Association of cholesteryl ester transfer protein gene, TaqIB polymorphism with the risk of type 2 diabetes mellitus in North Indian population

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ABSTRACT: Aim:*Cholesterol ester transfer protein (CETP) regulates plasma lipid distribution. The present study aimed to investigate the relationship of TaqIB (rs708272) polymorphism in the cholesteryl ester transport protein (CETP) gene with the risk of T2DM and various metabolic factors in North Indian population.*

Methods: *The present case-control study included a total of 605 participants (324 T2DM patients and 281 healthy controls). Various anthropometric, biochemical and genetic parameters were studied in all the subjects. Genotyping of Taq1B polymorphism in CETP gene was performed by PCR-RFLP analysis.*

Results: *The results of this study indicate significantly higher values of body mass index, waist circumference and waist to hip ratio in T2DM subjects than controls (p=<0.005). Dyslipidemia represented by higher levels of triglycerides and reduced values of high density lipoprotein (HDL) was more predominant in T2DM subjects compared to healthy subjects.The frequencies of B1B1, B1B2 and B2B2 genotypes of CETP gene TaqIB polymorphism in control subjects were 24.2%, 43.8% and 32 %, and in T2DM patients were 27.5%, 50 % and 22.5% respectively. The frequency of the B2B2 genotype was significantly lower in T2DM patients than in healthy controls (22.5% vs. 32%). Logistic regression analysis of the data showsan odds ratio with 95% CI for B2B2 genotype of TaqIB polymorphism as0.62 (0.39-0.97;p=0.034), indicating its protective effect against the development of T2DM in our study population.*

Conclusion: *A significant protective effect of the B2B2 genotype of CETP gene Taq1B polymorphism was observed against susceptibility of T2DM in North Indian population.*

Key Words: *CETP, Gene polymorphism, Type 2 diabetes mellitus, abdominal obesity, dyslipidemia.*

INTRODUCTION

Type 2 Diabetes Mellitus (T2DM) is a non-communicable disease characterized by chronic hyperglycemia, impaired insulin secretion or insulin resistance, which accounts for more than 95% of the total diabetic cases [1]. It is a major health problem of the society worldwide that causes severe acute and chronic complications resulting in increased morbidity, disability and early mortality [2]. There is a marked increase in the prevalence of T2DM in recent years, posing threat to global economy and has hasten the global ageing process. Presently, T2DM affects 285 million people across the globe and is predicted to rise to 642 million by 2040 [3].About 80% of the T2DM affected people are from middle- and low-income countries. The recent estimates by the International Diabetes Federation (IDF) indicate that so far 85 million individuals suffering from T2DM in India and this number is expected to rise to 109 million by year 2035 [4] thereby making it diabetic capital of the world. The health care expenditure associated with T2DM can significantly affect economy. Many influences affect the prevalence of disease throughout a country and identification of those factors is necessary to facilitate change when facing health challenges. T2DM is a polygenic metabolic disorder, in which both genetic and environmental factors along with dietary intake plays important role in its pathogenesis[5]. Increased consumption of western diet (refined grains and sugar-sweetened beverages) along with a sedentary lifestyle are the primary cause of T2DM [6, 7].The genome-wide association study (GWAS) has identified variants in many loci showing association with the risk of development of T2DM [8]. These genetic determinants together with various environmental factors would help people to better understand the underlying pathogenesis of a disease at the gene level, framing policies to counter the economic burden associated with the disease and pave a path for new improved and preventive therapeutic measures[9].

The increased risk of T2DM is associated with alterations in the lipid profile in plasma which is characterized by elevated triglyceride (TG) levels, small dense low-density lipoprotein-cholesterol (LDL-C) particles, and cholesterol, along with low levels of HDL cholesterol in T2DM patients [10]. Genetic polymorphisms of the enzymes and proteins involved in lipid metabolism like cholesteryl ester transfer protein (*CETP*) is associated with susceptibility to primary hyperlipidemia and T2DM [11].The human *CETP* geneis located on chromosome 16q21 and contains 25 kb genomic DNA with 16 exons [12, 13] and it regulates the transfer and exchange of cholesteryl ester (CE) and triglyceride (TG) between the plasma lipoproteins, and participates in high density lipoprotein- cholesteryl ester (HDL-CE) and apo A-I catabolism [14].Several studies have been conducted on the *CETP*polymorphism[15-21] which alters CETP activity; however, the results are inconsistent. Taq1B polymorphism has been the mostly studied. It is characterized by a silent base change in *CETP* gene at the 277th nucleotide in the intron 1, i.e., G to A that disrupts the TaqI restriction site [22]. This polymorphism has been associated with reduced CETP mass and raised levels of HDL-cholesterol [23]. Association of TaqIB polymorphism with HDL-C concentration is also influenced by various environmental factors such as alcohol consumption, smoking, exercise*etc.*which modulate HDL-C levels through the activities of lipases or lipid transfer proteins [24, 25]. Therefore, the present study was designed to investigate TaqIB polymorphism of the *CETP* gene in North Indian population, its relationship with T2DM and various environmental factors.

MATERIAL AND METHODS

Human Study subjects

The present study was commenced in year 2013 with the objectives designed to investigate the genetic and environmental risk factors associated with the T2DM in an Asian Indian population in north India. A total of 605 participants (324 T2DM patients and 281 healthy controls) were enrolled in this case-control study from north Indian population attending endocrinology OPD of PGIMER. The diagnosis of T2DM was done using criteria established by American Diabetes Association as follows: A medical record indicating either a fasting glucose levels >7.0 mmol/l or >126 mg/dl after a minimum 12-hour fast or 2-hour post glucose level (oral glucose tolerance test or 2-h OGTT) > 11.1 mmol/l or >200 mg/dl on more than one occasion with symptoms of diabetes. A standard questionnaire was used to collect information regarding demographic and socioeconomic characteristics. For all participants, data regarding age, sex, educational status, physical activity, dietary habits, family history of the disease and individual's smoking and alcohol use etc. were recorded.

Ethical considerations

The study protocol was approved by Institutional Ethics Committees of the participating institutes. A model consent form adhering to Indian, and International guidelines regarding the use of human subjects was used. After providing the necessary information to the study subjects, a written informed consent of the participation was obtained.

Inclusion/ Exclusion criteria

Patients or Healthy controls belonging to north Indian states only were included in this study. Subjects having family history of diabetes were not included as healthy controls. Controls were individuals with no clinically significant abnormal physical findings. They had normal blood pressure, heart rate and had normal fasting (<126 mg/dl) glucose level. All the subjects could communicate, competent and willing to give informed consent. The patients were clinically and biochemically confirmed as type 2 diabetes and were of age 25 years and above.

Anthropometric parameters

Standard anthropometric measurements were performed including stature, weight, waist and hip circumferences. Height was measured by wall mounted stature meter and weight using a portable balance beam scale. Waist and Hip circumferences were measured with a metal tape using standard procedures. Blood pressure was measured by Omron blood pressure machine in sitting position from the left arm resting on the table, with legs uncrossed and feet flat.

Biochemical characteristics

Venous blood samples were extracted from each subject after 12 hours of fasting. A serum sample was analyzed for fasting serum glucose, creatinine, and lipid profile [(triglycerides (TG), total cholesterol (TC), high density lipoprotein (HDL) cholesterol, low density lipoprotein (LDL) cholesterol, very low density lipoprotein (VLDL) cholesterol)]. Fasting and random blood glucose levels were measured using a portable glucometer (Abbott OptiumXceed, USA). Low density lipoprotein (LDL) level was calculated by using

Friedewald formula i.e. LDL-C = TC – [HDL-C – (TG in mg/dl/5)] [26].

Derived measures and phenotypic evaluations

Body mass index (BMI) was calculated according to Quetelet equation (BMI = weight in kilograms/height in meters squared). Waist to hip ratio (WHR) was calculated as ratio of abdomen to hip circumferences. The abdominal obesity was measured according to the new cut offs proposed for South Asian Indians as mentioned in our previous study [27] i.e. WHR >0.89 for men and > 0.81 for women. BMI <23 kg/m^2 has been proposed for low risk, 23-27.5 kg/m2 for increased risk and ≥27.5 kg/m2 for high risk for developing weight- related diseases in Asian populations. Body fat percentage (BF %) was calculated according to the method of Lean et al. [28] using following formulae; BF% for men = [(0.567 × waist circumference in cm) + (0.101 × age in years)] − 31.8; and BF% for women = [(0.438 × waist circumference in cm) + (0.221 × age in years)] − 9.4. High blood pressure or hypertension was defined by systolic blood pressure ≥140 mmHg and diastolic blood pressure ≥90 mmHg or or use of any antihypertensive medication.Dyslipidemia was defined according to National Cholesterol Education Program criteria [29] as the elevation of cholesterol (>200mg/dl), triglycerides (>150mg/dl) or both, or a low HDL level (<40 mg/dl in men and <50 mg/dl in women).

Genotyping of CETP gene

A fragment of 535 bp in intron 1 of the *CETP* gene was amplified by polymerase chain reaction (PCR) in a DNA Thermal Cycler as described by Fumeron*et al* [30] with the use of oligonucleotide primers. The forward primer sequence used was 5'- CACTAGCCCAGAGAGAGGAGTGCC-3' and the reverse primer sequence was 5'- CTGAGCCCAGCCGCACACTAAC-3'. The 25µl reaction mixture contained 200 ng genomic DNA, 1X Taq polymerase buffer, 1.5mM $MgCl_2$, 10pmol of each primer, 200µmol/l dNTPs and 1U of Taq DNA polymerase (Thermo). DNA amplification was carried out on thermal cycler (Eppendorf Mastercycler Nexus gradient) with an initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 58°C for 30 s, and extension at 72°C for 45 s, with a final extension step of 10 min at 72°C. The 535 bp fragment in the intron 1 of the CETP gene was amplified and digested overnight with Taq I restriction enzyme (Thermo Fischer scientific Inc.) at 65°C. The digestion included 5µl PCR product, 0.125µl endonuclease, 1.6µl 10X buffer, and 8.3µl DDW (total 15µl). The digested product was resolved using 2.5% Etbr stained agarose gel electrophoresis. The resulting fragments were 174 and 361 bp for B1 allele (presence of the restriction site) and 535 bp for B2 allele (absence of the restriction site).

Statistical Analysis

Results were expressed as mean ± SD. The genotypic distributions were tested for deviation from Hardy–Weinberg equilibrium. Chi-square analysis was applied to test the significance of differences in in genotypic and allelic frequencies. Group comparisons were done using unpaired t-tests. All the p-values <0.05 (two-tailed) were considered as significant difference. Logistic regression analyses were performed to correlate various clinical parameters with disease and to calculate odds ratios (ORs) and 95% CIs for each risk factor. Statistical analysis was performed using IBM-SPSS for Windows, version 20 (SPSS, Inc., Chicago, IL).

RESULTS

Basic characteristics of the study subjects

Table 1 and 2 shows the anthropometric and biochemical characteristics of the study subjects. There was a significant difference in the age of the diabetic and control subjects (47.13±11.7 vs. 55.3±11.3, p=0.001). T2DM subjects showed predominant abdominal obesity reflected by significantly higher values of mean BMI (26.92±4.14 vs. 25.8±4.6, p=0.002), waist circumference (93.43±9.96 vs. 87.35±10.98, p=0.000) and waist to hip ratio (0.97±0.06 vs. 0.92±0.08, p=0.000). No significant difference in mean body fat percent was observed in T2DM subjects compared to controls (34.12±10.36 vs. 32.58±9.30, p=0.057).Most the T2DM patients were suffering from hypertension as reflected by significantly higher values of systolic BP (136.21±18.83 vs. 115.56±7.00; p=0.000) and diastolic BP (81.36±11.55 vs. 75.80±6.81; p=0.000) and were taking anti-hypertensive drugs to limit their blood pressure levels to normal.

There was a significant difference observed in fasting serum triglycerides (TG), total cholesterol (TC), HDL-C, LDL-C and VLDL- C among type 2 diabetic and control subjects. The mean fasting blood glucose level in T2DM patients was significantly higher than the healthy controls (146.9 ± 53.4 mg/dl vs. 93.49 ± 10.85 mg/dl; p= 0.000). Although total cholesterol levels fall under the limit of borderline, yet there is significant difference between diabetic and non-diabetic. The triglyceride levels were higher in type 2 diabetic patients

than healthy controls (173.83 ± 91.9 vs 146.77 ± 50.61 mg/dl; p=0.000). Significantly low levels of HDL-C were observed in type 2 diabetic subjects compared to controls (41.36 vs 45.00 mg/dl). Alterations in clinical and anthropometric measurements pretend a risk for the development of cardiac diseases as demonstrated by higher TC/HDL, LDL/HDL and TG/HDL ratio in diabetic subjects compared to healthy controls (Table 2). Along with abdominal obesity evidenced by higher BMI and WHR, 12% patients were having dyslipidemia and were on lipid lowering drugs. The reduced HDL-cholesterol and elevated triglyceride values observed in T2DM patients may have contributed to the pathophysiology of the disease in the patients included in this study.

Table 1: Basic Characteristics of the Study Population.

Parameters	Subjects	N	Mean	SD	P-value
BMI (Kg/m2)	Healthy Control	281	25.80	4.60	0.002
	T2DM patient	324	26.92	4.14	
Waist circumference (cm)	Healthy Control	281	87.35	10.98	0.000
	T2DM patient	324	93.43	9.96	
Hip (cm)	Healthy Control	281	95.20	10.16	0.069
	T2DM patient	324	96.71	10.23	
Waist to Hip ratio (WHR)	Healthy Control	281	0.92	0.08	0.000
	T2DM patient	324	0.97	0.06	
Body fat (%)	Healthy Control	281	32.58	9.30	0.057
	T2DM patient	324	34.12	10.36	
Systolic BP (mm Hg)	Healthy Control	281	115.56	7.00	0.000
	T2DM patient	324	136.21	18.83	
Diastolic BP (mm Hg)	Healthy Control	281	75.80	6.81	0.000
	T2DM patient	324	81.36	11.55	
Cholesterol (mg/dl)	Healthy Control	272	176.58	30.46	0.008
	T2DM patient	311	185.76	49.40	
Triglycerides (mg/dl)	Healthy Control	272	146.43	50.61	0.000
	T2DM patient	311	173.83	91.92	
HDL-C (mg/dl)	Healthy Control	272	45.00	6.40	0.000
	T2DM patient	311	41.36	7.72	
LDL-C (mg/dl)	Healthy Control	272	102.29	27.90	0.019
	T2DM patient	311	109.63	44.18	
VLDL-C (mg/dl)	Healthy Control	272	29.29	10.12	0.000
	T2DM patient	311	34.77	18.38	
Total lipids (mg/dl)	Healthy Control	272	499.59	96.05	0.000
	T2DM patient	311	545.34	159.17	
Castelli's risk index I (TC/HDL)	Healthy Control	272	4.00	0.89	0.000
	T2DM patient	311	4.63	1.45	
Castelli's risk index II (LDL/HDL)	Healthy Control	272	2.33	0.76	0.000
	T2DM patient	311	2.73	1.20	
Atherogenic coefficient (TG/HDL)	Healthy Control	272	3.33	1.25	0.000
	T2DM patient	311	4.46	2.82	
Atherogenic Index	Healthy Control	272	0.49	0.16	0.000
	T2DM patient	311	0.58	0.23	
Creatinine (mg/dl)	Healthy Control	271	0.86	0.92	0.816
	T2DM patient	303	0.84	0.53	

Data values are represented as Mean ± SD. WC, Waist Circumference; HC, Hip Circumference; SBP, Systolic Blood Pressure; DBP, Diastolic Blood Pressure; WHR, Waist to Hip ratio; BMI, Body Mass Index. TC, Total Cholesterol; HDL-C, High Density Lipoprotein Cholesterol; LDL, Low Density Lipoprotein; VLDL, Very Low Density Lipoprotein. ∗P value <0.05 is considered as significant value.*Significant difference between T2DM and Control subjects.

Association of CETP Taq1B (rs708272) gene polymorphisms with T2DM

CETP genotyping (Taq1B) was performed by PCR followed by RFLP. The presence of product was verified on a 2% agarose gel stained with ethidium bromide, a band of 535bpwas observed as shown in Fig. 1. The PCR product was digested by TaqIrestriction enzyme and visualized by 3% agarose gelas shown in Fig. 2.Visualization of two DNA fragments of the TaqIB-treated amplicon at 174 and 354 bp indicates a B1 allele (presence of the restriction site), whereas an intact 535 bp indicates a B2 allele (absence of the restriction site).

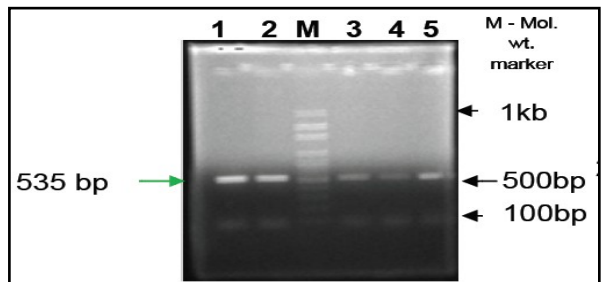

Fig 1. A 2% agarose gel showing 535 bp PCR amplified product of *CETP* gene; lane M: 100 bp ladder, all other lanes were loaded with amplified PCR product from the genomic DNA samples from different T2DM patients and healthy controls.

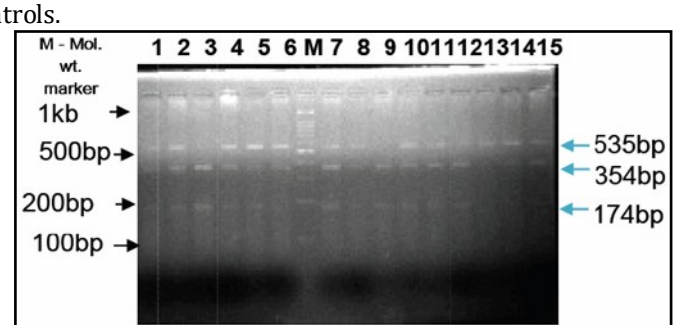

Fig 2:A 3% agarose gel showing TaqI restriction enzymedigested PCR products of *CETP* gene polymorphism. Lane M, 100 bp marker ladder; lanes 13- B2B2 genotype (535 bp); lanes 1,2,4,5,6,7,8,10,11,12,14-B1B2 genotype (535 bp, 354bp and 174bp); and lane 3,9- B1B1 genotype (354 bp and 174 bp).

The distribution of genotype and allelic frequencies for TaqIB polymorphism of *CETP* gene are shown in Table 2. The frequencies of B1B1, B1B2 and B2B2 genotypes of *CETP* gene Taq1B polymorphism in control subjects were 24.2%, 43.8% and 32 %, and in T2DM patients were 27.5%, 50 % and 22.5% respectively. The frequency of the B2B2 genotype was significantly lower in T2DM patients than in healthy controls (22.5% vs. 32%). Also, the allele frequency of B2 allele was higher in controls (0.54) and the frequency of B1 allele was higher in patients (0.52). The odds ratios of B1B2 and B2B2genotypes were calculated considering B1B1 genotype as reference genotype. Logistic regression analysis of the rs708272 polymorphism showed that odds ratio for B2B2 genotype was 0.62 (95% CI, 0.39-0.97; P=0.034) when compared with B1B1 genotype and was statistically significant and showed protective effect against the development of T2DM in North Indian population. Hence, we observed significant association of B2B2 genotype of *CETP* gene TaqIB polymorphism in protection against susceptibility of T2DM.

Table 2: Distribution of genotypes of *CETP* gene Taq1B polymorphism in healthy controls and T2DM subjects of North India.

Genotypes	Control Subjects N (%)	T2DM Subjects N (%)	Odd Ratio (OR) with 95% CI	p-value
B1B1	68 (24.2)	89 (27.5)	Reference	
B1B2	123 (43.8)	162 (50)	1.01 (0.68-1.49)	0.975
B2B2	90 (32)	73 (22.5)	0.62 (0.39-0.97)	0.034
Total	281	324		
Allele Frequencies				

B1	0.46	0.52		
B2	0.54	0.48		

Data is represented as number (%) unless otherwise stated. ∗P value <0.05 is considered as significant value.

Effect of CETP Taq1Bgene polymorphism on quantitative measures

Table 3 summarizes the comparative analysis of the anthropometric (BMI, WHR, systolic BP and diastolic BP) and biochemical (fasting plasma glucose, TC, TG, HDL, LDL, VLDL and Creatinine) characteristics of the T2DM patients and healthy controls according to different genotypes of Taq1B polymorphism of *CETP* gene i.e. B1B1, B1B2 and B2B2 genotype. But no significant association of these risk variables was observed among T2DM as well as control subjects carrying B1B1, B1B2 and B2B2 genotypes. However, multiple linear regression analysis showed a significant association of the genotypes with plasma glucose concentrations (p=0.008) and systolic BP (p=0.036) in control subjects. The results of biochemical parameters revealed that the glucose level, triglycerides, HDL-C and VLDL-C were comparable for T2DM patients as well as control subjects for B2B2 genotype, probably showing its protective effect against lipid variables.

Table 3: Distribution of metabolic characteristics in healthy controls and T2DM patients according to the genotypes of *CETP* gene Taq1B polymorphism.

		Control Subjects				T2DM Subjects			
Parameters	**Genotypes**	**N**	**Mean**	**SD**	**p-value**	**N**	**Mean**	**SD**	**p-value**
BMI (Kg/m2)	B1B1	68	25.77	4.04	0.988	89	26.39	4.21	0.338
	B1B2	123	25.84	4.72		162	27.19	4.22	
	B2B2	90	25.75	4.86		73	26.98	3.87	
Waist circumference (cm)	B1B1	68	86.13	12.29	0.486	89	93.02	8.83	0.392
	B1B2	123	88.12	9.40		162	94.15	10.68	
	B2B2	90	87.22	11.94		73	92.34	9.59	
Hip circumference (cm)	B1B1	68	94.04	10.20	0.485	89	95.44	8.95	0.193
	B1B2	123	95.25	9.35		162	97.72	11.07	
	B2B2	90	96.00	11.17		73	96.02	9.63	
Waist to Hip ratio (WHR)	B1B1	68	0.92	0.08	0.272	89	0.98	0.06	0.271
	B1B2	123	0.93	0.07		162	0.96	0.06	
	B2B2	90	0.91	0.08		73	0.96	0.07	
Body fat (%)	B1B1	68	33.20	9.07	0.781	89	33.20	9.77	0.051
	B1B2	123	32.21	9.86		162	35.48	10.99	
	B2B2	90	32.62	8.74		73	32.22	9.25	
Systolic BP (mm Hg)	B1B1	68	115.79	7.00	0.036	89	138.10	19.39	0.19
	B1B2	123	116.53	5.90		162	136.69	19.67	
	B2B2	90	114.06	8.11		73	132.85	15.78	
Diastolic BP (mm Hg)	B1B1	68	75.78	6.61	0.998	89	81.42	11.04	0.536
	B1B2	123	75.79	7.10		162	81.90	12.87	
	B2B2	90	75.84	6.63		73	80.08	8.79	
Glucose (mg/dl)	B1B1	65	89.38	10.97	0.008	88	149.62	54.43	0.578
	B1B2	118	94.20	10.40		161	144.62	48.76	

	B2B2	85	90.71	11.35		72	151.74	58.07	
Cholesterol (mg/dl)	B1B1	64	172.81	26.32	0.22	86	189.59	48.69	0.689
	B1B2	121	175.37	31.92		157	184.67	49.92	
	B2B2	87	181.04	31.03		68	183.40	49.55	
Triglycerides (mg/dl)	B1B1	64	143.97	47.61	0.832	86	186.79	103.09	0.306
	B1B2	121	148.43	50.40		157	169.30	87.11	
	B2B2	87	145.45	53.42		68	167.89	87.41	
HDL-C (mg/dl)	B1B1	64	45.62	8.51	0.118	86	40.56	7.64	0.271
	B1B2	121	44.11	5.21		157	42.06	8.06	
	B2B2	87	45.79	6.00		68	40.75	6.97	
LDL-C (mg/dl)	B1B1	64	98.40	25.43	0.224	86	111.67	44.89	0.88
	B1B2	121	101.57	29.00		157	108.76	43.85	
	B2B2	87	106.16	27.91		68	109.07	44.60	
VLDL-C (mg/dl)	B1B1	64	28.80	9.52	0.832	86	37.36	20.62	0.306
	B1B2	121	29.69	10.08		157	33.86	17.42	
	B2B2	87	29.09	10.68		68	33.58	17.48	
Total lipids (mg/dl)	B1B1	64	489.60	80.90	0.526	86	565.97	165.82	0.364
	B1B2	121	499.16	99.32		157	538.65	156.80	
	B2B2	87	507.53	101.85		68	534.69	155.96	
Creatinine (mg/dl)	B1B1	64	0.99	1.25	0.348	85	0.87	0.56	0.641
	B1B2	121	0.85	0.99		153	0.85	0.59	
	B2B2	86	0.77	0.30		65	0.79	0.32	
Castelli's risk index I (TC/HDL)	B1B1	64	3.89	0.81	0.53	86	4.85	1.59	0.219
	B1B2	121	4.03	0.90		157	4.51	1.37	
	B2B2	87	4.03	0.93		68	4.61	1.41	
Castelli's risk index II (LDL/HDL)	B1B1	64	2.24	0.73	0.515	86	2.88	1.37	0.36
	B1B2	121	2.35	0.77		157	2.65	1.11	
	B2B2	87	2.38	0.78		68	2.73	1.18	
Atherogenic coefficient (TG/HDL)	B1B1	64	3.24	1.15	0.491	86	4.83	2.82	0.357
	B1B2	121	3.43	1.28		157	4.30	2.88	
	B2B2	87	3.25	1.28		68	4.36	2.68	
Atherogenic Index	B1B1	64	0.48	0.16	0.429	86	0.62	0.23	0.162
	B1B2	121	0.51	0.16		157	0.56	0.24	

B2B2	87	0.48	0.17	68	0.58	0.23

Data values are represented as Mean ± SD. WC, Waist Circumference; HC, Hip Circumference; SBP, Systolic Blood Pressure; DBP, Diastolic Blood Pressure; WHR, Waist to Hip ratio; BMI, Body Mass Index. TC, Total Cholesterol; HDL-C, High Density Lipoprotein Cholesterol; LDL, Low Density Lipoprotein; VLDL, Very Low Density Lipoprotein. ∗P value <0.05 is considered as significant value.*Significant difference between T2DM and Control subjects.

DISCUSSION

Diabetes mellitus has become one of the biggest worldwide health crises of the 21st century. According to a World Health Organization (WHO), an estimated 3.4 million deaths are caused due to high blood sugar. Many risk factors contribute in the pathogenesis of the disease but their conclusive role is still not clear.T2DM is characterized by impaired insulin secretion, which can be attributed due to defective beta-cell mass and/or impaired beta-cell function [31, 32] in which both environmental and genetic factors interacts leading to development of insulin resistance [7, 33-35]. The present scenario is alarming as diabetes mellitus is amongst the top 10 causes of morbidity and mortality along with other major non-communicable diseases (NCDs) particularly cardiovascular disease, cancer and respiratory disease. India is currently observing an alarming rise in incidence of diabetes mellitus with the second highest number of diabetes cases globally. Therefore, assessment of risk factors is important. Hence, the current study emphasis on the evaluation of genetic (*CETP* polymorphism) and environmental risk factors associated with the T2DM in the North Indian population.

There is an increased prevalence of coronary artery disease, cerebral vascular disease) or arteriosclerosis obliterans in type 2 diabetic patients [36, 37]. This increased risk can be partly accounted for by the lipoprotein disorders linked to insulin resistance: elevated plasma triglycerides (TGs), TCand VLDL concentrations, a dense LDL phenotype and low levels of HDL-C [38, 39]. GWAS identified strong associations between *CET*P and plasma lipid concentrations in adults[40]. Association of the *CETP* genotypes with genetic variation in lipid and lipoprotein concentrations has been well documented in both hyperlipidemic or normolipidemic individuals [41, 42].*CETP* has a central role in the metabolism of HDL and polymorphism of the gene may be associated with complications of diabetes mellitus.HDL promotes cellular cholesterol efflux and reverse cholesterol transport (RCT) which transports cholesterol from peripheral cells and tissues to the liver for its metabolism and biliary excretion.

In the general population, the *CETP*TaqIB polymorphism, independentlyaffect both CETP activity and HDL-C concentrations. A previous study reported significant association of *CETP* genotype with CETP and HDL concentrations[43]. Highest concentration of HDL cholesterol was reported among B2B2 carriers and lowest in B1B1 carriers [44-48]. In the present study, the protective effect of a B2 allele against the development of T2DM was found and the association was statistically significant. The frequency of the B2B2 genotype was significantly lower in T2DM patients than in healthy controls (22.5% vs. 32%; p=0.034). Hence the B2 allele provides resistance against the susceptibility of T2DM in North Indian population. Our results are supported by the study conducted by Relvas*et al* who also found a higher prevalence of the B2B2 genotype of the *CETP* gene among T2DM than healthy controls [49]. Dedoussis*et al* also provided an evidence for a protective role of the B2B2 genotype of the *CETP*TaqIB polymorphism in Greek population (OR=0.27, p=0.02) [50]. In a study conducted in Iranian population, Heidari-Ben *et al* also reported that Taq1B polymorphism might have a protective effect on dyslipidemia (OR= 0.12, 95%CI: 0.07-0.20)[51]. Durlach*et al* showed that T2DM patients with the B2 allele had significantly less cardiovascular disease risk than those having the B1 allele of the *CETP* gene [52]. In contrast, a recent study found no association of *CETP* TaqIBpolymorphism with T2DM and its related metabolic parameters in Southern Thaipopulation [53]. The present study also did not find any link between TaqIB polymorphism and abnormalities in cardio-metabolic traits including BMI, WHR and dyslipidemia.Similarly, another study reported no association of *CETP* TaqIB polymorphism with the T2DM and also lipid levels did not show any correlation with it [54]. But the protective role of B2B2genotype for Taq1B polymorphism has also been reported against coronary arteriosclerosis [48]. Similarly, Cao *et al* in a meta-analysis study suggested that the B2B2 genotype of the *CETP*TaqIB polymorphism is a protective factor against the development of Myocardial infarction [55]. The frequency of the B2B2 genotype in MI patients was lower (OR= 0.87, 95% CI= 0.81–0.94). In a meta-analysis study conducted in Asians and Caucasians protective role of *CETP* Taq1B polymorphism against composite ischemic CVD risk was demonstrated[56]. Contrary, a study showed that homozygous *CETP* B1 variants were associated with increased risk of T2DM [57]. Klerkx*et al* also determined in a study that the TaqIB polymorphism is not an important factor in determining CETP or HDL-C levels [58]. Also, a study

conducted by Kalantar*et al* reported that relationship between *CETP* TaqIB polymorphism and HDL concentrations was affected by total dietary fat intake in normolipidemic T2DM patients [59]. Similarly, a study on Turkish population demonstrated that the *CETP* TaqIB gene polymorphism is associated with low HDL cholesterol levels in T2DM patients and healthy controls [60]. These contradictory studies give rise to two possibilities: TaqIB polymorphism is not the only determinant of HDL-cholesterol level or actual gene responsible/protective for T2DM may be different which is in linkage disequilibrium with *CETP* TaqIB polymorphism. *i.e.* the association may depend on gene-gene or gene-environment interactions. Therefore, large studies need to be conducted to assess gene-environment interactions and to determine whether a genetic predisposition can be one of the contributing factors in the pathogenesis of T2DM.

The present study did not show any statistical significance among the genotypes of TaqIB polymorphism in CETP gene and metabolic traits as shown in table 3. Similar results were observed in other studies where no significant difference in distribution among genotypes of *CETP* Taq1B polymorphism and lipid levels was observed[22, 61]. However, some studies reported the link between *CETP* gene TaqIB polymorphism and lipid levels including HDL, LDL and triglycerides[47, 62].

CONCLUSION

The present study demonstrated abdominal obesity and dyslipidemia as the independent risk factors for the development of T2DM. Genetic association study established the protectiveroleof TaqIBpolymorphism in *CETP* gene and type 2 diabetes mellitus in North Indian population.However, no significant influence of this polymorphism was observed with the various metabolic risk variables.

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CONFLICT OF INTERESTS

The authors have no conflicts of interest to declare.

AUTHORS CONTRIBUTION

Study Design and implementation: GKB, SS and JSB
Data Collection and Analysis: GKB, SKB
Manuscript Drafting: NK, GKB, JSB
Manuscript Revisions: All authors

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