

ORIGINAL ARTICLE

Association of PCSK-9 with the Biomarkers of Type-2 Diabetes and its Complications in the Indian Population: a Pilot Study

Mohd Waiz^{1, #}, Sahir Sultan Alvi^{1, #}, Saheem Ahmad², M. Salman Khan¹

[#] Both authors contributed equally

¹ IIRC-5, Clinical Biochemistry & Natural Product Research Lab, Department of Biosciences, Integral University, Lucknow, U.P., India

² Department of Medical Laboratory Sciences, College of Applied Medical Sciences, University of Hail, Saudi Arabia

SUMMARY

Background: Proprotein convertase subtilisin/kexin type-9 (PCSK-9) is a serine protease with profound effects on plasma LDL-C, the major risk factor for cardiovascular diseases (CVDs). However, plasma PCSK-9 level and its association with the biomarkers of CVDs, diabetes, and associated complications have not yet been reported in the northeastern population of India.

Methods: Of the total cohort (n = 233), we analyzed healthy controls (HC; n = 50), freshly diagnosed type-2-diabetes mellitus (T2DM-FD; n = 46), T2DM treated (T2DM-T; n = 49), diabetic nephropathy (T2DM-N; n = 43), and diabetic dyslipidemia (T2DM-DL; n = 45) subjects. Plasma PCSK-9 and other biological determinants associated with T2DM, CVD, and nephrotic dysfunction were assessed.

Results: The level of plasma PCSK-9 in HC, T2DM-FD, T2DM-T, T2DM-N, and T2DM-DL groups was found to be 184.1 ± 13.83 , $183.1 \pm 24.4.3$, 241.8 ± 75.42 , 403.7 ± 85.94 , and 641.3 ± 135.5 ng/mL, respectively, indicating its role in the severity of the here-mentioned complications. Moreover, plasma PCSK-9 levels further showed a significant correlation with the biomarkers of hyperglycemia, particularly HbA1c, as well as LDL-C in T2DM-FD, T2DM-N, and T2DM-DL subjects of the Indian population, while moderate association in T2DM-T subjects.

Conclusions: Our first-of-its-kind clinical study aiming to quantify the circulatory PCSK-9 level in the Indian population concluded that elevated PCSK-9 was significantly associated with the severity of diabetes and associated complications. Moreover, such elevation in PCSK-9 might be attributed to the lipid- and glucose-lowering medication-induced SREBP-2-dependent mechanisms. Since our conclusion is based on a pilot study, further cohort studies in larger populations of India are required to get a generalization regarding the role of PCSK-9 in DM and associated complications.

(Clin. Lab. 2024;70:xx-xx. DOI: 10.7754/Clin.Lab.2023.230602)

Correspondence:

M. Salman Khan, PhD
Associate Professor
Department of Biosciences
Integral University
Lucknow, 226026, U.P.
India
Phone: + 91 9044015790
Email: contactskhan@gmail.com

KEYWORDS

PCSK-9; diabetes mellitus (DM), diabetic nephropathy, diabetic dyslipidemia, LDL-C

LIST OF ABBREVIATIONS

ApoB - Apolipoprotein-B
BMI - Body mass index
CVDs - Cardiovascular diseases
DM - Diabetes Mellitus
DL - Dyslipidaemia
FBG - Fasting blood glucose
FD - Freshly diagnosed GLUT-1 or 4

Glucose transport-1 or 4
 HC - Healthy controls
 HDL - High-density lipoproteins
 HbA1c - Glycated Hemoglobin
 HMG-CoA - 3-Hydroxy-3-methylglutaryl-coenzyme A reductase
 HOMA-IR - (Homeostatic Model Assessment of insulin resistance)
 LDL - Low-density lipoproteins
 LDL-R - Low-density lipoprotein receptor
 N - Nephropathy
 PCSK-9 - Proprotein convertase subtilisin/kexin type 9
 T1DM - Type 1 diabetes mellitus
 T2DM - Type 2 diabetes mellitus
 T - Treatment
 VLDL - Very low-density lipoproteins

INTRODUCTION

Diabetes mellitus (DM) results from altered carbohydrate metabolism, impaired insulin action, and lifestyle factors, such as urbanization, aging, obesity, and physical inactivity [1]. In 2021, ~537.0 million adult people were living with DM worldwide, with over 6.0 million fatalities, half of which were still undiagnosed [2]. This figure was projected to increase up to ~645 and ~785 million by 2030 and 2045, respectively [2]. India was also ranked 2nd amongst the top countries with the highest number of adults facing the burden of diabetes in 2021 after China (74.2 vs. 140.9 million, respectively) and these stats are projected to increase by 2045 (124.9 vs. 174.4 million, respectively) [2].

T2DM accounts for more than 90% of all diabetes cases. The importance of this issue stems from DM patients' increased vulnerability to subsequent microvascular and macrovascular complications, such as nephropathy (20 - 40%) and cardiovascular disease (CVD: 32.2%) [3,4]. These complications stem from perturbed carbohydrate and lipid metabolism in both systemic circulation and specific tissues. The regulation of DM relies on the functionality of carbohydrate metabolizing enzymes (i.e., α -amylase and α -glucosidase) [5], insulin [6], and glucose transporters (i.e., GLUT-1 and GLUT-4) [1]. However, cholesterol homeostasis is orchestrated via HMG-CoA reductase activity [7] and the clearance of atherogenic LDL-C [8], which is pivotal in atherosclerotic plaque formation and subsequent CVD events. Higher organisms have evolved with a receptor-mediated mechanism to remove undesired ApoB-containing LDL-C from the circulation into the cells or target tissues, utilizing the LDL-receptor (LDL-R) [9]. In order to lower cholesterol levels, the majority of therapeutic approaches focus on both HMG-CoA reductase activity and LDL-R expression. Statins have been the drug of choice for the last five decades [8,10]. Interestingly, the discovery of PCSK-9 challenged the world once again due to its ability to promote lysosome-mediated intracellular degradation of LDL-R, emerging as a crucial

target in CVD management [11]. Recent reports have established that the here-mentioned ailments are interconnected, and PCSK-9 is proposed as the pivotal link bridging these metabolic disorders. Briefly, the level of PCSK-9 has been correlated with the etiology of distinct ailments, i.e., DM [12], renal dysfunction [13], and cancer [14]. Based on our literature search and to the best of our knowledge, it has been apparent that there is no report on the association of PCSK-9 with diabetes, associated complications, and coronary heart disease markers from India. Therefore, the present study aims to decipher the possible association of PCSK-9 with different specific biochemical markers of diabetes, diabetes-linked hyperlipidemia, and diabetic nephropathy and to delineate its role in the prognosis of these comorbidities.

MATERIALS AND METHODS

Study design and participants

All the subjects with informed consent were originally recruited from the IIMS&R, Integral University, Lucknow. The current cohort (n = 233) was divided into five groups. Group-I: (n = 50) healthy control (HC); Group-II: (n = 46) freshly diagnosed T2DM subjects not receiving any type of medications (T2DM-FD); Group-III: (n = 49) T2DM subjects on treatment (T2DM-T); Group-IV: (n = 43) T2DM subjects with nephropathy (T2DM-N); and Group-V: (n = 45) T2DM subjects with dyslipidemia (T2DM-DL).

Inclusion/exclusion criteria

Subjects with T2DM (as per the WHO criteria), aged between 20 - 75 years with signed informed consent (either by the subject or their representative) were included in the current study. However, the subjects suffering from any chronic disease (except for diabetes), any acute infection, pregnant women, and those who did not give informed consent were excluded from the study.

Collection of blood and plasma

The subjects attending the OPD/IPD in IIMS&R, Lucknow, India, previously diagnosed and marked as T2DM with impaired renal and cardiac functions were selected for the present study. Blood (5 mL) was drawn from the subjects after overnight fasting from a cubital vein by a professional phlebotomist into heparinized tubes, and the plasma was separated via centrifugation at 2,500 rpm for 10 minutes and stored at -80°C for further experimental use. The plasma from healthy subjects served as control subjects.

Anthropometric data collection

According to international standards, body weight and height were measured by a research nurse. BMI was calculated by dividing the body weight (kilograms) by the height in meters squared. As per the WHO standards, values of BMI from 18.5 - 24.9 kg/m² are consid-

ered as healthy (normal weight), BMI values ≤ 25.0 to < 30.0 kg/m² indicated overweight, and BMI value is greater than 30.0 kg/m² was considered as obese. Socio-demographic data were collected by a pre-designed questionnaire and patient history including smoking, alcohol intake, disease duration, and medication, recorded at the time of their recruitment for the current study. The systolic and diastolic blood pressure was measured three times consecutively after ten minutes in the sitting position and the average reading was taken for the study.

Assessment of clinical data

Fasting blood glucose (FBG), glycated hemoglobin (HbA1c), total cholesterol (TC), low-density lipoproteins (LDL-C), high-density lipoproteins (HDL-C), triglycerides (TG), very low-density lipoproteins (VLDL), urea, uric acid, and creatinine were evaluated in central pathology of IIMS&R Lucknow by the professional pathologist. The average reading was taken after three consecutive measurements. Furthermore, the HOMA-IR was calculated by using the formula described by Matthews et al. [15]. The estimated glomerulation filtration rate was computed using the MDRD equation [16].

Assessment of plasma PCSK-9 and insulin level

Plasma PCSK-9 (Cat. No: E-EL-H1579) and insulin (Cat. No. E-EL-H2665) levels were determined by the commercially available Enzyme-linked immunosorbent assay (ELISA) kit, procured from Elabscience Biotechnology Inc. The conducted tests were standardized. These assays were based on the Sandwich-ELISA principle and the results have been expressed as ng/mL and μ IU/mL for PCSK-9 and insulin, respectively. The tests were performed in triplicate and expressed as mean \pm SD.

Statistical analysis

The common laboratory variables were represented as mean \pm SD and $p < 0.05$ was considered significant throughout the study. The linear correlation between two factors was performed using Spearman's correlation (area under 95% confidence intervals) test. The statistical significance among different study groups (i.e., HC, FD-T2DM, T2DM-T, T2DM-N, and T2DM-DL) was done by implementing the Mann-Whitney test and Kruskal Wallis test with Dunn's multiple comparison post-test, where appropriate. A general linear model (ANCOVA) was applied to conduct adjusted comparisons between the study groups. The results were evaluated using GraphPad Prism 8.4.0 for Windows (GraphPad Software, San Diego, CA, USA).

RESULTS

Anthropometric data

The baseline characteristics of HC, T2DM-FD, T2DM-T, T2DM-N, and T2DM-DL subjects have been repre-

sented in Table 1. First, the BMI of the subjects with diabetic complications (i.e., T2DM-N and T2DM-DL) was significantly different in comparison to HC, whereas no significant difference was observed in T2DM-FD and T2DM-T. Moreover, the HC subjects neither smoked nor drank alcohol, whereas approximately 13.2%, 16.4%, 11.8%, and 33.5% of the subjects in T2DM-FD, T2DM-T, T2DM-N, and T2DM-DL groups, respectively, were smokers. However, according to the obtained results from the questionnaire, none of the subjects were alcoholics (Table 1).

Biochemical parameters

Plasma PCSK-9 level

PCSK-9 is a serine protease with profound effects on plasma LDL-C, a major risk factor for cardiovascular diseases (CVDs). The mean PCSK-9 level was found to be 184.1 ± 13.83 , $183.1 \pm 24.4.3$, 241.8 ± 75.42 , 403.7 ± 85.94 , and 641.3 ± 135.5 ng/mL in HC, FD-T2DM, T2DM-T, T2DM-N, and T2DM-DL subjects, respectively (Figure 1).

Correlation of FBG with PCSK-9

Statistically, FBG levels in T2DM-FD ($p \leq 0.0001$), T2DM-N ($p \leq 0.0001$), and T2DM-DL ($p \leq 0.0001$) were significantly elevated, whereas a moderate difference was observed in T2DM-T ($p \leq 0.001$) subjects when compared to HC. However, this increased concentration of sugar (fasting) in T2DM-T subjects is within the recommended range under the diabetes state. Spearman's rank correlation analysis revealed that elevated PCSK-9 level was correlated with FBG across all the diabetic groups and showed a substantial positive association with FBG in T2DM-FD ($r = 0.6185$, $p \leq 0.0001$), T2DM-N ($r = 0.6563$, $p \leq 0.0001$), and T2DM-DL ($r = 0.7096$, $p \leq 0.0001$), whereas it was moderately associated in T2DM-T ($r = 0.3319$, $p = 0.0198$) subjects. However, no correlation was observed in HC ($r = 0.2101$, $p = 0.1430$) subjects (Figure 2).

HbA1c and its association with PCSK-9

All the subjects except HC and T2DM-T, showed a higher percentage of HbA1c (Figure 1). Further correlation analysis determines that the elevated percentage of HbA1C positively corresponded with the circulatory PCSK-9 that was reported in T2DM-FD ($r = 0.6625$, $p \leq 0.0001$), T2DM-N ($r = 0.6647$, $p \leq 0.0001$), and T2DM-DL ($r = 0.6371$, $p \leq 0.0001$) subjects. On the other hand, a moderate association was also observed in the T2DM-T subjects ($r = 0.4219$, $p = 0.0025$) while no significant correlation was reported in HC subjects ($r = 0.0800$, $p = 0.5805$) (Figure 2).

Correlation of insulin with PCSK-9

The insulin level was significantly elevated in T2DM-FD, T2DM-N, and T2DM-DL subjects when matched with HC subjects, whereas no remarkable difference was observed in the T2DM-T subjects (Figure 1). Additionally, we found that an increased level of insulin

Table 1. Anthropometric characteristics of HC, T2DM-FD, T2DM-T, T2DM-N, and T2DM-DL subjects.

Characteristics	HC	T2DM-FD	T2DM-T	T2DM-N	T2DM-DL
Number (n)	50	46	49	43	45
Age (years)	40.71 ± 10.21	41.78 ± 11.23 ^{ns}	48.47 ± 11.64 [*]	60.26 ± 12.5 ^{**}	65.07 ± 10.85 ^{****}
Duration (years)	NA	NA	1.8 ± 0.8 ^{***}	8.3 ± 2.8 ^{****}	11.03 ± 3.2 ^{****}
Smokers (n)	0	6	8	5	15
Alcohol intake (n)	0	0	0	0	0
BMI (kg/m ²)	22.64 ± 4.15	23.6 ± 3.23 ^{ns}	26.34 ± 5.13 ^{ns}	30.2 ± 4.1 [*]	32.1 ± 5.12 [*]
Systolic BP (mmHg)	116.99 ± 6.2	121.33 ± 7.6 ^{ns}	118.23 ± 5.6 ^{ns}	158.45 ± 10.32 ^{****}	145.24 ± 9.42 ^{****}
Diastolic BP (mmHg)	70.66 ± 5.88	76.38 ± 8.6 ^{ns}	75.76 ± 7.9 ^{ns}	93.11 ± 6.8 ^{****}	82.21 ± 7.3 ^{***}

BMI - Body Mass Index.

Data are expressed as ± SD.

The level of significance was defined as ^{ns} - p > 0.05, ^{*} - p < 0.05, ^{**} - p ≤ 0.01, ^{***} - p ≤ 0.001, ^{****} - p ≤ 0.0001 vs. HC as determined by the Mann-Whitney U test.

Table 2. Biochemical characteristics of HC, T2DM-FD, T2DM-T, T2DM-N, and T2DM-DL subjects.

Characteristics	HC	T2DM-FD	T2DM-T	T2DM-N	T2DM-DL
Number (n)	50	46	49	43	45
FBG (mg/dL)	85.34 ± 8.23	179.2 ± 18.36 ^{****}	139.4 ± 42.81 ^{***}	250.25 ± 69.85 ^{****}	315 ± 83.15 ^{****}
HbA1c (%)	4.81 ± 0.75	8.04 ± 0.71 ^{****}	6.97 ± 0.60 ^{***}	11.65 ± 1.81 ^{****}	14.26 ± 1.82 ^{****}
Insulin (μIU/mL)	19.16 ± 5.99	29.33 ± 8.04 ^{****}	22.86 ± 9.77 ^{ns}	31.92 ± 14.0 ^{****}	35.96 ± 13.74 ^{****}
HOMA-IR	4.06 ± 1.41	13.09 ± 4.13 ^{****}	7.94 ± 5.45 ^{**}	20.97 ± 12.60 ^{****}	29.88 ± 15.82 ^{****}
LDL-C (mg/dL)	78.18 ± 5.50	80.92 ± 5.32 ^{ns}	102.8 ± 11.54 ^{****}	159.6 ± 17.19 ^{****}	218.5 ± 15.02 ^{****}
HDL-C (mg/dL)	44.30 ± 2.97	45.14 ± 4.07 ^{ns}	43.42 ± 4.16 ^{ns}	36.90 ± 5.94 ^{****}	30.18 ± 4.90 ^{****}
VLDL (mg/dL)	23.17 ± 1.90	31.09 ± 2.32 ^{****}	31.67 ± 2.65 ^{****}	36.60 ± 2.16 ^{****}	75.24 ± 4.63 ^{****}
TG (mg/dL)	115.9 ± 9.49	155.5 ± 11.6 ^{****}	158.4 ± 13.27 ^{****}	183.0 ± 10.83 ^{****}	376.2 ± 23.16 ^{****}
TC (mg/dL)	145.7 ± 37.26	157.2 ± 8.45 ^{ns}	177.9 ± 13.53 ^{****}	233.1 ± 17.19 ^{****}	323.9 ± 15.42 ^{****}
TC/HDL	3.2 ± 0.21	3.4 ± 0.25 ^{ns}	4.13 ± 0.46 ^{****}	6.4 ± 1.16 ^{****}	11.02 ± 1.90 ^{****}
PCSK-9 (ng/mL)	184.1 ± 13.83	183.1 ± 24.43 ^{ns}	241.8 ± 75.42 ^{***}	403.7 ± 85.94 ^{****}	641.3 ± 131.5 ^{****}
Urea (mg/dL)	12.52 ± 4.09	19.03 ± 3.56 ^{***}	13.59 ± 5.06 ^{ns}	47.86 ± 11.69 ^{****}	29.93 ± 9.275 ^{**}
Creatinine (mg/dL)	0.89 ± 0.16	1.04 ± 0.24 ^{ns}	0.99 ± 0.28 ^{ns}	2.17 ± 0.64 ^{****}	1.57 ± 0.48 ^{****}
Uric Acid (mg/dL)	3.37 ± 0.59	4.23 ± 1.23 ^{ns}	3.90 ± 0.78 ^{ns}	13.06 ± 3.42 ^{****}	7.21 ± 1.71 ^{****}
eGFR (mL/minute/1.73m ²)	108.65 ± 8.23	96.56 ± 10.23 ^{ns}	92.54 ± 7.32 ^{ns}	36.73 ± 4.73 ^{****}	57.52 ± 10.76 ^{**}

FBG - fasting blood glucose, HbA1c - glycated hemoglobin, HOMA-IR - homeostasis model assessment of insulin resistance, eGFR - estimated glomerulation filtration rate.

Data are expressed as ± SD.

The level of significance was defined as ^{ns} - p > 0.05, ^{*} - p < 0.05, ^{**} - p ≤ 0.01, ^{***} - p ≤ 0.001, ^{****} - p ≤ 0.0001 vs. HC as determined by the Mann-Whitney U test.

positively correlated with the circulatory elevated level of PCSK-9 in T2DM-FD (r = 0.6155, p = < 0.0001), T2DM-N (r = 0.7074, p = < 0.0001) and T2DM-DL (r = 0.7207, p = < 0.0001) subjects. Although a slight positive association was observed in T2DM-T (r = 0.4950,

p = 0.0003) subjects, there was no significant correlation reported in HC (r = 0.2531, p = 0.0762) subjects.

PCSK-9 Adversely Correlates with the Diabetes Markers

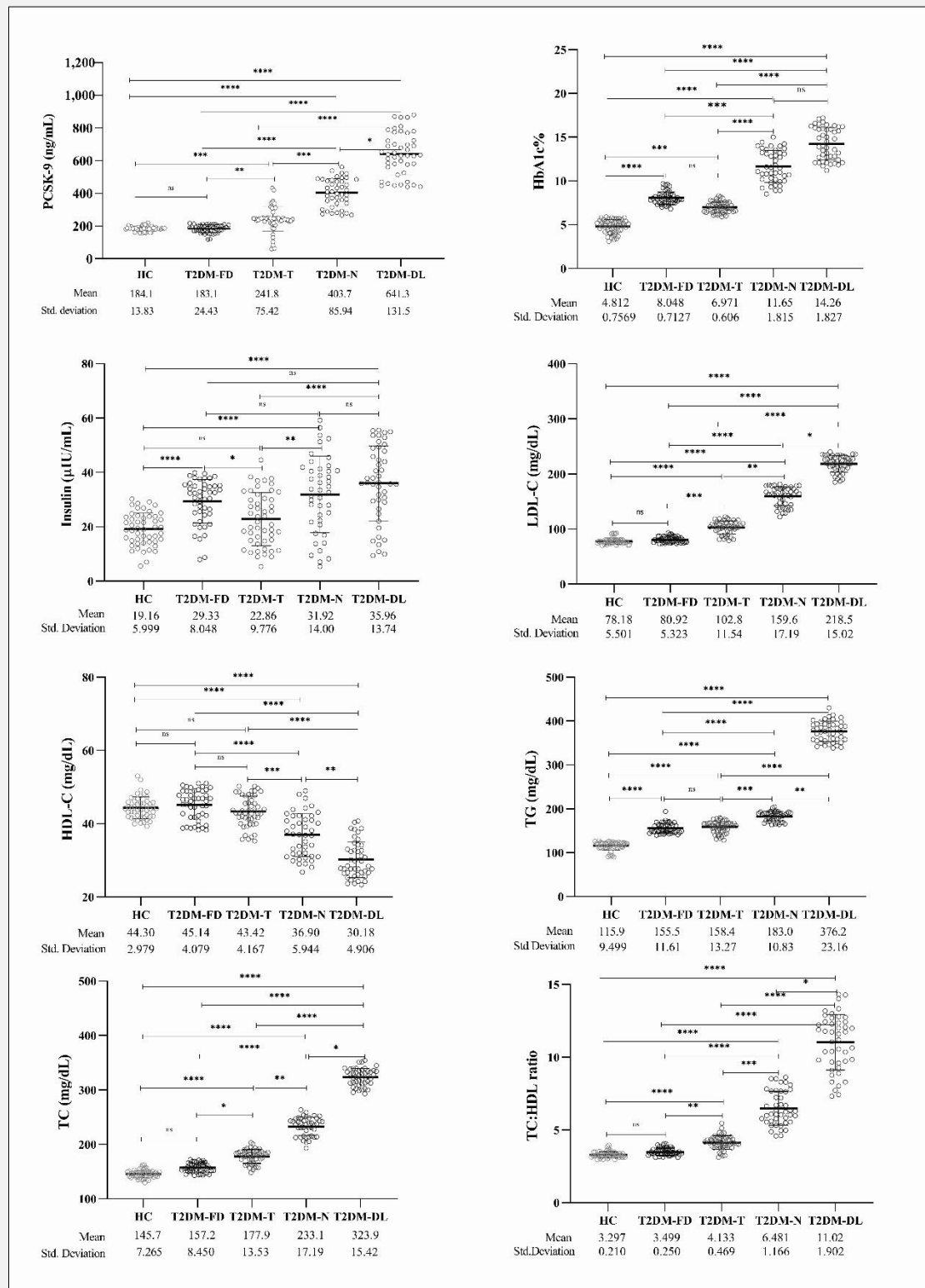


Figure 1. Scatterplot of plasma PCSK-9, diabetic, and lipid parameters in HC (n = 50), T2DM-FD (n = 46), T2DM-T (n = 49), T2DM-N (n = 43), and T2DM-DL (n = 45) groups.

Data mean \pm SD are shown with significance as determined by the Kruskal-Wallis test.

ns - $p > 0.05$, * - $p < 0.05$, ** - $p \leq 0.01$, *** - $p \leq 0.001$, **** - $p \leq 0.0001$.

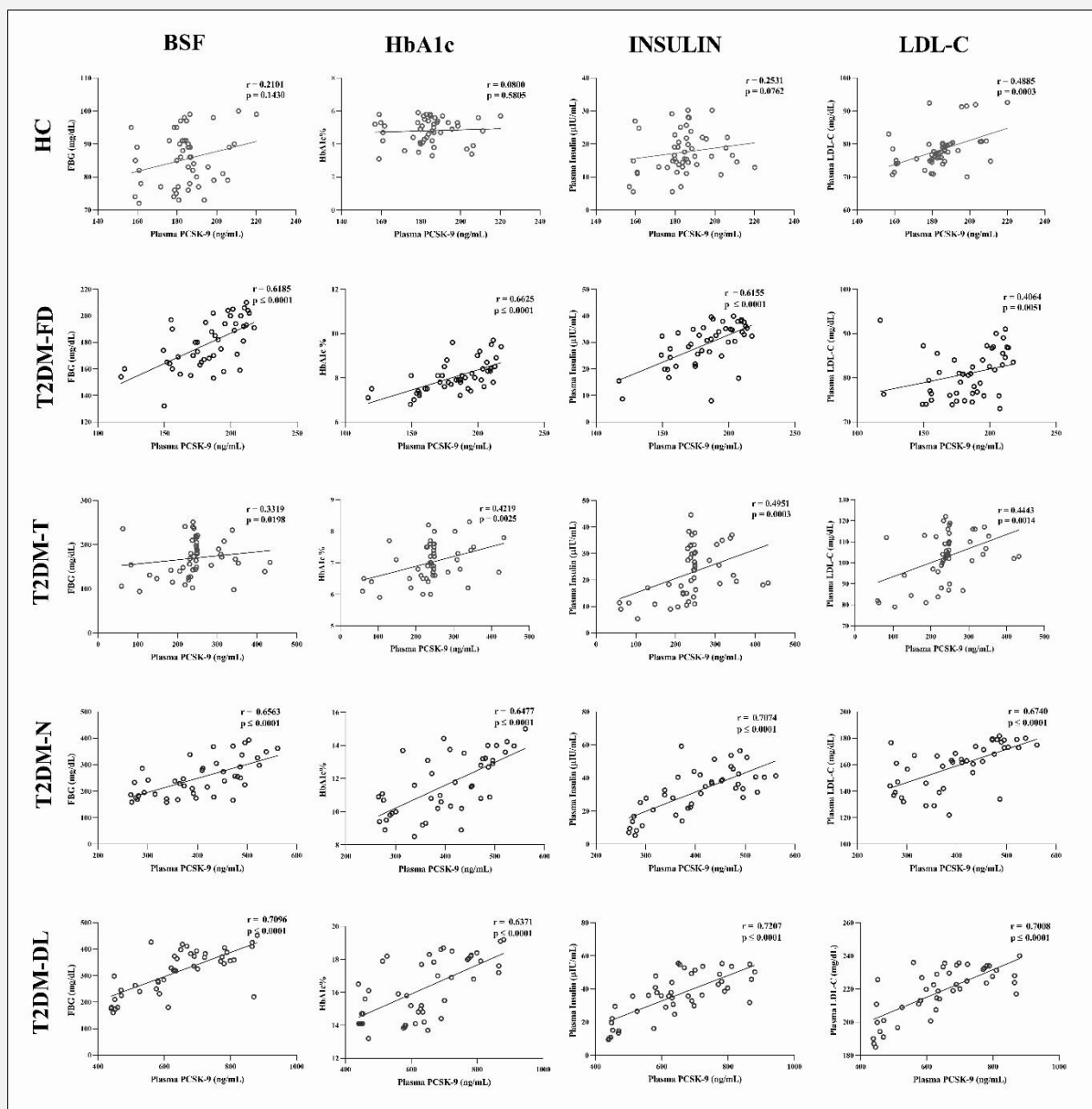


Figure 2. The relationship between plasma PCSK9 and FBG, HbA1c, insulin, and TC in HC, T2DM-FD, T2DM-T, T2DM-N, and T2DM-DL groups.

Spearman’s correlation coefficients were determined and significance was defined as $p < 0.05$.

Lipoproteins and their association with PCSK-9

The level of LDL-C, HDL-C, VLDL-C, TGs, and TC were evaluated in all the assigned group subjects (Figure 1). Interestingly, it is a well-known fact that PCSK-9 is involved in the regulation of LDL-C [8]. Herein we also observed a strong positive association between PCSK-9 and LDL-C in all the designated groups, i.e., HC ($r = 0.4885$, $p = 0.0003$), T2DM-FD ($r = 0.4064$, $p = 0.0051$), T2DM-T ($r = 0.4443$, $p = 0.0014$), T2DM-N

($r = 0.6740$, $p \leq 0.0001$), and T2DM-DL ($r = 0.7008$, $p \leq 0.0001$) subjects (Figure 2).

Moreover, HDL-C was inversely associated with PCSK-9 in T2DM-N ($r = -0.3104$, $p = 0.0428$) and T2DM-DL ($r = -0.5982$, $p \leq 0.0001$) subjects, whereas no association was reported in T2DM-FD ($r = 0.2249$, $p = 0.1330$) and HC ($r = 0.1555$, $p = 0.2808$) subjects (Figure 3). On the other hand, the levels of TGs varied significantly across T2DM-FD, T2DM-T, T2DM-N, and

PCSK-9 Adversely Correlates with the Diabetes Markers

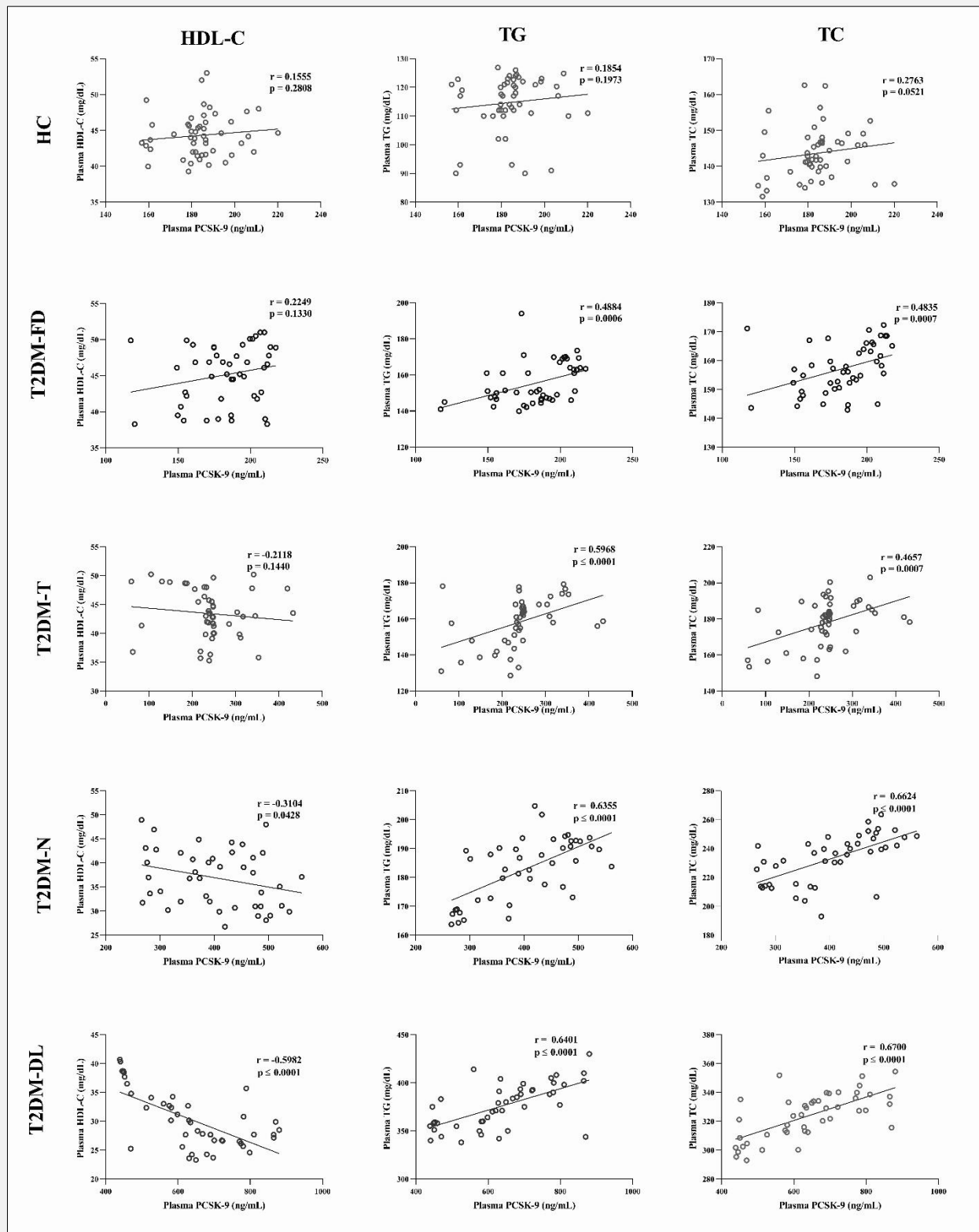


Figure 3. The relationship between plasma PCSK9 and LDL, HDL, and TG in HC, T2DM-FD, T2DM-T, T2DM-N, and T2DM-DL groups.

Spearman's correlation coefficients were determined and significance was defined as $p < 0.05$.

T2DM-DL subjects in comparison to the HC subjects. We have found a positive association between PCSK-9 and TGs in T2DM-FD ($r = 0.4884$, $p = 0.0006$), T2DM-T ($r = 0.5968$, $p \leq 0.0001$), T2DM-N ($r = 0.6355$, $p \leq 0.0001$), T2DM-DL ($r = 0.6401$, $p \leq 0.0001$) cases, whereas in HC ($r = 0.1854$, $p = 0.1973$) the association was not significant (Figure 3).

TC, which is a direct reflection of these lipoproteins, was assessed in all the subjects. An elevated level of TC was significantly associated with PCSK-9 in T2DM-N ($r = 0.6624$, $p \leq 0.0001$) and T2DM-DL ($r = 0.6700$, $p \leq 0.0001$) subjects, whereas it was moderately associated in T2DM-FD ($r = 0.4835$, $p = 0.0007$) and T2DM-T ($r = 0.4657$, $p = 0.0007$) subjects. No correlation was reported in HC ($r = 0.2763$, $p \leq 0.0521$) subjects (Figure 3).

DISCUSSION

Our study investigated the circulating plasma PCSK-9 level and its correlation with FBG, HbA1c, insulin, and lipid parameters in HC, T2DM-FD, T2DM-T, T2DM-N, and T2DM-DL subjects. Our results demonstrated that the PCSK-9 was significantly associated with FBG when matched with HC (Figure 2). These findings are in line with the previously published report that also observed similar correlation between the level of PCSK-9 and FBG in DM [17]. However, the mechanism of this association is still unknown. Although, existing data of the epidemiological, preclinical, and clinical investigations suggested a positive association of PCSK-9 with glycemic parameters [18].

FBG is notably impacted by the secretion of insulin and receptor-mediated glucose uptake in cells/target tissues. [19]. On the other hand, elevated blood sugar or food intake triggers insulin, insufficient to encounter hyperglycaemia, since either it was abnormally secreted or the inability of glucose transporters (GLUT-4) to translocate the glucose into the cells, a state called insulin resistance [20]. Our results demonstrated that elevated insulin was positively associated with the PCSK-9 in T2DM-FD, T2DM-T, T2DM-N, and T2DM-DL subjects when compared to the HC. However, according to several experimental studies high level of insulin does not influence the levels of PCSK-9 in both diabetic and nondiabetic subjects [21]. Furthermore, other researchers found no change in the PCSK-9 level between diabetic subjects taking insulin or not [22,23]. The high level of PCSK-9 in our study may be attributed to the elevated level of insulin in diabetic patients which is based on the fact that insulinemia modulates the PCSK-9 expression via a sterol regulatory element-binding protein-(SREBP-1c) pathway-dependent manner [19,24, 25]. Several previous studies have also found a positive correlation of PCSK-9 with insulin [26,27]. However, previously, it was also reported that treatment with glucose and lipid-lowering drugs further elevates the level of PCSK-9 in T2DM patients [28]. In the same study, the investigators also reported that taking insulin alone

as well as in combination with statins also increases the level of PCSK-9.

Persistent hyperglycemia causes specific structural and functional changes in proteins, mainly covalent modifications. Serum proteins such as hemoglobin undergo increased carbonyl vulnerability leading to non-enzymatic glycation to form glycated hemoglobin (HbA1c) [29]. The larger proportion of HbA1c in T2DM-FD, T2DM-N, and T2DM-DL cases was significantly associated with elevated PCSK-9 level compared to HC, whereas it was moderately associated in T2DM-T. A high level of HbA1c infers the severity of the disease as the formation of glycated hemoglobin is directly proportional to the glucose content as well as its duration of exposure [6,30]. Prior studies have also demonstrated the positive correlation of HbA1c with PCSK-9 in the T1DM and T2DM subjects population [22,31,32], whereas others observed no correlation [33]. Our findings are consistent with prior research [23].

In contrast, diabetes is well known to trigger the establishment of hypercholesterolemia via altered lipid synthesis as well as clearance by LDL-R [1]. PCSK-9 binds to the EGF-A domain of LDL-R and leads to its lysosomal degradation hence lowering the value of LDL-R [11]. A strong association of PCSK-9 with LDL-C in T2DM-N and T2DM-DL patients was also reported in our study. Interestingly, the same was also observed in HC, T2DM-FD, and T2DM-T subjects. The observed correlation between PCSK-9 and LDL-C is well supported by the previous report [23]. This rise in LDL-C may be attributed to the PCSK-9-mediated degradation of LDL-R, which ultimately resulted in compromised LDL-C clearance for circulation [9,34]. Moreover, the high functionality of PCSK-9 in T2DM-DL subjects may further be linked to hyperinsulinemia, a well-known factor for PCSK-9 upregulation [19]. On the other hand, the high level of PCSK-9 in T2DM-DL subjects in our study, irrespective of the statin therapy, may also be accredited to the ability of statins to fuel the PCSK-9 expression via SREBP-2 and hepatocyte nuclear factor-1-dependent mechanism as documented previously [8,35]. It was also found that treatment with statins decreases the HDL-C level in diabetes [36].

Contrary to LDL-C, elevated HDL-C level promotes healthy cardiovascular functioning and lipid homeostasis owing to its ability to reverse cholesterol transport as well as an inbuilt aryl-esterase-dependent antioxidant potential [10,35]. Herein, significantly diminished level of HDL-C was reported in T2DM-N and T2DM-DL subjects, that was inversely associated with elevated PCSK-9 level when compared to the HC, T2DM-FD subjects. A slight negative association was also observed in T2DM-T subjects as they have a slight hike in PCSK-9. Our observations are well justified by the previous studies documenting an inverse association between the level of PCSK-9 and HDL-C in diabetic dyslipidemia [9,36-38]. Similarly, the TG level exhibited a strong positive association with PCSK-9. This rise in TG in T2DM-N and T2DM-DL subjects in this study

was possibly achieved as a consequence of compromised lipoprotein lipase (LPL) activity [35] where the LPL functionality is greatly influenced by the action of apolipoprotein C-III [35]. The level of PCSK-9 has previously been shown to exhibit a positive correlation with TGs [39,40]. The same pattern was observed in the case of TC in our study which is a direct reflection of the level of lipoproteins.

To the best of our knowledge, our study for the first time demonstrated the level of PCSK-9 and its correlation with the markers of diabetes and its associated complication including dyslipidemia in the northeastern population of India. However, the conclusion is based on our pilot study. Further cohort studies on a larger population with multiple stratifications are needed for a better understanding of the role of PCSK-9 in the establishment, early diagnosis, and therapeutic management of diabetes as well as its complications. Although the exact mechanism underlying the pathophysiological role of PCSK-9 in diabetes and its complications has not yet been delineated, elevated level of PCSK-9 in diabetic dyslipidemic subjects indicates its direct role in the progression of diabetes and its complications.

CONCLUSION

Considering the detrimental role of PCSK-9 in the etiology of CVD and other metabolic disorders, herein we quantified the level of PCSK-9 in HC, T2DM-FD, T2DM-T, T2DM-N, and T2DM-DL subjects and assessed its correlation with the cardiometabolic variables in Indian population. Our first-of-its-kind clinical study aiming to quantify the circulatory PCSK-9 level in the Indian population concluded that PCSK-9 was elevated in T2DM-T, T2DM-N, and T2DM-DL subjects, and high levels of PCSK-9 significantly correlated with the severity of diabetes and associated complications. Moreover, such elevation in PCSK-9 might be attributed to the lipid- and glucose-lowering medication-induced SREBP-2-dependent mechanisms. Our findings might be helpful in the early diagnosis and management of these ailments by targeting the functionality of PCSK-9. Further cohort studies in larger populations are required to get a generalization regarding the role of PCSK-9 in DM and associated complications.

Ethical Approval:

Prior approval for the current study was obtained from IEC, IIMS&R, Lucknow, India (File No. IEC/IIMS&R/2017/38). The work was done according to the Declaration of Helsinki.

Informed Consent:

All the participants of this study were aware of all the procedures and aims of this study and written informed consent was obtained from all participants.

Source of Funds:

The Department of Health Research (DHR), MoHFW, Govt. of India, is highly acknowledged for awarding the Young Scientist Grant (YSS Project No. 12014/13/2019-HR) to Dr. S. S. Alvi. The authors are thankful to DST for infrastructural support to the Department of Biosciences, Integral University under the FIST program. This paper has Integral University manuscript communication no. IU/R&D/2022-MCN0001662.

Declaration of Interest:

The authors declare that there is no conflict of interest.

References:

1. Nabi R, Alvi SS, Saeed M, Ahmad S, Khan MS. Glycation and HMG-CoA Reductase Inhibitors: Implication in Diabetes and Associated Complications. *Curr Diabetes Rev* 2019 Apr 1;15(3): 213-23. (PMID: 30246643)
2. Sun H, Saedi P, Karuranga S, et al. IDF Diabetes Atlas: Global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045. *Diabetes Res Clin Pract* 2022 Jan;183: 109119. (PMID: 34879977)
3. Dronavalli S, Duka I, Bakris GL. The pathogenesis of diabetic nephropathy. *Nat Clin Pract Endocrinol Metab* 2008;4(8):444-52. (PMID: 18607402)
4. Einarson TR, Acs A, Ludwig C, Panton UH. Prevalence of cardiovascular disease in type 2 diabetes: A systematic literature review of scientific evidence from across the world in 2007-2017. *Cardiovasc Diabetol* 2018;17(1):83. (PMID: 29884191)
5. Ahmad P, Alvi SS, Iqbal J, Khan MS. Identification and evaluation of natural organosulfur compounds as potential dual inhibitors of α -amylase and α -glucosidase activity: an in-silico and in-vitro approach. *Med Chem Res* 2021;30(12):2184-202. <https://doi.org/10.1007/s00044-021-02799-2>
6. Nabi R, Alvi SS, Shah A, et al. Ezetimibe attenuates experimental diabetes and renal pathologies via targeting the advanced glycation, oxidative stress and AGE-RAGE signalling in rats. *Arch Physiol Biochem* 2021. (PMID: 33508970)
7. Alvi SS, Iqbal D, Ahmad S, Khan MS. Molecular rationale delineating the role of lycopene as a potent HMG-CoA reductase inhibitor: in vitro and in silico study. *Nat Prod Res* 2016;30(18): 2111-4. (PMID: 26548547)
8. Waiz M, Alvi SS, Salman Khan M. Potential dual inhibitors of PCSK-9 and HMG-R from natural sources in cardiovascular risk management. *EXCLI J* 2022;21:47-76. (PMID: 35221836)
9. Alvi SS, Ansari IA, Khan I, Iqbal J, Khan MS. Potential role of lycopene in targeting proprotein convertase subtilisin/kexin type-9 to combat hypercholesterolemia. *Free Radic Biol Med* 2017 Jul; 108:394-403. (PMID: 28412198)
10. Ahmad P, Alvi SS, Iqbal D, Khan MS. Insights into pharmacological mechanisms of polydatin in targeting risk factors-mediated atherosclerosis. *Life Sciences* 2020 Aug 1;254:117756. (PMID: 32389832)
11. Goldstein JL, Brown MS. A century of cholesterol and coronaries: from plaques to genes to statins. *Cell* 2015;161(1):161-72. (PMID: 25815993)

12. Peng J, Zhu CG, Li JJ. The predictive utility of circulating PCSK9 levels on diabetes mellitus. *Cardiovasc Diabetol* 2021 Feb 13;20(1):45. (PMID: 33581713)
13. Konarzewski M, Szolkiewicz M, Sucajtys-Szulc E, et al. Elevated Circulating PCSK-9 Concentration in Renal Failure Patients is Corrected by Renal Replacement Therapy. *Am J Nephrol* 2014; 40(2):157-63. (PMID: 25171595)
14. Mahboobnia K, Pirro M, Marini E, et al. PCSK9 and cancer: Rethinking the link. *Biomed Pharmacother* 2021 Aug;140:111758. (PMID: 34058443)
15. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis Model Assessment: Insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985 Jul;28(7):412-9. (PMID: 3899825)
16. Levey AS, Eckfeldt JH. Estimating Glomerular Filtration Rate Using Serum Creatinine. *Clin Chem* 2017;63(6):1161-2. (PMID: 28381407)
17. Han E, Cho NH, Moon SS, Cho H. Comparison of serum PCSK9 levels in subjects with normoglycemia, impaired fasting glucose, and impaired glucose tolerance. *Endocrinol Metab (Seoul)*. 2020; 35(2):480-3. (PMID: 32615732)
18. Xu JN, Wang TT, Shu H, Shi SY, Tao LC, Li JJ. Insight into the role of PCSK9 in glucose metabolism. *Clin Chim Acta* 2023 Jul; 547:117444. (PMID: 37315725)
19. Miao J, Manthena PV, Haas ME, et al. Role of insulin in the regulation of proprotein convertase subtilisin/kexin type 9. *Arterioscler Thromb Vasc Biol* 2015;35(7):1589-96. (PMID: 26023080)
20. Leto D, Saltiel AR. Regulation of glucose transport by insulin: traffic control of GLUT4. *Nat Rev Mol Cell Biol* 2012 136;13(6): 383-96. (PMID: 22617471)
21. Kappelle PJWH, Lambert G, Dullaart RPF. Plasma proprotein convertase subtilisin-kexin type 9 does not change during 24h insulin infusion in healthy subjects and type 2 diabetic patients. *Atherosclerosis* 2011;214(2):432-5. (PMID: 21122860)
22. Bojanin D, Vekic J, Milenkovic T, et al. Association between proprotein convertase subtilisin/kexin 9 (PCSK9) and lipoprotein subclasses in children with type 1 diabetes mellitus: Effects of glycemic control. *Atherosclerosis* 2019 Jan 1;280:14-20. (PMID: 30453116)
23. Yang SH, Li S, Zhang Y, et al. Positive correlation of plasma PCSK9 levels with HbA1c in patients with type 2 diabetes. *Diabetes Metab Res Rev* 2016;32(2):193-9. (PMID: 26377225)
24. Momtazi AA, Banach M, Pirro M, Stein EA, Sahebkar A. PCSK9 and diabetes: is there a link? *Drug Discov Today* 2017 Jun;22(6): 883-95. (PMID: 28111330)
25. Cui CJ, Li S, Li JJ. PCSK9 and its modulation. *Clin Chim Acta* 2015;440:79-86. (PMID: 25444750)
26. Guo W, Gong Y, Gu Y, et al. Circulating PCSK9 levels and 2-hPG are positively correlated in metabolic diseases in a Chinese Han population. *Lipids Health Dis* 2018;17(1):15. (PMID: 29343301)
27. Hasan H, Attlee A, Raigangar V, Madkour M, Awadallah S. Proprotein convertase subtilisin/kexin type 9 (PCSK9) and metabolic syndrome components among young adult females. *Diabetes Metab Syndr* 2017 Nov;11 Suppl 1:S337-41. (PMID: 28283395)
28. Awadallah S, Taneera J, Mohammed AK, Unnikannan H, Sulaiman N. Combined intake of glucose-and lipid-lowering medications further elevates plasma levels of PCSK9 in type 2 diabetes patients. *Diabetes Metab Syndr* 2020 Nov-Dec 1;14(6): 2087-92. (PMID: 33142230)
29. Nabi R, Alvi SS, Shah MS, et al. A biochemical & biophysical study on in-vitro anti-glycating potential of iridin against d-Ribose modified BSA. *Arch Biochem Biophys* 2020;686:108373. (PMID: 32325089)
30. Akhter F, Alvi SS, Ahmad P, Iqbal D, Alshehri BM, Khan MS. Therapeutic efficacy of *Boerhaavia diffusa* (Linn.) root methanolic extract in attenuating streptozotocin-induced diabetes, diabetes-linked hyperlipidemia and oxidative-stress in rats. *Biomed Res Ther* 2019;6(7):3293-306. <http://bmrar.org/index.php/BMRAT/article/view/556>
31. Laugier-Robiolle S, Vergès B, Le Bras M, et al. Glycaemic control influences the relationship between plasma PCSK9 and LDL cholesterol in type 1 diabetes. *Diabetes Obes Metab* 2017;19(3): 448-51. (PMID: 27804190)
32. Levenson AE, Shah AS, Khoury PR, et al. Obesity and type 2 diabetes are associated with elevated PCSK9 levels in young women. *Pediatr Diabetes* 2017;18(8):755-60. (PMID: 28093849)
33. Brouwers MCGJ, Trout JS, van Greevenbroek MMJ, et al. Plasma proprotein convertase subtilisin kexin type 9 is not altered in subjects with impaired glucose metabolism and type 2 diabetes mellitus, but its relationship with non-HDL cholesterol and apolipoprotein B may be modified by type 2 diabetes mellitus: The CODAM study. *Atherosclerosis* 2011 Jul;217(1):263-7. (PMID: 21497351)
34. Libby P, Tokgözoğlu L. Chasing LDL cholesterol to the bottom-PCSK9 in perspective. *Nat Cardiovasc Res* 2022;1(6):554-61. <https://doi.org/10.1038/s44161-022-00085-x>
35. Alvi SS, Ansari IA, Ahmad MK, Iqbal J, Khan MS. Lycopene amends LPS induced oxidative stress and hypertriglyceridemia via modulating PCSK-9 expression and Apo-CIII mediated lipoprotein lipase activity. *Biomed Pharmacother* 2017;96:1082-93. (PMID: 29174038)
36. Vergès B, Florentin E, Baillet-Rudoni S, et al. Rosuvastatin 20 mg restores normal HDL-apoA-I kinetics in type 2 diabetes. *J Lipid Res* 2009;50(6):1209-15. (PMID: 19168444)
37. Haase CL, Tybjerg-Hansen A, Nordestgaard BG, Frikke-Schmidt R. HDL cholesterol and risk of type 2 diabetes: A mendelian randomization study. *Diabetes* 2015;64(9):3328-33. (PMID: 25972569)
38. Adiels M, Olofsson SO, Taskinen MR, Borén J. Diabetic dyslipidaemia. *Curr Opin Lipidol* 2006;17(3):238-46. (PMID: 16680028)
39. Brouwers MCGJ, Van Greevenbroek MMJ, Konrad RJ, Trout JS, Schaper NC, Stehouwer CDA. Circulating PCSK9 is a strong determinant of plasma triacylglycerols and total cholesterol in homozygous carriers of apolipoprotein ε2. *Clin Sci (Lond)* 2014; 126(9):679-84. (PMID: 24308640)
40. Chernogubova E, Strawbridge R, Mahdessian H, et al. Common and low-frequency genetic variants in the pcsk9 locus influence circulating PCSK9 levels. *Arterioscler Thromb Vasc Biol* 2012; 32(6):1526-34. (PMID: 22460556)