levels.[20] These evidences suggest that though the risk of CAD is reduced with dietary and physical activity targeted interventions, PCKS9 levels remain unaltered.

Evolocumab and Airocumab are the monoclonal antibodies, which inhibit PCSK9, that were made available recently for use in patients with high cholesterol levels but were non responding to treatment with statins and other drugs aimed at lipid level control. An association between PCSK9 levels and Diabetes Mellitus (DM) was reported earlier.[21]Also, PCSK9 activity was found to have a positive correlation with HOMA-IR, irrespective of diabetes state,[22] which could be because PCSK9 expression is regulated by insulin via the sterol regulatory element-binding protein I-C.[22,23]Though it is now well known that PCSK9 has an important role in lipid metabolism and pathogenesis of CAD, the interpretation of serum PCSK9 level remains under scrutiny by researchers and were not well documented till date. Further research works are required to study the effects of increased levels of PCSK9 on CAD in the general population. The present study was an attempt to determine PCSK9 enzyme levels in patients having CAD with Diabetes

Mellitus and to correlate the enzyme levels with severity of CAD based on angiogram reports.

Material and Methods

The present study was undertaken as a hospital based cross sectional study, in a tertiary care teaching hospital in Puducherry, a cost town in South India. The hospital caters to population of different socioeconomic backgrounds from Puducherry and neighbouring districts of Tamil Nadu. Individuals of either sex in the age group of 30-65 years who were already diagnosed to have CAD with Diabetes Mellitus. The minimum required sample size was calculated to be 40, using the software Open Epi version 3.0. Convenient non probability sampling technique was used to select study participants from all those who were eligible to participate in the study. The study was carried out during the period starting from May 2018 to November 2018. Patients without Diabetes Mellitus, Dyslipidemia and recent occurrence of acute coronary syndrome, cardiac failure, cancer, and acute or chronic liver or renal failure were excluded from the study. Blood sample of 5ml was collected for estimation of fasting plasma glucose, post prandial glucose and lipid profile. In addition, Electrocardiogram, Two-dimensional echocardiography, Coronary angiogram and PCSK9 Enzyme levels were investigated for all the study patients.

Institute ethical committee approval was obtained before beginning the study. Informed written consent was obtained from all the patients, before including them in the study. Data entry was carried out using MS Excel 2016 and data analysis was done using SPSS (Statistical Package for Social Sciences) version 22.0. Means and proportions were calculated for continuous categorical variables. Continuous variables were subjected to tests of normality and appropriate statistical tests were applied. Difference in means between two independent groups having non parametric distribution was tested using Mann Whitney U test and in case of more than two independent groups, Kruskal Wallis test was applied. A p value <0.05 was considered statistically significant.

Results

Majority of the study participants were in the age group of 46-60 years and the mean age was 55±10 years and majority of them were males (55.5%). More than 60% of the study participants had higher blood sugar levels. Abnormal lipid profile parameters were observed in in 15-30% of the study participants. Nearly 57.5% were found to have triple vessel disease in angiogram and 15% had double vessel diseases. Mean PCSK9 levels were found to significantly high among the patients with raised blood sugar levels and among those with triple vessel disease (Table 1).

Mean PCSK9 levels were found to be considerably high in patients with triple vessel disease as compared to those patients with one or two vessel involvements (Figure 1).

Area under the Curve (AUC) in Receiver Operating Characteristics (ROC) curve analysis of PCSK9 levels for triple vessel disease, revealed considerably higher values (AUC – 0/991) (Figure 2).

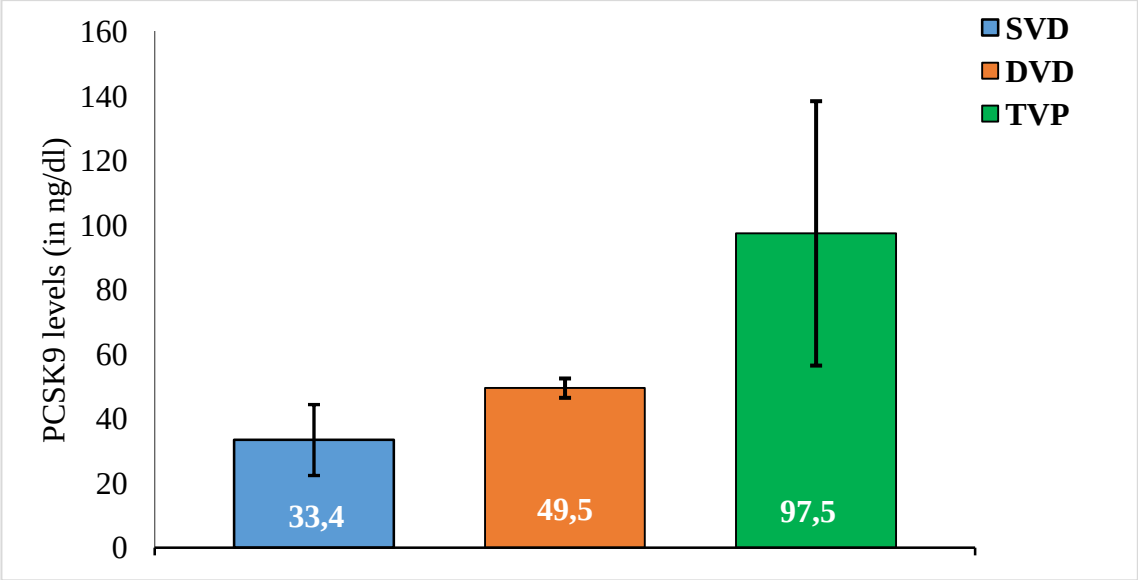
For a PCSK9 cut off value of 51.5 ng/dl, the sensitivity and specificity for triple vessel disease was calculated to be 88.24% (65.7% -96.7%) and 95.65% (79%-99.2%), respectively (Table 2).

Table 1 Distribution of study participants based on Demographic characteristics and respective PCSK9 levels (n=40).

Parameter	Frequency	Percentage	PCSK9 levels (in ng/dl)	
			Median (IQR)	p value
Age				
30-45	10	25.0	48.5(23.7-77.2)	**0.014**#
46-60	20	50.0	53(43.5-85.7)	
>60	10	25.0	94.5(66.5-107.5)	
Sex				
Male	31	77.5	69(47-92)	0.610*
Female	9	22.5	47(44-99)	
Random Blood Sugar (RBS)				
≤140 mg/dl	9	22.5	53(35-97.5)	0.588*
>140 mg/dl	31	77.5	69(47-92)	
Fasting Blood Sugar (FBS)				
≤110 mg/dl	12	30.0	42.5(25-65)	**0.004***
>110 mg/dl	28	70.0	79.5(47.7-103)	
Post Prandial Blood Sugar (PPBS)				
≤200 mg/dl	16	40.0	44(26.2-53)	**<0.001***
>200 mg/dl	24	60.0	85(56-105.7)	
Total Cholesterol				
≤220 mg/dl	32	80.0	68(45.5-95.7)	0.584*
>220 mg/dl	8	20.0	59(43.2-86.5)	
Triglycerides				
≤150 mg/dl	28	70.0	68(47-92)	0.493*
>150 mg/dl	12	30.0	50(41.2-94.2)	
High Density Lipoprotein (HDL)				
≥40 mg/dl	11	27.5	65(45-92)	0.765*
<40 mg/dl	29	92.5	67(45-94.5)	
Low Density Lipoprotein (LDL)				
≤165 mg/dl	34	85.0	68(44.5-99)	0.541*
>165 mg/dl	6	15.0	59(40.2-75.5)	
Very Low Density Lipoprotein (VLDL)				
≤30 mg/dl	28	70.0	68(47-92)	0.493*
>30 mg/dl	12	30.0	50(41.2-94.2)	
Coronary Angiogram Findings				
Single Vessel Disease	11	27.5	35(25-43)	**<0.001#**
Double Vessel Disease	6	15.0	48.5(47-53)	
Triple Vessel Disease	23	57.5	90(70-106)	

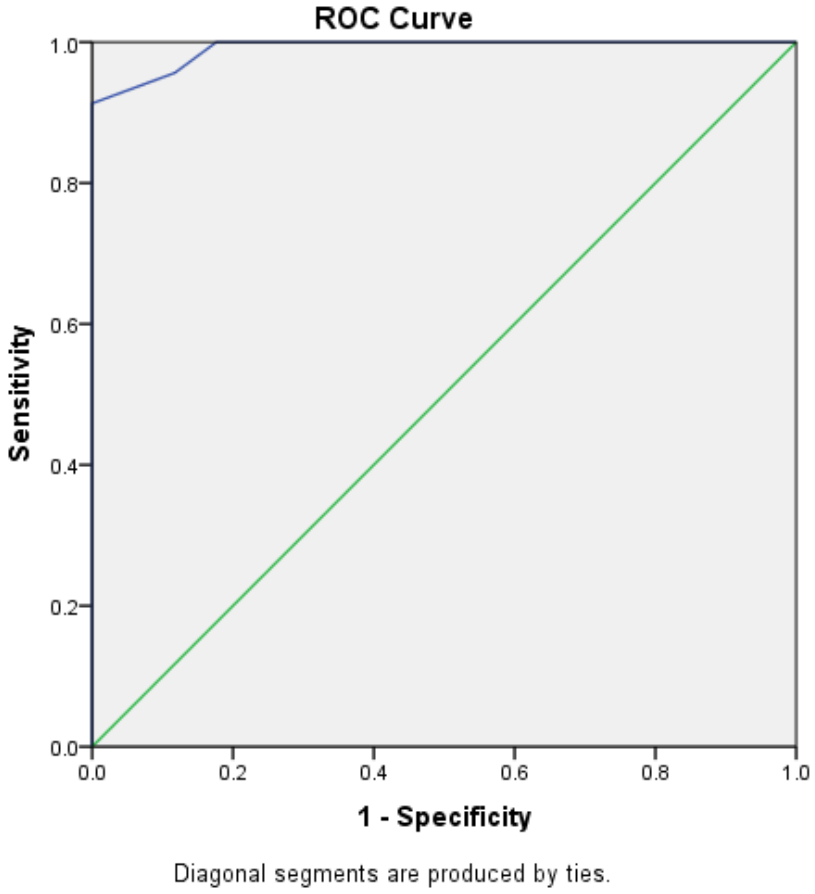
Kruskal Wallis test; * Mann Whitney U test.

Figure 1 Mean PCSK9 levels among patients with different severities of vessel block (n=40).



*Vertical bars indicate standard deviation

Figure 2 ROC Curve for PCSK9 levels and presence of Triple vessel disease on Angiogram



Area Under the Curve (AUC) = 0.991.

Table 2 Predictive accuracy of PCSK9 levels on presence of Triple vessel disease (n=40)

PCSK9 levels	Triple Vessel Disease		Total n (%)	p value*	Odds Ratio (95% CI)
	Present n (%)	Absent n (%)			
>51.5 ng/dl	15(93.8)	1(6.3)	16(100.0)	<0.001	165 (13.7- 1987)
≤51.5 ng/dl	2(8.3)	22(91.7)	24(100.0)		
Total	17(42.5)	23(57.5)	40(100.0)		
Sensitivity	88.24% (65.7% -96.7%)				
Specificity	95.65% (79%-99.2%)				
PPV	93.75% (71.7%- 98.9%)				
NPV	91.67% (74.1%- 97.7%)				

*Chi Square test was applied to test statistical difference in proportions

Discussion

PCSK9 was initially proposed to be a biomarker for predicting adverse cardiovascular events among patients with both risk factors and among general population as well.[24] Since discovery, the association between PCSK9 and DM has been of interest to researchers. The present study was an attempt to determine PCSK9 enzyme levels in patients having CAD with Diabetes Mellitus and to correlate the enzyme levels with severity of CAD based on angiogram reports. Mean PCSK9 levels were found to be considerably high in patients with triple vessel disease as compared to those patients with one or two vessel involvements in the present study. Despite the fact that both CAD and DM were individually associated with PCSK9, it was noted that there was no published research material that studies PCSK9 levels in patients with both CAD and DM. However, there exists sufficient evidence for possible etiopathogenesis of such an association. Expression of PCSK9 in endothelial cells are triggered by reactive oxygen species. Further, PCSK9 is found to increase expression of oxidised LDL (oxLDL) receptors, which leads to continuous uptake of cholesterol by endothelial cells.[25]

PCSK9 is found to activate MAP kinase pathways (p38), which might trigger apoptosis throughbcl-2/bax and caspase 3 ultimately augments lectin like oxLDL-1 (LOX-1) receptor dependent apoptosis in endothelial cells.[26] Expression of ABCA1gene is restricted by PCSK9 leading to a atherosclerosis favouring state of macrophages in vessel walls.[27] The enzyme is also found to exhibit various LDL receptor dependent actions in endothelial cells, and cardiomyocytes. It plays a vital role in increasing lysosomal and endosomal degradation by binding to LDL receptor. To further the role, it was found that ventricular cardiomyocytes express PCSK9 and its oxLDL-dependent activities could be antagonized by PCSK9 neutralisation.[28] Apart from expression of PCSK9 in cardiomyocytes, it is also secreted by myocardium and it is found to correlated to size of the infarct and cardiac function among patients after an acute myocardial infarction.[29] Further investigations also revealed that PCSK9 promote inflammation, by activating NF-kB signaling leading to increased secretion of pro-inflammatory cytokines by macrophages, worsening cardiomyocyte damage. [30]

Deepa PK et al documented in the study findings that circulation PCSK9 levels were high in patients with CAD and it associated significantly with other conventional risk factors of CAD, like, fasting blood sugar, total cholesterol, triglycerides, HDL and HDL. A similar correlation was observed in the present study as well. Peng J et al[21] in their study reported that PCSK9 correlated significantly with total cholesterol, LDL, and HbA1C. It was also observed in their study that higher PCSK9 levels were associated with increased change of major adverse cardiac events (MACE) in patients with stable coronary artery disease and diabetes mellitus. These results were similar and identical to that of those observations obtained in the present study. In contrast to these results, in a systematic review it was reported that diabetes status did not affect the association between PCSK9 levels and MACE.[31] One of the important limitation of the present study is that exercise, smoking, alcohol consumption and lipid lower drugs are also found to affect PCSK9 levels,[32] however, the same was not studied in the present research work. To complicate the association, a case report has documented increase in HbA1C levels after PCSK9 inhibitor therapy, with Alirocumab, and HbA1C reversed to lower levels on stopping the drug.[33] These findings, necessitates large scale cohort studies to evaluated the role of PCSK9 and its inhibitors in patients with CAD and DM.

Conclusion

PCSK9 levels correlated significantly with number of vessels involved in CAD, as evaluated by angiography, among patients with CAD and DM. A PCSK9 cut off level of 51.5 ng/dl had remarkable sensitivity and specificity for triple vessel disease.

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