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Original Article

Interlukin-10 gene polymorphisms (-819T/C and -1082A/G) and Type 2 diabetes mellitus in North Indian population

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Abstract

Diabetes is a metabolic disorder characterized by chronic hyperglycemia and impaired cytokine levels leading to inflammation. Interlukin-10 (IL-10) is an anti-inflammatory cytokine which acts as macrophage deactivator affecting the synthesis of TNF-a, IL-1, IL-6, IL-8 and GM-CSF. Single-nucleotide polymorphisms (SNPs) viz. -592A/C, -819T/C and -1082A/G in IL-10 promoter are associated with IL-10 production. Low IL-10 levels in T2DM cases may be regulated by such gene variants. The present study was undertaken to evaluate the association of two genetic polymorphisms viz. IL-10 -819T/C and -1082A/G with T2DM in a North Indian population. Blood samples from 402 subjects (201 each of controls and T2DM cases) were collected after ethical approval and individual written consent. All subjects were genotyped by polymerase chain reaction-restriction length polymorphism (PCR-RFLP) using specific primers and restriction enzymes. Genotypic, allelic, carriage rate frequencies were calculated and haplotypic analysis performed by SPSS (version 21.0) and SHEsis (online version). All biochemical parameters except WHR and TG showed significant association with T2DM (P<0.005). In the study population 'TC' genotype of -819T/C and 'GG' genotype of -1082A/G showed significant association with T2DM (P< 0.0001, OR=2.195; P<0.038, OR=1.946 respectively) while allelic frequencies did not show association. Individuals with genotypic combinations viz. CT/AG (+/-) and CT/GG (+/-, +/+) showed 2-4 times higher risk of developing T2DM while haplotype analysis did not show any association. All genotypes of IL-10 polymorphism showed significant association with biochemical parameters viz. BPS, BPD, F, PP and TC (P<0.007). The IL-10 gene polymorphisms therefore play important roles in determining diabetes susceptibility.

Introduction

Type 2 diabetes mellitus (T2DM) is a metabolic syndrome with 90% share of total diabetic population and the major cause of complications like cardiovascular diseases, atherosclerosis, obesity etc. Worldwide diabetes population was 387 million in 2014 and is expected to increase to 592 million by 2035. India has the second largest diabetic population in the world with 65.1 million people in 2013 which will reach up to 109.0 million by 2035 (1).

Studies have suggested that subclinical systemic inflammation which takes part in deregulation of innate immune system with change in cytokine expression is highly associated with T2DM (2-4). Interlukin-10 (IL-10) is a pleiotropic cytokine, mainly produced by the Th2 subset of CD4+ helper cells and certain subsets of activated T and B cells (5,6). IL-10 plays a crucial role in regulating other anti-inflammatory cytokines which play an important role in innate immune system (7-12). IL-10 is capable of inhibiting synthesis of pro-inflammatory cytokines such as IFN-γ, IL-2, IL-3, TNFα and GM-CSF synthesized by macrophages and regulatory T-cells (12-15).

The gene encoding IL-10 has been mapped to chromosome 1q (1q31-q32) and is composed of five exons (6,16). Several polymorphic sites within the promoter region have been described, including two microsatellite and three biallelic polymorphisms at positions -592A/C (rs1800872), -819T/C (rs1800871) and -1082A/G (rs1800896) (4,5,12,17). Cytokine production appears to be related to inter-individual differences resulting from allelic polymorphisms in the respective genes (5,18). T2DM is associated with low production of IL-

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10 in peripheral blood cells, which is strictly regulated at the transcription level. Studies have suggested that IL-10 level is determined by genetic variants in the IL-10 promoter region which may be responsible for the endothelial dysregulation associated with T2DM (5,19). It has also been proposed that variable production of Th2 cytokines including IL-10 may influence the degree of β cell destruction and age of clinical onset (5,20). IL-10 is known to affect the cyclooxygenase 2 (COX2) pathway as well i.e in the absence of IL-10, COX2 expression increases and activates thromboxane receptor which ultimately leads to vascular endothelial and cardiovascular dysfunction (21-23). An attempt has been made to study the association of two IL-10 promoter polymorphisms -819T/C and -1082A/G with T2DM in a north Indian population.

Materials and Methods

Patient selection and clinical evaluation

Five ml blood samples were collected from T2DM cases (n=201) and age/sex matched controls (n=201) from Balarampur Hospital, Lucknow, India under the supervision of expert clinicians. The study was approved by Institutional Ethics Committee and a written informed consent was obtained from all subjects enrolled in the study. Controls showing a normal oral glucose tolerance test were included in the study whereas those having family history of coronary artery disease or other metabolic disorders were excluded. Cases included in the diabetes group showed a fasting glucose concentration of ≥126 mg/dl or ≥200 mg/dl after 2-h of 75-g oral glucose intake. The inclusion and exclusion criteria for patients and controls were according to American Diabetic Association (ADA) and World Health Organization (WHO) norms (24-26). Medical records were further reviewed for diabetic complications.

Anthropometric measurements

Systolic and diastolic blood pressures (BPS/BPD) were measured in sitting position. Height, weight, waist and hip circumferences were used for calculation of body mass index (BMI) and waist hip ratio (WHR). BMI and WHR were calculated by using the following formulae:

BMI=Weight (in kg)/Height (in meter)

WHR=Waist circumference (in cm)/ Hip circumference (in cm)

Estimation of biochemical parameters

Blood (without EDTA) was centrifuged at 1157 x g (3000 RPM) for 10 min, sera were collected and stored at -200C until further use. Estimations of plasma glucose (mg/dl), serum creatinine (SCRT), lipid profile viz. total cholesterol (TC), high density lipoprotein cholesterol (HDL-C) and triglyceride (TG) were carried out using commercially available Ecoline kits (Merck, USA) by double beam spectrophotometer at different wavelengths as per manufacturer's instructions. According to Friedewald et al (1972) low density lipoprotein (LDL) and very low density lipoprotein (VLDL) were calculated by using the following formulae (27).

LDL= TC- (TG/5+HDL)

VLDL= TC-(LD+HDL)

DNA extraction and genotyping

Genomic DNA was extracted from peripheral blood leucocytes using the standard salting out method (28)

Table 1. Clinical characteristics of controls and T2DM cases

Clinical parameters	Controls (n=201)	T2DM cases (n=201)	P value
Age (years)	46.64± 0.79	48.42±0.61	0.089
Waist hip ratio	0.93±0.01	0.95±0.03	0.472
Body mass index (Kg/m2)	23.11±0.14	24.32±0.25	0.008
Systolic blood pressure (mmHg)	122.40±0.40	134.90±1.25	<0.0001
Diastolic blood pressure(mmHg)	81.26±0.23	88.19±0.93	<0.0001
Fasting (mg/dl)	84.16±0.77	173.00±3.98	<0.0001
Postprandial (mg/dl)	136.20±0.96	268.80±6.23	<0.0001
Total cholesterol (mg/dl)	161.80±3.58	210.60±2.38	<0.0001
Triglyceride (mg/dl)	124.80±5.38	114.80±1.15	0.207
HDL (mg/dl)	50.11±1.64	46.22±0.47	0.0056
LDL (mg/dl)	52.40±2.13	172.30±3.49	0.001
VLDL (mg/dl)	27.91±1.90	20.79±0.39	0.0005
SCRT (mg/dl)	**1.08±0.01**	1.81±0.04	<0.0001

Table 2. Genotype, allele and carriage rate frequencies (F) of IL-10 SNPs - 819T/C and -1082A/G in controls and T2DM cases.

	Controls (n=201) (%)	Cases (n=201) (%)	P value	OR (95% CI)
IL-10 -819T/C				
TT	82 (40.8)	55 (27.4)		1.0 (Ref.)
TC	91 (45.2)	134 (66.6)	<0.0001	2.20 (1.424-3.385)
CC	28 (14.0)	12 (6.0)	0.247	0.64 (0.300-1.363)
Alleles				
T	255 (63.4)	244 (60.7)		1.0 (Ref.)
C	147 (36.6)	158 (39.3)	0.424	1.123 (0.845-1.494)
Carriage rate				
T+	173 (86.1)	189 (94.0)		1.0 (Ref.)
T-	28 (13.9)	12 (6.0)	0.009	0.39 (0.193-0.796)
C+	119 (59.2)	146 (72.6)		1.0 (Ref.)
C-	82 (40.8)	55 (27.4)	0.005	0.55 (0.360-0.831)
IL-10-1082A/G				
AA	84 (41.8)	84 (41.8)		1.0 (Ref.)
AG	98 (48.8)	80 (39.8)	0.347	0.82 (0.535-1.246)
GG	19 (9.4)	37 (18.4)	0.038	1.95 (1.037-3.658)
Alleles				
A	266 (66.2)	248 (61.7)		1.0 (Ref.)
G	136 (33.8)	154 (38.3)	0.186	1.22 (0.91-1.62)
Carriage rate				
A+	182 (90.5)	164 (81.6)		1.0 (Ref.)
A-	19 (9.5)	37 (18.4)	0.011	2.16 (1.195-3.907)
G+	117 (58.2)	117 (58.2)		1.0 (Ref.)
G-	4 (41.8)	84 (41.8)	1.00	1.00 (0.673-1.486)

with slight modifications (26). Quality and quantity of DNA were estimated by biophotometer (Eppendorf, Germany).

Two different single nucleotide polymorphisms (SNPs) viz. -819T/C and -1082A/G at IL-10 promoter region were analyzed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Primers were designed with the help of Primer 3 software (version online) and further checked by in-silico PCR (UCSC genome browser). The forward and reverse primer sequences for -819T/C and -1082A/G SNPs were 5'TCAACTTCTTCCACCCCATC3' and 5'AGTGAGCAAACTGAGGCACA3'; 5'TTCCCCAGGTAGAGCAACAC3' and 5'CCATGACCCCTACCGTCTCT3' respectively. Restriction enzymes (REs) were selected with the help of NEB cutter (version online). Amplification was performed in a 25µl reaction mixture containing genomic DNA (100-150 ng), 10 pmol of each primer, 200 µM dNTPs, buffer (100 mM Tris, pH 9.0, 500 mM KCl, 15 mM MgCl2, 0.1% gelatine) and 0·3U of Taq DNA polymerase (MBI-Fermentas, USA) using a gradient master cycler (Eppendorf, Germany). PCR products were checked on 2% agarose gel and digested with Ms1I and HpyAV restriction enzymes respectively. Digested products were resolved on 15% polyacrylamide gels, visualized with ethidium bromide (EtBr) and documented in gel documentation system (Vilber Lourmat, France).

Statistical analysis

The continuous variables of each group were summarized as mean±SD and compared by Student's t-test. The Hardy-Weinberg equilibrium at individual locus was assessed by Chi-square ($\chi 2$) test. QUANTO software (version 1.2.4) was used for sample size calculation for each SNP taking minor allele frequency (MAF) and prevalence of disease into consideration. Allele frequencies and carriage rates of alleles in both groups were compared using a 2x2 contingency table and genotype frequencies in a 2x3 contingency table by $\chi 2$ and Fisher's exact test. Differences were considered statistically

Table 3. Distribution of double combinations of IL-10 SNPs -819T/C and -1082A/G in controls and T2DM cases.

	Controls (n=201) (%)	Cases (n=201) (%)	P value	OR (95% CI)
TT/AA				
-/-	87 (43.3)	93 (46.3)		1.0 (Ref.)
-/+	32 (15.9)	53 (26.4)	0.104	1.55 (0.924-2.625)
+/-	30 (14.9)	24 (11.9)	0.358	0.75 (0.406-1.379)
+/+	52 (25.9)	31 (15.4)	0.032	0.56 (0.328-0.950)
TT/AG				
-/-	47 (23.4)	87 (43.3)		1.0 (Ref.)
-/+	72 (35.8)	59 (29.4)	0.001	0.44 (0.270-0.726)
+/-	56 (27.9)	34 (16.9)	<0.0001	0.32 (0.188-0.571)
+/+	26 (12.9)	26 (12.9)	0.016	0.44 (0.222-0.854)
CT/AA				
-/-	54 (26.9)	35 (17.4)		1.0 (Ref.)
-/+	56 (27.9)	32 (15.9)	0.685	0.88 (0.480-1.619)
+/-	63 (31.3)	82 (40.8)	0.011	2.01 (1.173-3.437)
+/+	28 (13.9)	52 (25.9)	0.001	2.87 (1.532-5.359)
CT/AG				
-/-	64 (31.8)	41 (20.4)		1.0 (Ref.)
-/+	46 (22.9)	26 (12.9)	0.692	0.88 (0.474-1.641)
+/-	39 (19.4)	80 (39.8)	<0.0001	3.20 (1.851-5.538)
+/+	52 (25.9)	54 (26.9)	0.083	1.62 (0.938-2.800)
CT/GG				
-/-	102 (50.7)	58 (28.9)		1.0 (Ref.)
-/+	8 (4.0)	9 (4.5)	0.183	1.98 (0.724-5.407)
+/-	80 (39.8)	106 (52.7)	<0.0001	2.33 (1.510-3.596)
+/+	11 (5.4)	28 (13.9)	<0.0001	4.48 (2.076-9.652)

significant for P<0.05. The association of various combinations of SNPs with T2DM was studied by logistic regression analysis using SPSS (version 21.0). Genotypic association with biochemical parameters was statistically analyzed by Students t-test and haplotype analysis was performed using SHEsis (version online).

Results

Anthropometric and Biochemical analysis

Except WHR and TG all parameters viz. BPS, BPD, F, PP, TC, LDL and SCRT showed significant increase in T2DM while HDL and VLDL showed significant decrease in T2DM cases when compared to controls (Table 1.).

Genetic analysis

Two SNPs (-819T/C and -1082A/G) of IL-10 gene were successfully genotyped in 201 each of cases and controls. Their genotype patterns are shown in Figures 1 and 2. Genotype, allele and carriage rate frequencies are shown in Table 2. The prevalence of 'TC' genotype of -819T/C polymorphism showed significantly higher frequency in cases as compared to controls and the disease risk was 2.195 times higher (P<0.0001) (Table 2). The 'GG' genotype in -1082A/G was higher in T2DM as compared to controls. It was significantly associated with T2DM and showed 1.946 folds higher risk (P<0.038) (Table 2).

Out of nine possible double combinations between the two SNPs, five were found with frequencies between 2 to 50% in the study population (Table 3). The genotypic combinations with TT/AA (+/+) and TT/AG (-/+, +/-, +/+) showed significantly higher frequency in normal individuals than those with T2DM (P<0.05) (Table 3) whereas CT/AA (+/-, +/+), CT/AG (+/-) and CT/GG

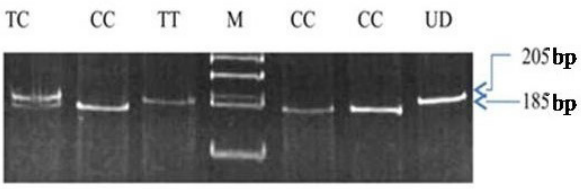

Figure 1. Polyacrylamide gel (12%) showing the genotypes of IL-10-819T/C polymorphism. UD-undigested; M-pUC19/*MspI* digest.

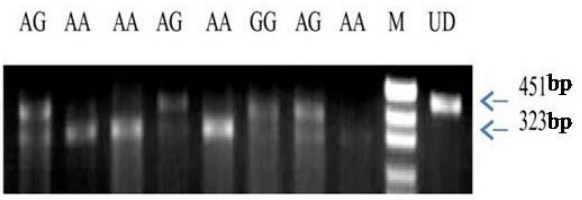

Figure 2. Agarose gel (2.5%) showing the genotypes of IL-10-1082A/G polymorphism. UD- undigested; M-pUC19/*MspI* digest.

(+/-, +/+) combinations showed 2-4 folds significantly higher odds ratio (OR) with T2DM (Table 3).

Haplotype analysis of IL-10 -819T/C and -1082A/G gene variants did not show any association with T2DM (Table 4).

All the three genotypes of -819T/C polymorphism showed significant association with most of the biochemical parameters viz. BPS, BPD, F, PP, TC, HDL, LDL, and SCRT in T2DM cases when compared to controls (Table 5). In addition 'TC' genotype also showed significant association with age and BMI (Table 5). 'AA' genotype of -1082A/G showed significant association with BPS, BPD, F, PP, TC, HDL, LDL and SCRT while 'AG' genotype showed significant association with all parameters except BMI and TG. Homozygous recessive genotype 'GG' of -1082A/G showed significant association with age, BPS, BPD, F, PP, TC, and SCRT (Table 5).

Discussion

IL-10 also called cytokine inhibitory factor acts as a key regulator in immune responses and inhibits the production of various cytokines (8,19). Therefore, it is known to be associated with various disorders viz. nephritic syndrome, cancer multiple sclerosis, alzheimer, acute coronary syndrome, type 1 diabetes mellitus (T1DM) and T2DM (5,19,29). Studies showed that hypertriglyceridemia and insulin resistance are induced by pro-inflammatory cytokine treatment (10,30). The different cytokine gene polymorphisms lead to variation in immune responses (31-32). Cytokine expression levels are defined by genetic variants in their promoter regions which play crucial roles in disease condition. Several polymorphic studies in IL-1β, IL-1Ra, IL-4, IL-6, IL-18 cytokines have been reported to be associated with T2DM (33-34). Three major IL-10 gene promoter polymorphisms viz. -592A/C, -819T/C and -1082A/G were found to be associated with obesity and T2DM (4,16,31,35). These SNPs in the promoter region might be affecting the transcription factor recognition site and resulting in altered IL-10 levels (35-36).

Previous studies reported that 'GG' genotype of IL-10 promoter polymorphism -1082A/G showed significant association with T2DM (16,31,37-38). They also found synergistic effect of 'GA+GG' genotype combination on T2DM risk (16). A study from South India reported significant association with T2DM in case of 'GG' genotype of IL-10 -1082A/G polymorphism (39). Similar results were observed in the present study where 'GG' genotype was more frequent in cases as compared to controls and showed 1.95 folds marginal risk to T2DM. However, this polymorphism did not show association with T2DM in the European population (37).

A recently published meta-analysis also revealed the association of IL-10 promoter region polymorphisms. IL-10 -1082A/G polymorphism was found to be strongly associated whereas the -819T/C did not show any association with T2DM risk (38). Like IL-10 -1082A/G, no association of -819T/C polymorphism was found in the European population (37). However, some studies found that -819T/C was associated with T2DM, T1DM and nephrophathy (5,12,16,31). In the present study, 'TC' genotype of IL-10 -819T/C was more frequent in T2DM

Table 4. Haplotype analysis of IL-10 -819 T/C and -1082A/G gene variants and their association with controls and T2DM cases

	Controls (n=201) (%)	Cases (n=201) (%)	Chi 2	Fisher's P	Pearson's P	OR (95% CI)
C A*	66.89 (0.17)	72.38 (0.18)	0.264	0.607	0.607	1.10 (0.764-1.586)
C G*	80.13 (0.20)	85.62 (0.21)	0.229	0.632	0.632	1.09 (0.772-1.530)
T A*	199.13 (0.49)	175.62 (0.45)	2.763	0.097	0.096	0.79 (0.599-1.043)
T G*	55.87 (0.14)	68.38 (0.17)	1.491	0.222	0.222	1.27 (0.865-1.865)

Table 5. Association of anthropometric/ biochemical parameters with IL-10 -819T/C and -1082A/G genotype
(y; years, WHR; waist hip ratio, BMI; body mass index, BPS; blood pressure systolic, BPD; blood pressure diastolic, F; fasting, PP; post-prandial)

IL-10 Genotypes		Age	BMI	WHR	SBP	DBP	F	PP
-819 T/C								
TT	Controls	45.45±11.84	23.36±1.31	0.99±0.11	123.32±7.08	82.10±4.29	94.48±27.78	140.87±31.04
	Cases	49.13±8.94	23.39±4.32	1.00±0.001	132.89±18.01	86.60±13.92	178.38±73.34	267.18±97.73
	P value	0.052	0.944	0.415	<0.0001	0.007	<0.0001	<0.0001
TC	Controls	42.54±11.60	22.99±1.99	0.99±0.01	122.08±4.38	80.78±3.51	89.62±21.63	140.78±32.00
	Cases	48.72±10.07	24.13±4.14	0.98±0.15	136.16±18.06	88.99±13.06	171.40±5.93	265.84±97.89
	P value	<0.0001	0.016	0.224	<0.0001	<0.0001	<0.0001	<0.0001
CC	Controls	40.50±12.31	23.52±1.44	0.99±0.02	121.04±4.33	80.36±4.29	85.96±7.86	132.14±9.20
	Cases	47.67±11.07	24.69±4.92	0.92±0.29	129.67±3.28	86.67±9.85	136.00±39.65	227.92±78.52
	P value	0.091	0.255	0.147	0.001	0.007	<0.0001	<0.0001
-1082 A/G								
AA	Controls	45.65±12.38	23.42±1.21	0.99±0.01	123.06±5.22	82.19±4.16	91.30±27.48	141.99±29.02
	Cases	49.20±8.90	23.96±4.20	1.00±0.001	136.05±16.64	89.21±14.05	169.98±78.04	260.64±106.13
	P value	0.034	0.273	0.319	<0.0001	<0.0001	<0.0001	<0.0001
AG	Controls	42.21±11.63	23.07±1.98	0.99±0.10	122.18±6.08	80.54±3.56	91.02±20.96	138.16±31.23
	Cases	48.64±10.15	23.69±4.54	0.97±0.16	134.21±19.08	87.00±13.04	170.98±63.65	266.81±91.32
	P value	<0.0001	0.227	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
GG	Controls	40.00±9.40	23.06±1.75	0.99±0.02	121.00±5.26	80.84±4.47	90.53±12.58	136.58±22.39
	Cases	48.08±11.13	24.57±3.25	0.95±0.23	133.65±17.56	88.46±11.10	174.46±59.25	265.11±88.39
	P value	0.009	0.066	0.361	0.003	0.006	<0.0001	<0.0001

Table 5 (continued). Association of anthropometric/ biochemical parameters with IL-10 -819T/C and -1082A/G genotype
(TC; total cholesterol , TG; triglyceride, HDL; high density lipoprotein, LDL; low density lipoprotein, VLDL, very low density lipoprotein , SCRT; serum creatinine.)

IL-10 Genotypes		TC	TG	HDL	LDL	VLDL	SCRT
-819 T/C							
TT	Controls	166.64±49.48	121.64±49.12	50.30±16.13	92.00±50.23	24.33±9.82	1.34±0.55
	Cases	227.18±28.83	114.30±20.25	42.94±3.28	162.9±25.24	18.52±10.41	1.84±0.63
	P value	<0.0001	0.296	0.001	<0.0001	0.001	<0.0001
TC	Controls	177.31±26.64	123.31±39.23	49.33±13.62	103.32±31.07	24.66±7.85	1.28±1.14
	Cases	223.64±41.48	118.79±22.86	45.32±6.70	156.79±46.52	21.40±7.86	1.81±0.67
	P value	<0.0001	0.279	0.004	0.004	0.003	<0.0001
CC	Controls	183.59±24.54	127.74±42.93	48.10±8.06	109.96±29.63	25.55±8.59	1.11±0.32
	Cases	221.08±42.15	115.23±21.48	45.55±4.18	152.48±43.29	22.51±5.65	1.92±0.80
	P value	0.001	0.346	0.308	0.232	0.269	<0.0001
-1082 A/G							
AA	Controls	167.50±46.98	120.68±37.68	49.20±15.72	94.16±48.25	24.14±7.53	1.22±0.47
	Cases	216.12±39.96	116.94±24.57	44.63±5.59	148.63±43.64	20.71±7.85	1.93±0.67
	P value	<0.0001	0.448	0.014	0.33	0.004	0.027
AG	Controls	178.62±30.04	128.24±32.09	49.94±13.67	103.04±34.4	25.64±10.20	1.36±1.14
	Cases	229.84±32.09	117.34±18.87	44.18±5.43	162.75±33.29	20.16±8.86	1.64±.62
	P value	<0.0001	0.072	0.01	0.006	<0.0001	0.051
GG	Controls	177.16±23.15	108.79±19.99	49.13±7.42	106.25±25.03	21.75±3.99	1.16±0.37
	Cases	231.72±44.03	118.28±23.16	46.00±7.50	163.95±51.63	21.74±9.72	1.97±0.65
	P value	<0.0001	0.137	0.145	0.084	0.996	<0.0001

cases as compared to controls, showing significant association and 2.20 folds higher risk (P< 0.001) (Table 2).

A study was carried out in a Chinese population wherein no association was seen in case of -819T/C polymorphism (16). Although 'C' allele was found to be very rare in Taiwanese population it appeared to be significantly associated with T2DM cases (35). However, 'C' allele of -819T/C was higher in Caucasian, Australian and German populations (12,29,35). As mentioned earlier the contribution of genetic differences is responsible for variation in IL-10 secretion during diseases (35,40). The 'C' allele of -819T/C and 'G' allele of -1082A/G are responsible for low levels of IL-10 production (41). Yaghini et al (2011) also found that IL-10 cytokine level was significantly decreased in T2DM cases as compared to controls (9.53±2.27 and 16.11±2.27 respectively)(11). Our studies suggested that allelic frequencies of IL-10 -819T/C and -1082A/G polymorphisms do not have any significant association with T2DM. It has been reported that haplotypes i.e. 'ATA' and 'GCC' of -592A/C, -819T/C and -1082A/G SNPs were significantly associated with low and high levels of IL-10 production respectively (35-36). Haplotype analysis of the two SNPs in the present study did not show any significant association although three out of four haplotype combinations showed minor disease susceptibility (OR=1.00) (Table 4). In case of double combinations of genotypes, TT/AA (+/+) and TT/AG (-/+, +/-) showed significant difference and act as protective genotypic combinations (P=0.001). CT/AA (+/-, +/+), CT/AG (+/-) and CT/GG (+/-, +/+) genotypic combinations were significantly associated with T2DM and showed 2-4 times higher risk (Table 3).

BMI levels were raised in T2DM cases as compared to controls although not significantly and only 'TC' genotype of -819T/C seems to have significant association with BMI (Table 5). Individuals with 'AG' genotype of -1082A/G showed significant association with WHR as well. All genotypes of -819T/C and -1082A/G SNPs showed significant association with other biochemical parameters viz. BPS, BPD, F, PP and TC. All genotypes except 'AA' and 'GG' showed significant association with HDL. All genotypes, except 'CC' of -819T/C, 'AA' and 'GG' of -1082A/G showed significant association with LDL. 'TT' and 'TC' genotypes of -819T/C as well as 'AA' and 'AG' of -1082A/G showed significant association with VLDL (Table 5). All genotypes of -819T/C and -1082A/G except 'AG' of -1082A/G showed significant association with SCRT.

Conclusion

The present study was undertaken to explore the association of IL-10 gene polymorphisms with T2DM and a sincere effort was made to determine whether specific genotypes/haplotypes of SNPs could actually be useful in predicting the susceptibility risk of individuals. Individuals with genotypes such as 'TC' and 'GG' or combinations CT/AA (+/-, +/+), CT/AG (+/-) and CT/GG (+/-, +/+) showed higher susceptibility to T2DM. Also, few combinations such as TT/AA (+/+) and TT/AG (-/+, +/-) may be protective. However, further studies are required in more number of samples in order to confirm the correlation between IL-10 gene polymorphisms and T2DM cases from north India. SNP studies have shown a considerable level of variation amongst various ethnic populations around the world. Therefore, it is essential to perform association studies/SNP analyses in individual populations. Individuals at risk will be able to take prior precautionary measures and avoid or delay the onset of the disease.

Declaration of Interest: The authors declare no conflict of interest

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