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Research Article

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Association of Pro Protein Convertase Subtilisin / Kexin 9, Apo B and HbA1c in Coronary Artery Disease with and without Diabetes Mellitus

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Abstract: Pro-protein convertase subtilisin/Kexin type 9 (PCSK9) is a soluble protein synthesized as a zymogen that influences plasma levels of low-density lipoprotein cholesterol (LDL-C). Understanding the association is very essential to develop adequate treatment strategies. Hence,the present study aims to explore the relationship between plasma levels of PCSK9, Apo B, and HbA1C in patients with coronary artery disease (CAD) with Diabetes Mellitus. All patients had no history of using lipid-lowering medication. Of these 62patients (40 male and 22 female, age group 30 -70 years), all had angiographically diagnosed CAD.25 are CAD with DM, and 37 are CAD without DM. Plasma PCSK9 and Apo B were measured using an enzyme-linked immunosorbent assay (ELISA). HbA1 c Levels are measured by using the HPLC method. In this work, we compared plasma PCSK9 and Apo B in CAD with DM and CAD without DM with the help of the HbA1c value. Both plasma levels of PCSK9 (0.040 (<0.050) and 0.036 (<0.05)) and Apo B (0.021 (< 0.05) and [(0.425 (> 0.05)] HbA1C were significantly higher in patients with CAD and DM as compared with those with CAD without DM. In Group with CAD without DM there is no elevation of HbA1C but PCSK 9 and Apo B are significantly elevated. PCSK 9 and Apo B are independent markers of CAD. Correlation analysis showed plasma level of PCSK9 was significantly correlated (P<0.05) with that of Apo B in both patients with CAD and without DM. However, multivariate regression analysis after adjustment for age, gender, smoking, alcohol, hypertension, and hyperlipidemia showed that only in CAD patients with diabetes mellitus, there was a significant positive correlation (p<0.05) between plasma levels of PCSK9 and HbA1C.

Keywords: Diabetes mellitus, HbA1c, The Tamil population, Metabolic diseases, Coronary arterial diseases

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1. INTRODUCTION

Coronary artery diseases (CAD) are affecting globally a large number of individuals.[1] India is not an exemption, as there are a large number of cases in India also and further yearly raise in the cases morbidity and mortality.[1] When there are high levels of triglycerides (TG) or Low-density lipoproteins (LDL) or decreased levels of high-density lipoproteins (HDL), it is labeled as hyperlipidemia. There is a day-by-day increase in the cases of hyperlipidemia in the Indian population. This is dangerous as it leads to the accumulation of fat in the blood vessels and leads to the development of atherosclerosis and further to coronary arterial diseases. And hence, there is an increase in the awareness of lowering cholesterol to protect the population from death due to coronary diseases.[2]The proprotein convertase subtilisin/Kexin type 9 (PCSK9) gene belongs to the family of proprotein convertase that plays an important role in cholesterol metabolism.[3]PCSK9 overexpression increases plasma LDL-C levels by downregulating LDL receptor (LDLR) expression after transcription. PCSK9 is not only involved in lipid metabolism but also participates in liver regeneration and neural differentiation and inhibits LDL-R expression. Cardiac disease that develops as a direct consequence of DM in patients with type 1 DM (T1DM) or type 2 DM (T2DM) is known as diabetic heart disease. Diabetic heart disease is a conglomeration of coronary artery disease (CAD), cardiac autonomic neuropathy (CAN), and diabetic cardiomyopathy (DCM), and these diseases are characterized by molecular, structural, and functional changes in the myocardium.[4] Type 1and Type 2 diabetes is associated with a 10-fold and 2- to 4-fold increase in CVD, respectively, over that of people without diabetes.[5,6] Type 2 diabetes and insulin resistance in elder people are mainly related with increased triglycerides, decreased high-density lipoprotein (HDL) cholesterol levels, dense low-density lipoprotein (LDL), and increased Apolipoprotein B (apo B).[7,8] This chief objective of this study was to assess the relationship of circulating PCSK9 levels with Apo B and HbA1C of coronary artery patients with and without diabetes mellitus.[9] The relationship between PCSK9 and T2DM has become an amusing issue since its finding.[10] It was first noticed that plasma PCSK9 levels were higher in patients with T2DM.[11] Moreover, the results obtainable from epidemiology, preclinical, and clinical studies put forward a positive association of plasma PCSK9 concentration with diabetic parameters and risks of T2DM.[12] Meanwhile, a new animal study has disclosed that downregulating PCSK9 can enhance lipid and glucose metabolism. In humans, circulating PCSK9 was related to the breakdown of Apo B,[13-14] increased LDL breakdown, and lowering plasma level of LDL-C. The present study was undertaken to observe the relationship between Pro Protein Convertase Subtilisin / Kexin 9, Apo B and HbA1C in Coronary Artery Disease with and without Diabetes Mellitus in South Indian Tamil Population.

2. MATERIALS AND METHODS

2.1 Subjects and Study design

The present study was a case-control study.The present study was conducted at the Department of Biochemistry, Saveetha Medical College, Hospital and Research Centre, Thandalam, Chennai, India. The present study comprises sixty-two cases of angiographically diagnosed coronary artery disease patients, recruited from the outpatient department of Saveetha Hospital, Thandalam, Chennai. The recruited participants were further grouped with diabetes mellitus (n=25) and without diabetes mellitus (n=37). Sixty-two aged gender-matched healthy individuals were recruited as controls. The following criteria were used to recruit cases. Angiographically diagnosed coronary artery disease male and female patients, within the age group of 30-70 years willing to participate in the study were included in the study. Unwilling participants and participants with any severe complications were excluded from the study. The present study protocol was approved by the institutional human ethical committee of Saveetha Medical College and Hospital, Chennai (007/09/2019/IEC/SMCH) on 24th Sep 2019. Informed consent was obtained from all the participants. All the procedures were followed as per the guidelines of ICMR. Confidentiality of data was maintained.

2.2 Assessment of severity of CAD

The study subjects were subjected to the diagnosis of CAD, which was performed as per the literature using the standard Judkin's technique with the filming of multiple views of each vessel according to our previous studies. The severity of CAD was assessed as per the guidelines of standard methods like SYNTAX, Gensini, and Jeopardy scoring systems.[32-34]

2.3 Methods

After recruitment, all the participants underwent a detailed physical examination which enabled us to collect their clinical history. Informed consent was obtained from all the participants before recruitment.

2.4 Biochemical tests

Following the literature standard procedures and precautions,5ml of venous blood was collected from all the participants in pre-cooled EDTA tubes. These blood samples were subjected to centrifugation with a speed of 3000rpm for 15 minutes at four degrees centigrade. Plasma aliquots were stored at −80 ◦C. Fasting plasma glucose, Post-prandial blood sugar, and lipid profile was estimated with the help of Randox fully automated biochemistry analyzer. Glycated hemoglobin was estimated in the D10 bio rad instrument (HPLC method). PCSK9 levels and Apo B were estimated using a commercially available ELISA kit (Human Diagnostics, Germany). [24,25]

3. STATISTICAL ANALYSIS

Data was analyzed using SPSS 20.0 version. The sample size was calculated by an expert bio-statistician based on previous studies.The required sample size for each arm is 62.T-test was done to compare the PCSK9 levels and conventional risk factors like FPG, HBA1C, TC, TG, HDL cholesterol, and LDL cholesterol (Direct) between cases and controls. The level of statistical significance was made with a p-value of < 0.05. Pearson correlation was used to find out the correlation between PCSK9 levels with other conventional risk factors.

4. RESULTS

Figure 1 presents the correlation between Diabetic CAD PCSK 9 and Apo B. Pearson correlation was used to find out the correlation. There was a significant positive correlation between the parameters (figure 1).

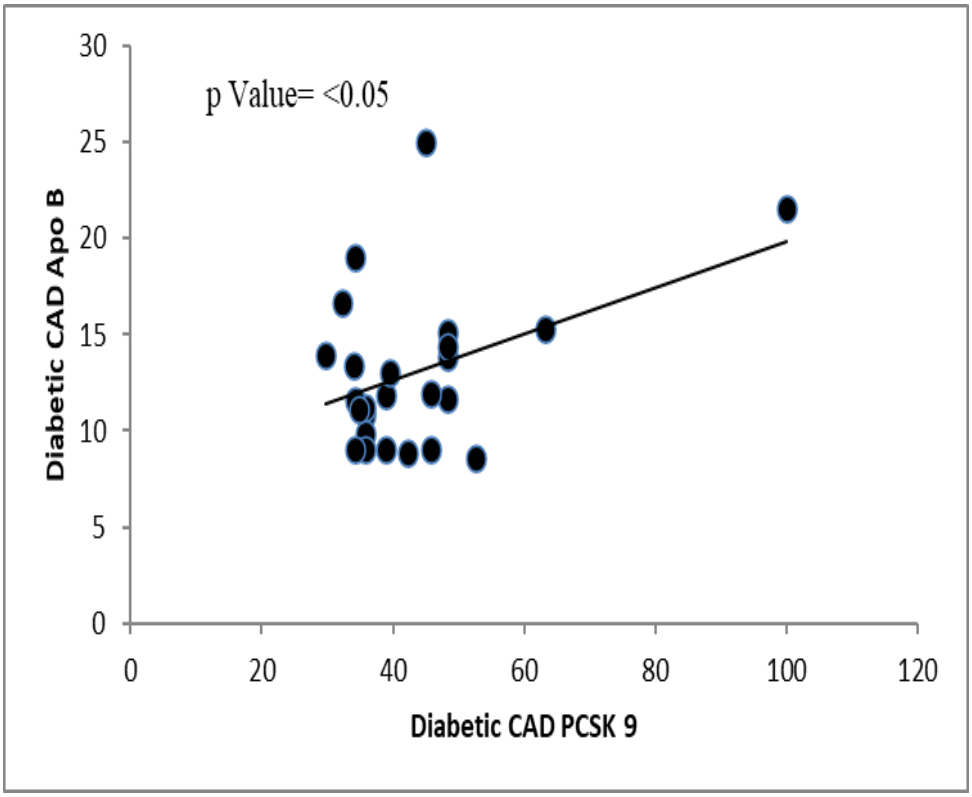

Fig 1: Correlation between Diabetic CAD PCSK 9 and Apo B.Pearson correlation was used to find out the correlation. There was a significant positive correlation between the parameters.

Figure 2 presents the correlation between diabetic HbA1C and PCSK 9. Pearson correlation was used to find out the correlation. There was a significant positive correlation between the parameters (figure 2).

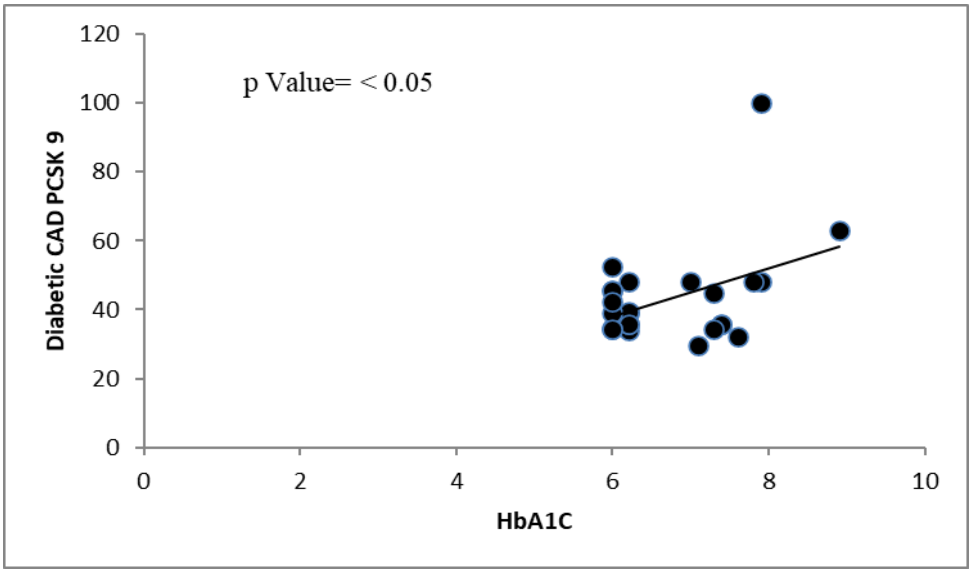

Fig 2: Correlation between diabetic HbA1C and PCSK 9. Pearson correlation was used to find out the correlation. There was a significant positive correlation between the parameters.

Figure 3 presents the correlation between Diabetic CAD HbA1C and Apo B. Pearson correlation was used to find out the correlation. There was a significant positive correlation between the parameters (figure 3).

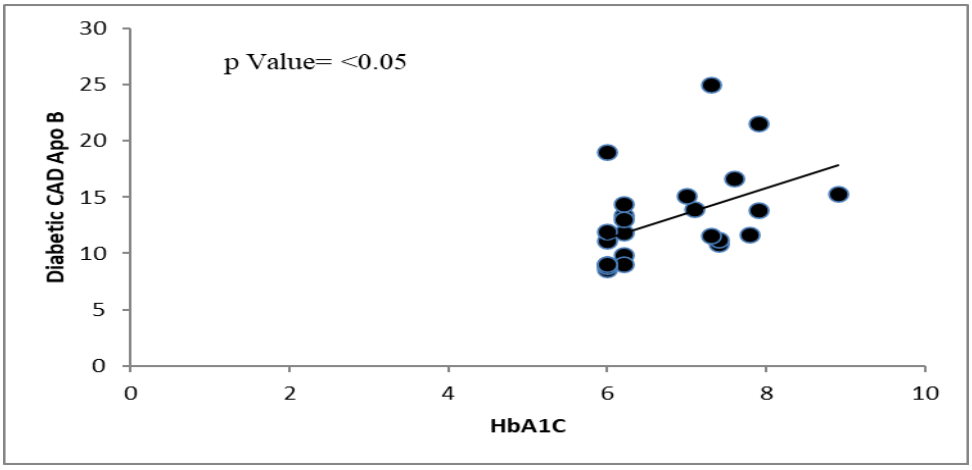

Fig 3: Correlation between Diabetic CAD HbA1C and Apo B.Pearson correlation was used to find out the correlation. There was a significant positive correlation between the parameters.

Figure 4 presents the correlation between Non- diabetic CAD PCSK 9 and Apo B. Pearson correlation was used to find out the correlation. There was a significant positive correlation between the parameters (figure 4).

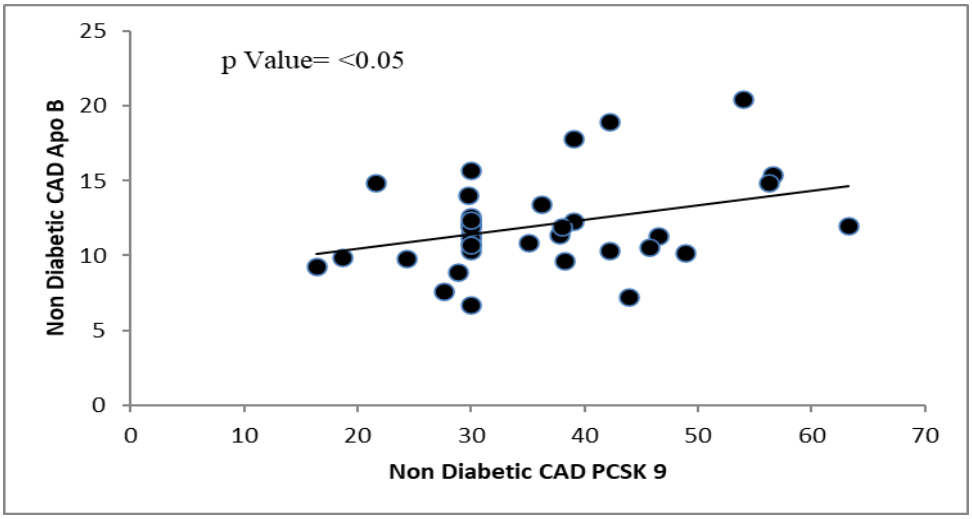

Fig 4: Correlation between Non-diabetic CAD PCSK 9 and Apo B. Pearson correlation was used to find out the correlation. There was a significant positive correlation between the parameters.

Figure 5 presents the correlation between Non-diabetic CAD HbA1C and PCSK 9. Pearson correlation was used to find out the correlation. There was a significant positive correlation between the parameters (figure 5).

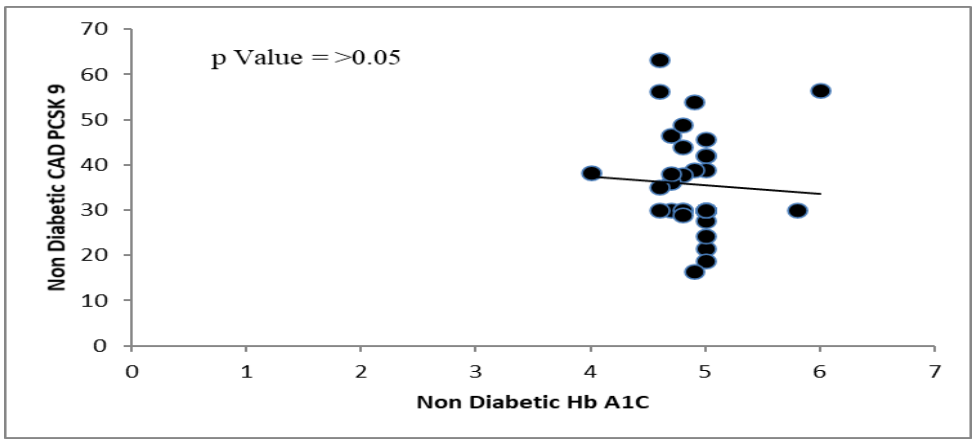

Fig 5: Correlation between Non-diabetic CAD HbA1C and PCSK 9. Pearson correlation was used to find out the correlation. There was a significant positive correlation between the parameters.

Figure 6 presents the correlation between Non-Diabetic HbA1C and Apo B.There was a significant positive correlation between the parameters.

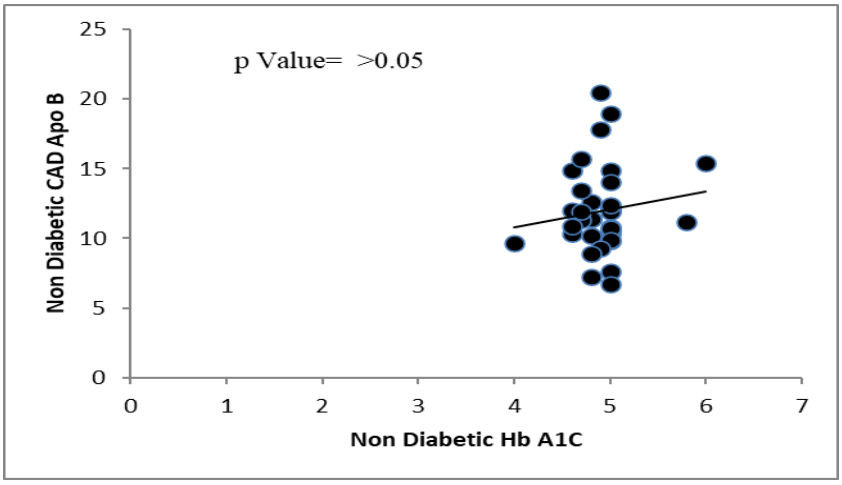

Fig 6: Correlation between Non-Diabetic HbA1C and Apo B.Pearson correlation was used to find out the correlation. There was a significant positive correlation between the parameters.

Table 1 presents the comparison of the significance of the difference between the fasting blood glucose and HbA1c levels of case groups and controls groups (figure 6). Data were presented as mean and SD. **P<0.01 is significant between the parameters.

Table 1: Fasting blood glucose and HbA1c levels of case groups and controls groups.

Diabetic Data	CAD(n= 62)	CONTROL(n = 62)	P value
FBS	139.1 ± 52.3	90.3 ± 5.5	< 0.01**
HbA1C	5.66 ± 1.08	4.75 ± 0.39	< 0.01**

Data were presented as mean and SD. **P<0.01 is significant.

Table 2 presents the comparison of the significance of the difference between the lipid profile values of case groups and control groups (table 2). Data were presented as mean and SD. **P<0.01 is significant.

Table 2: Lipid profile values of case groups and control groups.

Lipid profile	CAD	CONTROL	P Value
TC [mg/dl]	229.8 ± 27.4	176.9 ± 13.8	<0.01**
TG [mg/dl]	173.6±26.96	120.0±19.95	<0.01**
HDL [mg/dl]	36.3±2.83	47.65±4.69	<0.01**
LDL [mg/dl]	157.5±24.9	110.7±10.6	<0.01**

Data were presented as mean and SD. **P<0.01 is significant.

Table 3 presents the correlation of PCSK 9, Apo B, and HbA1 c in patients with CAD with and without diabetes. Pearson correlation was used to find out the correlation (table 3).

Table 3: Correlation of PCSK 9, Apo B and HbA1 c in patients with CAD with and withoutdiabetes.Pearson correlation was used to find out the correlation.

Parameters	Patient with CAD and diabetes(n= 25)		Non diabetic CAD patients(n = 37)	
	R value	P - value	R value	P- value
HbA1c and PCSK 9	0.42	0.036 (<0.05)	-0.056	0.743 (>0.05)
PCSK 9 and Apo B	0.413	0.040(<0.05)	0.347	0.036 (< 0.05)
Apo B andHbA1 c	0.459	0.021 (< 0.05)	0.135	0.425 (> 0.05)

5. DISCUSSION

The present study was undertaken to observe the relationship between Pro Protein Convertase Subtilisin / Kexin 9, Apo B and HbA1C in Coronary Artery Disease with and without Diabetes Mellitus in South Indian Tamil Population. There was a significant positive correlation observed between the parameters. In this study, we compared the levels of circulating PCSK9 concentration, Apo B, HbAlc, and lipid profile in a group of normal healthy subjects, patients with diabetic CAD and without Diabetic CAD. The outcomes of the instant study reported that levels of PCSK9, HbA1c, Apo, B and lipid profiles were significantly high in CAD patients compared to normal controls. But HbA1C levels are low in Patients without DM and CAD. Circulating PCSK9 level was significantly correlated with Apo B and lipid profiles in both case groups. Coronary artery disease (CAD) is regularly associated with glucose alterations. Many studies have investigated the effect of abnormal glucose metabolism on the risk of atherosclerosis or CVD.[15] HbA1c is an important pointer of long-term glycemic control with the ability to reveal the combined glycemic history of the leading two to three months. HbA1c not only gives a reliable test of chronic hyperglycemia but also correlates strongly with the risk of long-term diabetes complications. According to Sherwani et al.,[16] elevated PCSK 9 and Apo B has also been regarded as an independent risk

factor for coronary heart disease and stroke in subjects with or without diabetes. HbA1C is considered as an independent risk factor in CAD patients with DM. The valuable information provided by a single HbA1c test has rendered it a reliable biomarker for the diagnosis and prognosis of diabetes. Our results are well in agreement with the above studies as we have high FPG, HbA1c values in CAD patients in the DM group. Guidelines for the control of patients with hyperlipidemia firstly focus on exploiting of goal low-density lipoprotein cholesterol (LDLC) levels for coronary heart disease (CHD) risk decrease (NCEP-ATP III) (Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. Epidemiological studies have observed an inverse connection between HDL-C and coronary heart disease.[17] There is rising attention to high-density lipoprotein cholesterol (HDL-C) as a second-line target of therapy. APO B directly uplifts the number of plasma atherogenic lipid profile parameters.[17] Many studies reported that PCSK9 plasma levels were related to the brutality of coronary injuries in patients with acute coronary syndrome and myocardial infarction.[18,19] Similarly, most studies focusing on the association between plasma PCSK9 and early coronary atherosclerosis described a not clear direct relationship.[20] Other studies reported that those increased levels of circulating PCSK9, combined with rather higher levels of Apo B.[21-23] In our line of work, we noticed that circulating PCSK9 level was correlated with Apo B, HbA1c, and lipid profiles in patients with diabetic CAD. We also observed a significant correlation between PCSK 9 and Apo B in patients with CAD without DM but these parameters are inversely correlated with HbA1C in this group.Earlier studies explained that especially the relationship between circulating PCSK9 levels and metabolic and lipid parameters in healthy subjects.[26-28] The present study divided the subjects with and without diabetes mellitus and assessed the parameters. The present study assessed the importance of Pro Protein Convertase Subtilisin / Kexin 9, Apo B, and HbA1Cin Coronary Artery Disease with and without Diabetes Mellitus. Another study reported that there was a effect of statin therapy that can modify these parameters.[29] Hence, the authors recommend further studies in the context of dividing the participants with statin therapy also. In the context of management of dyslipidaemia, it is very essential to change the lifestyle along with the therapy. Further, it is recommended by earlier studies to estimate the genes in the diagnosis.[30-36]

6. CONCLUSION

The present study was undertaken to observe the relationship between Pro Protein Convertase Subtilisin / Kexin 9, Apo B, and HbA1C in Coronary Artery Disease with and without Diabetes Mellitus in South Indian Tamil Population. Both plasma levels of PCSK9 and Apo B and HbA1C were significantly higher in patients with CAD and DM as compared with those with CAD without DM. In Group with CAD without DM there is no elevation of HbA1C but PCSK 9 and Apo B are significantly elevated. PCSK 9 and Apo B are independent markers of CAD. Correlation analysis showed plasma level of PCSK9 was significantly correlated with that of Apo B in both patients with CAD and without DM. However, multivariate regression analysis after adjustment for age, gender, smoking, alcohol, hypertension, and hyperlipidemia showed that only in CAD patients with diabetes mellitus, there was a significant positive correlation between plasma levels of PCSK9 and HbA1C. There was a significant positive correlation observed between the parameters. The present study recommends further detailed studies in this area for a better understanding of the relationship between the parameters.

7. ACKNOWLEDGEMENT

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8. AUTHOR'S CONTRIBUTION STATEMENT

Deepa P K, Kedari G S R, Resmi C R**,** conceptualized and designed the study. Deepa P K collected the data and prepared the final draft. All authors verified and approved the final draft.

9. CONFLICT OF INTEREST

Conflict of interest declared none.

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