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RESEARCH

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CD36 gene variant rs1761667(G/A) as a biomarker in obese type 2 diabetes mellitus cases

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Abstract

Background Several reports discussed a connection between CD36 genotypes associated with obesity, influencing the development of Type 2 diabetes mellitus (T2DM). Therefore, this study examines the prognostic value of CD36 polymorphism rs1761667 (G/A) in individuals with obese T2DM. The investigation also explores the correlation between this genetic variation and the clinical/biochemical parameters of the subjects.

Methods Blood samples of a total of 475 subjects from north India were collected from the outpatient unit (OPD), Department of Medicine, KGMU, Lucknow as per inclusion/exclusion criteria. Anthropometric details of study subjects were recorded and biochemical parameters were estimated in 250 T2DM cases, 75 obese T2DM cases, and 150 controls. The CD36 gene variant rs1761667 (G/A) was subject to genotypic analysis using the polymerase chain reaction-restriction fragment length polymorphism (PCR–RFLP) method, utilizing specific primers and *HhaI* enzyme. All statistical analysis was done using SPSS (ver. 21.0) and Prism (5.01) software.

Results Fasting plasma glucose (FPG), systolic blood pressure (SBP), post-prandial glucose (PPG) were significant in T2DM subjects. Lipid profile such as Total Cholesterol (TC), Low-Density Lipoprotein (LDL) and Very Low-Density Lipoprotein (VLDL) were also found significantly associated with obese T2DM cases. GA and AA genotypes of rs1761667 (G/A) showed significant associations in obese T2DM cases. The GA genotype demonstrated a considerable association ($P < 0.001$) with a 2.77-fold increased susceptibility to the high risk of T2DM. The AA genotype was found to be significantly associated ($P = 0.008$) with 2.94-fold higher risk of T2DM in obesity while 9.33 folds significant risk of developing obesity in T2DM cases.

Conclusions The risk of obesity in T2DM cases can be assessed by genotyping the *CD36* genetic variant rs1761667 (G/A). However, raised FPG, PPG, TC, LDL, and VLDL showed poor prognosis in obese T2DM cases. *CD36* gene variant can be proposed as a prognostic biomarker for risk prediction of T2DM and obesity, while anthro-biochemical risk factors as preventive biomarker.

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Highlights

- GA genotype of rs1761667 is significantly associated with T2DM with 2.77 folds higher risk.3
- FPG, PPG, TC, LDL, and VLDL also showed significant association with T2DMObese cases.
- rs1761667 can be proposed as a prognostic biomarker for risk prediction of T2DM and obesity.
- Anthro-biochemical risk factors can be used as a preventive biomarker.

Keywords CD36 gene variants, Obesity, Prognostic biomarker, Risk assessment, Type 2 diabetes mellitus, Biochemical risk factors

Background

In terms of fatality rates, Diabetes mellitus (DM) is the world's ninth most common disease. In the year 2021, 6.7 million people died from diabetes worldwide, among which 537 million people were diagnosed while 231.9 million remained undiagnosed [1]. The worldwide incidence of type 2 diabetes mellitus (T2DM) has risen dramatically, compromising people's health, and adding to societal financial challenges. [2]. Although a variety of organs can malfunction and cause glucotoxicity, pancreatic β-cell dysfunction that is crucial pathophysiology of diabetes. Thus, targeting and preventing β-cell dysfunction is a vital part of the T2DM treatment strategy [3].

Upon exposure to an abundance of nutrients, pancreatic β-cells exhibit an initial rise followed by a subsequent decline in insulin secretion. There are several hypotheses, including oxidative stress, ER (endoplasmic reticulum) stress, mitochondrial malfunction, lipotoxicity, glucotoxicity and glucolipotoxicity (a combination of both). However, the exact cause of metabolic alteration is still unknown. Though insulin secretion is stimulated by free fatty acid (FFA), chronically increased FFA leads to β-cell dysfunction both in vivo and in vitro, causing lipotoxicity [4].

CD36 (Cluster Determinant 36) is a 36 kb gene, situated at the long arm of chromosome 7 at 11.2 band (7q11.2), and contain 17 exons and 16 introns [5]. *CD36* gene encodes a CD36 membrane glycoprotein found on the plasma membrane and made up of a solitary polypeptide chain that weighs 88-kDa and is composed of 472 amino acids [6]. The *CD36* gene is a component of the active FFA transport system and has been found in a variety of tissues including the liver, muscle, and pancreatic cells. [4]. Previous studies have been reported that *CD36* induction increase FFA uptake in several cells linked with insulin resistance in diabetes [7, 8]. Apart from FFAs, CD36 binds with lipoproteins and plays major role in cholesteryl esters, cholesterol and oxidized low/high-density lipoproteins [9–11]. Therefore, by virtue of its capacity to bind with diverse ligands and modulate their functions, CD36 could potentially impact various metabolic syndromes, including insulin resistance, atherosclerosis, diabetes, and inflammation [12, 13]. In recent times, research has revealed that a lack of CD36 reduces oxidative stress in the heart due to obesity by lowering the activity of NADPH oxidase [14]. Additionally, there are reports that link CD36 genotypes to obesity, which consequently associated with the risk T2DM [15].

Moreover, CD36 polymorphisms are linked to increased hypertension, and high-density lipoprotein (HDL) and also showed protection against metabolic syndrome [16, 17]. Several previous studies led us to explore the prospect of a particular single nucleotide polymorphism (SNP) rs1761667 (G > A), in the *CD36* gene as a biomarker in obese T2DM cases [18–22].

Materials and methods

Selection of study subjects

The study was carried out after permission from the Institutional Ethics Committee. (1294/Ethics/2020 Dated: 09.12.2020) at King George's Medical University, Lucknow, India, and after written informed consent from all study subjects. Cases and controls were selected from the outpatient unit (OPD), Department of Medicine, KGMU, Lucknow, as per inclusion and exclusion guideline by American Diabetes Association (ADA) guidelines [4]. The study involved a total of 475 participants, including 250 individuals with T2DM, 75 with obesity-related T2DM, and 150 age- and sex-matched controls.

Clinical evaluation and sample collection

Anthropometric measurements, such as waist-hip ratio (WHR), age (in years), body mass index (BMI), diastolic blood pressure (DBP), and systolic blood pressure (SBP) were collected [23].

The venous blood from all the subjects were collected in 2 different vials (5 ml), 3 ml in EDTA and 2 ml in plain vials for serum separation under the supervision of expert clinicians. Biochemical parameters were estimated in all study subjects. Blood samples (in plain vials) were centrifuged at 1157 × g (3000 RPM) for 10 min, sera was collected and stored at − 20°C until further use. Measurements of lipid profile (mg/dl) and plasma glucose (mg/dl)

including high-density lipoprotein (HDL), total cholesterol (TC), triglyceride (TG), and low-density lipoprotein (LDL) were carried out using commercially available kits (Meril, India) and analyzed by Spectra Blood Analyzer (Merck, India).

Genotyping of CD36 rs1761667 (G > A)

Genomic DNA of each individual was isolated from venous blood leucocytes via standard salting out method with minor modifications. [24, 25]. The quantity and quality of DNA were estimated using a Bio-Photometer (Eppendorf, Germany) and agarose gel electrophoresis, respectively (Fig. 1).

The SNP rs1761667 (G > A) CD36 was genotyped by PCR–RFLP technique in controls and cases using specific primers. The forward (5′-CAA AAT CAC AAT CTA TTC AAG ACCA-3′) and reverse (5′-TTT TGG GAG AAA TTC TGA AGA G-3′) primers were designed using Primer3 software (Online version) and validated using the UCSC genome browser. A reaction mixture (15 µl) was prepared, consisting of genomic DNA (100–150 ng), 200 µM dNTPs, 10 pmol of primer, buffer (0.1% gelatin, 100 mM Tris, 500 mM KCl, pH 9.0, 15 mM MgCl2), and Taq polymerase (0.3U) (GeNei, Bangalore). The mixture was processed in a thermocycler (Eppendorf, Germany).

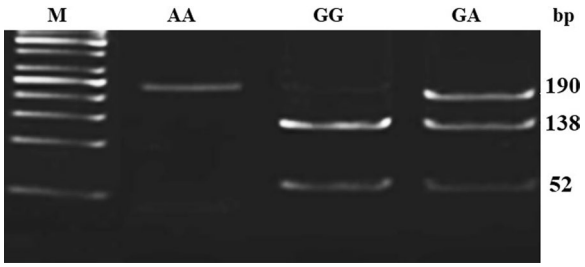

Fig. 1 Polyacrylamide gel showing the genotypes of CD36 rs1761667 (G/A) variant; M, 50 bp ladder. *M Marker

After amplification, the products of PCR were digested by the endonuclease *HhaI* selected by NEB cutter (ver. Online) and the digested products were checked on 12% polyacrylamide gel electrophoresis (PAGE).

Statistical analysis

Each group's continuous variables were summarized as Mean ± SD and compared using the Student's t-test. The calculation of sample size was done utilizing QUANTO software (version 1.2.4). A comparison of allele frequencies and carriage rates between the two groups was performed using a 2×2 contingency table, while genotype frequencies were analyzed using a 2×3 contingency table with χ2 and Fisher's exact tests. $P < 0.05$ was used to determine statistical significance.

Results

Genotyping of rs1761667 of CD36 was performed in a total number of 475 individuals, including 250 cases of T2DM, 75 cases of Obese T2DM, and 150 age/sex-matched controls. The PCR product (190 bp) was cleaved by *HhaI* endonuclease into two fragments, 138 and 52 bp representing the GG genotype, whereas three fragments viz.,190, 138 and 52 bp represented the GA genotype and the undigested 190 bp represented the AA genotype. The allelic and genotypic frequencies are calculated as represented in Tables 1, 2, and 3. All allelic and genotypic frequencies were consistent with Hardy–Weinberg equilibrium (HWE).

The analysis of genotypes indicated a marked increase in the prevalence of the heterozygous genotype 'GA' in T2DM cases compared to the healthy controls, with a significant difference ($P < 0.001$) (Table 1). Furthermore, the recessive genotype 'AA' exhibited a notable distinction (P value = 0.034) between T2DM cases and healthy controls. Genotypic and allelic frequencies of recessive homozygous genotype 'AA' ($P = 0.008$) and allele 'A'

Table 1 Genotypic and allelic frequencies of *CD36* gene polymorphism in controls ($n = 150$) and cases ($n = 250$)

***CD36* (rs1761667)**				
Genotype frequency				
	Controls (%) ($n = 150$)	Cases (%) ($n = 250$)	*P*	OR (CI = 95%)
GG	60 (40)	56 (22)	–	1.0 (Ref)
GA	73 (49)	189 (76)	**< 0.001**	2.77(1.76–4.40)
AA	17 (11)	05 (02)	**0.034**	0.31(0.12–0.85)
Allele frequency				
	Controls (%) ($n = 300$)	Cases (%) ($n = 500$)	*P*	OR (CI = 95%)
G	193 (64)	301 (60)	0.260	1.19(0.89–1.61)
A	107 (36)	199 (40)		

Bold font indicates statistically significant values

*T2DM: Type 2 diabetes mellitus; OR: Odds Ratio

Table 2 Genotypic and allelic frequencies of *CD36* gene polymorphism in healthy controls ($n=150$) and T2DMObesecases ($n=75$)

***CD36* (rs1761667)**				
Genotype frequency				
	Controls (%) ($n=150$)	T2DMObese cases (%) ($n=75$)	*P*	OR (CI = 95%)
GG	60 (40)	30 (40)	–	1.0 (Ref)
GA	73 (49)	20 (26.7)	0.097	0.54 (0.28–1.06)
AA	17 (11)	25 (33.3)	**0.008**	**2.94** (1.42–6.45)
Allele frequency				
	Healthy Controls (%) ($n=300$)	T2DMObese cases (%) ($n=150$)	*P*	OR (CI = 95%)
G	193 (64)	80 (53.3)	**0.031**	**1.58** (1.06–2.34)
A	107 (36)	70 (46.7)		

Bold font indicates statistically significant values

*T2DM: Type 2 diabetes mellitus; OR: Odds Ratio

Table 3 Genotypic and allelic frequencies of *CD36* gene polymorphism in T2DM ($n=250$) and T2DMObese ($n=75$) cases

***CD36* (rs1761667)**				
Genotype frequency				
	T2DM Cases (%) ($n=250$)	T2DMObese cases (%) ($n=75$)	*P*	OR (CI = 95%)
GG	56 (22)	30 (40)	–	1.0 (Ref)
GA	189 (76)	20 (26.7)	**< 0.001**	0.19(0.11–0.36)
AA	05 (02)	25 (33.3)	**< 0.001**	9.33(3.21–23.79)
Allele frequency				
	T2DM (%) ($n=500$)	T2DMObese cases (%) ($n=150$)	*P*	OR (CI = 95%)
G	301 (60)	80 (53.3)	0.156	1.32(0.91–1.91)
A	199 (40)	70 (46.7)		

Bold font indicates statistically significant values

*T2DM: Type 2 diabetes mellitus; OR: Odds Ratio

($P=0.031$) were found to be statistically significant as compared to healthy controls and T2DMObese cases (Table 2). Genotypic difference for 'GA' and 'AA' genotypes was statistically significant ($P<0.001$) among T2DM and T2DMObese cases (Table 3).

The anthropometric analysis in all three groups, i.e., healthy controls, T2DM and T2DMObese cases is shown in Table 4. The mean age of controls was 46.11 ± 11.20, T2DM cases was 48.21 ± 10.24 and that of T2DMObese cases was 48.27 ± 5.65. The waist-hip ratio (WHR) of controls, T2DM cases, and T2DMObese cases was 0.94 ± 0.04, 0.95 ± 0.51, and 0.99 ± 0.17, respectively. The Body Mass Index (BMI) of T2DM cases (24.53 ± 4.55) was slightly higher than controls (22.83 ± 3.75), whereas the BMI of T2DMObese cases was as much higher (31.61 ± 1.39). Systolic blood pressure (SBP) was significantly high in T2DM cases as compared with healthy controls (***P* = 0.002**).

Biochemical parameters were analyzed in all three groups (Healthy controls, T2DM cases, and T2DMObese cases) and values are represented as Mean ± SD in Table 4. A significant increase in Fasting Plasma Glucose (FPG), Post Prandial Glucose (PPG), Total Cholesterol (TC), and Very-Low Density Lipoprotein (VLDL) (***P* = 0.001**) was observed in both T2DM and T2DMO cases as compared with healthy controls. Triglycerides (TGL) was significantly increased in T2DMO cases (***P* = 0.001**). Low-Density Lipoprotein (LDL) was significantly increased in both T2DM (***P* = 0.001**) and T2DMO cases (***P* = 0.034**).

Biochemical parameters were examined across all three groups (Healthy controls, T2DM cases, and T2DMObese cases), and the values are presented as Mean ± SD in Table 4. A noteworthy elevation in Post Prandial Glucose (PPG), Total Cholesterol (TC), Fasting Plasma Glucose (FPG), and Very-Low Density Lipoprotein (VLDL) ($P=0.001$) was noted in both T2DM and T2DMO cases in comparison to healthy controls. Triglycerides (TGL) exhibited a significant increase in T2DMO cases ($P=0.001$). Moreover, Low-Density Lipoprotein (LDL) showed a significant rise in both T2DM ($P=0.001$) and T2DMO cases ($P=0.034$).

Table 4 Anthropometric and biochemical parameters in normal healthy controls, T2DM and T2DMObese individuals

Parameters	Controls ($n=150$)	T2DM ($n=250$)	*P*	T2DMObese cases ($n=75$)	*P* Value
Anthropometric measurements (Mean ± SD)					
Age (Years)	46.11 ± 11.20	48.21 ± 10.24	0.999	48.27 ± 5.65	0.999
Waist-Hip Ratio (WHR)	0.94 ± 0.04	0.95 ± 0.51	0.999	0.99 ± 0.17	0.999
Body Mass Index (BMI) (Kg/m2)	22.83 ± 3.75	24.53 ± 4.55	0.999	31.61 ± 1.39	0.148
Systolic Blood Pressure (SBP)	123.48 ± 11.96	136.52 ± 13.92	**0.002**	129.07 ± 7.38	0.633
Diastolic Blood Pressure (DBP)	85.94 ± 7.92	86.04 ± 8.78	0.999	81.87 ± 5.38	0.999
Biochemical Parameters (Mean ± SD) (mg/dl)					
Fasting Plasma Glucose (FPG)	96.52 ± 25.78	189.60 ± 47.52	**0.001**	179.16 ± 36.64	**0.001**
Post Prandial Glucose (PPG)	139.93 ± 9.92	283.75 ± 110.21	**0.001**	297.01 ± 51.44	**0.001**
Total Cholesterol (TC)	191.47 ± 14.60	210.90 ± 41.86	**0.001**	204.71 ± 36.47	**0.009**
Triglycerides (TGL)	119.68 ± 18.88	115.35 ± 27.46	0.554	190.27 ± 43.57	**0.001**
High-Density Lipoprotein (HDL)	43.80 ± 13.17	46.66 ± 9.30	0.999	52.04 ± 12.28	0.196
Low-Density Lipoprotein (LDL)	61.89 ± 19.59	135.36 ± 54.72	**0.001**	73.23 ± 16.66	**0.034**
Very Low-Density Lipoprotein (VLDL)	54.35 ± 2.10	23.80 ± 6.75	**0.001**	79.44 ± 41.49	**0.001**
Serum Creatinine (SC)	1.06 ± 0.13	1.05 ± 0.11	0.999	1.04 ± 0.11	0.999

Bold font indicates statistically significant values

*T2DM: Type 2 diabetes mellitus

Discussion

The CD36 is a transmembrane glycol protein serve as fatty acid translocase receptor associated with lipid metabolism, insulin resistance, obesity, inflammation, atherosclerosis, and thrombosis through the functional and physical interactions of proteins [5, 15, 26]. The transmembrane receptor CD36 is a scavenger receptor of class B that is expressed on a variety of cells including myocytes, adipocytes, hepatocytes, and macrophages. [27]. The SNP rs1761667 (G/A), a commonly reported promoter variant of *CD36* located on the upstream region of exons 1A and 1B has been associated to promote serum FFA. The receptor was also associated with susceptibility to coronary artery disease in T2DM subjects [28]. Alteration in CD36 gene polymorphisms (SNPs) have been linked with the pathogenesis of insulin resistance. and other metabolic diseases, such as cardiovascular disease, atherosclerosis, and obesity [29].

Alter polymorphism of *CD36* gene can cause various physiological conditions, such as diabetes, coronary, heart disease, sensory perception, lipid metabolism, etc. [30, 31]. Altered CD36 function is associated with reduced the potential to uptake of oxidized low-density lipoproteins (LDLs) in macrophages [32]. It is previously reported that the deficiency of CD36 is linked with high serum lipids with a poor metabolic profile in diabetic patients [33].

CD36 has been identified as a key mediator for fatty acid uptake in skeletal muscle cells, implying a strong relationship between obesity and muscle regeneration. [34]. The *CD36* gene (SNP) rs1761667 (A/G) is very common, its allele 'A' is responsible for downregulate its protein expression, leading to a significant association with taste perception [35]. A case–control study of an Egyptian population including metabolic syndrome cases and normal healthy controls reported that the SNP rs1761667 (G/A) is associated with the high risk of metabolic syndrome [29]. Another study in a Moroccan population including renal disease cases and healthy controls found that rs1761667 (G/A) would be a strong predictor of the onset of kidney disease [36].

Mutations in the *CD36* gene have been resulting in low adiponectin levels and altered insulin homeostasis in T2DM cases [37]. According to Enciso-Ramírez and Mayra et al., 2020, the *CD36* gene rs1761667 (G/A) has a significant connection with overweight and obesity in Mexican children. According to Heart and Aging Research in Genomic Epidemiology consortium, a genome-wide association study (GWAS) of 15 SNPs of *CD36* gene demonstrated its linked with the risk of stroke [38]. Another study of a Mexican American population reported that *CD36* gene is also associated with HDL [39]. However, Banerjee et al. [18] 2010 found that the GA genotype of *CD36* rs1761667 (G/A) was significantly associated in North Indian T2DM cases. Another study in a Chinese Han population reported that rs3211928 of *CD36* gene polymorphism is also susceptible to ischemic stroke [40]. Lee et al. [41] 2021 demonstrated that the SNP rs1761667 of *CD36* gene is significantly related with stroke and T2DM cases in the Korean population,

especially in male cases. In *CD36* SNP rs1761667, the homozygous AA genotype was reported more prevalent in the obese group than in the normal-weight group [42]. Bajit et al. [43] 2020 detected that oleic acid level was much increased in AG and AA genotypes in obese when compared with normal-weight subjects. A study in Tunisian women performed a lipid taste perception threshold to see the association with *CD36* (rs1761667) genes. The results confirmed that the AA genotype linked with high level of gustatory fat in obese women with [44].

Chu Y and team in 2017, in a Chinese Han population of postmenopausal females, indicated that the presence of the CT genotype of rs12998782 the GA genotype of rs1761667 and in *CD36* might be associated with an elevated susceptibility to the development of carotid atherosclerosis [45]. Pioltine et al. [46] 2016 found no association between rs1761667 genotypes and obesity risk. However, the study found a significant genetic relationship between the A allele of the *CD36* rs1761667 genotype and fat intake. A reduced fat and sugar intake was observed in obese children and adolescents. A study suggested that the AA genotype of *CD36* rs1761667 is related to increased fat intake as compared to AG and GG genotypes in LF (liver fibrosis) in CHC (chronic hepatitis C) cases [47]. In a prior study, we identified a noteworthy correlation between the CD36 promoter SNP rs1761667 G/A polymorphism and T2DM (Banerjee et al., 2010). Heterozygous genotype 'GA' rs1761667 G/A was found as more prevalent in T2DM cases and showed a significant association with diabetes (Table 1). Genotypic frequency distribution of 'GA' was quite higher among healthy controls and T2DM cases with Obesity (T2DMObese) but was not statistically significant (Table 2). Mutant homozygous genotype 'AA' of rs1761667 G/A polymorphism showed significant differences when compared to controls and T2DM cases ($P=0.034$), controls and T2DMObese ($P=0.008$) and T2DM cases with T2DMObese ($P<0.001$).

Several studies have established BMI as a highly reliable predictor of T2DM [48, 49]. According to Solakivi et al. [50] (2011), the homozygous 'AA' genotype of rs1761667 exhibited a significant association with reduced BMI compared to the GG and AG genotypes (with respective *P* values of 0.001, 0.005, and 0.013) at the ages of 40, 45, and 50 years. This study also discovered no link between CD36 rs1761667 and hypertension. Within our study cohort, a noteworthy difference in SBP was observed in comparison to the control group, with a significant *P* value of 0.002 (Table 4). Comparison of clinical profiles concerning VLDL, PPG, TC, FPG, and LDL was found as statistically significant among healthy controls, T2DM and T2DMObese subjects (Table 4). The TGL profile demonstrated a notable increase in T2DM obese individuals when compared to both controls and T2DM cases, with a significant *P* value of 0.001 (Table 4). Therefore, we assume that in our study population TGL can be considered as a strong predictor for obesity-related T2DM progression.

Conclusion

Individuals carrying the GA genotype of rs1761667 are prone to 2.77 times increased risk of developing T2DM, whereas those with the AA genotype show 2.94 times higher risk of developing T2DM accompanied by obesity. No notable associations were observed among anthropometric parameters and T2DM except Systolic Blood Pressure (SBP). Elevated levels of FBG, PPG, TC, and LDL were observed in both T2DM and T2DM obese cases.

Therefore, it can be concluded that rs1761667(G/A) polymorphism within the *CD36* gene could be a potential prognostic biomarker for differential susceptibility of the study population to T2DM and T2DM with Obesity. However, a population studies on a larger scale may be required to validate these findings.

Abbreviations

BMI	Basal metabolic index
CD36	Cluster of differentiation 36
EDTA	Ethyl diamine tetra acetic acid
FFA	Free fatty acid
FPG	Fasting plasma glucose
GWAS	Genome-wide association study
HDL	High density lipoproteins
HWE	Hardy–weinberg equilibrium
LDL	Low density lipoproteins
NEB	New England biolabs
PCR	Polymerase chain reaction
PPG	Post prandial glucose
RFLP	Restriction fragment length polymorphism
SBP	Systolic blood pressure
SNPs	Single nucleotide polymorphisms
T2DM	Type2 diabetes mellitus
T2DMObese	T2DM patients with obesity
TC	Total cholesterol
VLDL	Very low-density lipoproteins
WHO	World Health Organization

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Author contributions

AKS and AS have performed the experiments, analysis and prepared the manuscript. ASK designed the experiment, carried out data curation, validation and analysis. KU and SS helped in clinical data collection. MB conceptualized, edited, reviewed the manuscript and provided all laboratory facilities.

Availability of data and materials
This article contains all the data that was produced or examined during the study.

Declarations

Ethics approval and consent to participate
The research was conducted following the endorsement from the Institutional Ethics Committee (Approval Number: 1294/Ethics/2020 Dated: 09. 12. 2020) at King George's Medical University, Lucknow, India. Additionally, written informed consent was obtained from all participants involved in the study.

Competing interests
The authors declare no conflicting financial interests or personal relationships that might have impacted the work presented in this paper.

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