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CRP Gene Polymorphism and Their Risk Association With Type 2 Diabetes Mellitus

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Abstract

BACKGROUND: C-reactive protein (CRP) is an inflammatory marker associated with T2DM, obesity, insulin resistance, and cardiovascular disease.

AIM: The present study evaluates the association of CRP +1059 G/C polymorphism of the *CRP* gene in 100 T2D cases and 100 healthy controls.

METHODS: Present study was done by allele specific PCR method to study the CRP gene polymorphism in study subjects.

RESULTS: Study found that *CRP* (+1059 G/C) genotype distribution among case and controls was found to be significant (p=0.001), Higher *CRP* C allele frequency (0.16) was observed compared to controls (0.04). *CRP* +1059 GC and CC had 2.72 (1.12-6.61), 20.56 (1.16-362.1) risk for T2D. It has been observed, HTN, Obesity, Smoking and alcoholism was found to be associated with increased risk of T2D, and a significant difference was observed in biochemical parameters.

CONCLUSION: Study concluded that *CRP* gene polymorphism was found to be associated with risk of Type 2 Diabetes and risk was linked with heterozygosity and mutant homozygosity. Hypertension, Obesity, Smoking and alcoholism increases the risk of occurrence of Type 2 Diabetes.

Introduction

Type 2 diabetes mellitus (T2DM) is one of the major health-related global health problems [1]. Genetic alterations played an important role in determining why some diabetic patients develop these complications while others do not [2]. Several evidence showed the importance of inflammation in the pathogenesis of diabetic complications, especially enhancement of atherosclerosis and complications [3]. According to the International Diabetes Federation revealed that currently, around 415 million people with diabetes all over the world are likely to increase to 642 million by 2040 [4]. T2D accounts for high morbidity and mortality due to complications like renal failure, amputations, cardiovascular disease, and cerebrovascular events [5]. In 2015 there were approximately 5.0 million deaths by diabetes worldwide [4]. C-reactive protein (CRP) is an inflammatory marker associated with T2DM, obesity, insulin resistance, and cardiovascular disease [6].

Furthermore, according to recent data, CRP is not an acute phase protein only, but it is a mediator of atherogenesis, serving as a marker for cardiovascular disease (CVD) [7]. CRP levels display extensive interindividual variability. Although plasma C reactive protein levels are influenced by sociodemographic,

behavioural, and lifestyle factors and obesity and type 2 diabetes, twin and family studies have estimated that genetic factors could contribute up to 35–50% of the variation of CRP [8] [9]. Several population-based association studies have shown that common genetic variants at the *CRP* locus are significantly associated with plasma CRP levels [10]. CRP gene polymorphisms of were found to be in mild to moderate association with the basal CRP levels in healthy men and women [11].

Thus, the current study aimed to evaluate the role of *CRP* (+1059G/C) polymorphism in type 2 diabetic patients.

Material and Methods

The present case-control study enrolled a total of 200 individuals. There were 100 newly diagnosed type 2 diabetes and 100 healthy controls collected. This study was approved by the institutional ethical committee, Jamia Millia University. Written informed consent was obtained from all individual participants included in the study.

All the clinical parameter such as fasting glucose and postprandial glucose were taken care as per standard criteria. Total 3 ml of blood sample were withdrawn, 1 collected in EDTA vials and 2 ml in plain vials from all the study subjects included in the study.

DNA isolation was done by a phenol-chloroform method from blood samples collected in EDTA vials from cases as well as controls. Genomic DNA was analysed on 1% agarose gel to confirm and observed under UV transilluminator.

Isolated DNA was then amplified to determine the genotypes of CRP by allele-specific primers forward: 5′ CATTTGTACAAGCTGGGAGT 3′, 2. Allele C specific reverse: 5′ ATGGTGTTAATCTCATCTGGTGGG 3′, Allele G specific reverse: 5′′TGGTGTTAATCTCATCTGGTGGC 3′ using thermocycler. PCR was performed in 25 µl reaction volume containing 3 µl of 100 ng template DNA, 0.25 µl of 25 pmol each primer, 2.5 µl of 10 mM dNTPs, 1.5 µl of 20mM MgCl2, 0.3 µl of 5 U/µl Taq polymerase with 2.5 µl of 10X Taq Buffer (Fermantas) and 14.7 µl of nuclease-free ddH_2O. The PCR was performed with initial denaturation at 94°C for 10 minutes, followed by 40 cycles of denaturation at 95°C for 40 seconds, annealing at 58°C for 40 seconds, extension at 72°C for 40 seconds and the final extension was at 72°C for 10 minutes. The amplified product of 237 bp was visualised under UV transilluminator.

Genotype frequencies between the cases and controls were evaluated using the Chi-square test and values ≤ 5 were analysed by Fisher exact test. Allele frequency was calculated by the Hardy–Weinberg Equilibrium (HWE) equation. The associations between *CRP* genotypes and risk of T2D were estimated by computing the odds ratios (ORs) with 95% confidence intervals (CIs). The parametric and nonparametric test was used to analyse the quantitative data, p-value less than 0.05 considered to be statistically significant.

Results

Demographic characteristic of study subjects investigated in the present study has been summarised in Table 1.

Table 1: Distribution of selected characteristics among T2D patients and healthy controls

Variables	T2D patients n (%)	Healthy controls n (%)
Total no.	100 (100%)	100 (100%)
Gender		
Males	62 (62%)	64 (64%)
Females	38 (38%)	36 (36%)
Age at diagnosis (Years)		
≤ 50	61 (%)	66 (%)
> 50	39 (%)	34 (%)
Mean age (years)	49.82	47.89
HTN		
Yes	63 (63%)	28 (28%)
No	37 (37%)	72 (72%)
Obesity		
Yes	20 (20%)	5 (5%)
No	80 (80%)	95 (95%)
Smoking status		
Yes	58 (58%)	30 (30%)
No	42 (42%)	70 (70%)
Alcoholism		
Yes	49 (49%)	26 (26%)
No	51 (51%)	74 (74%)

The difference observed in genotype among cases and controls was found to be significant (p = 0.001) (Table 2). It was observed that high percentage of heterozygous GC 16 (16%) and mutant homozygous CC 8 (8%) genotype was found in patients compared to controls, heterozygous GC 8 (8%) and CC 0 (0%) while lower GG 76 (76%) genotype in patients compared to control homozygous GG 90 (90%) genotype. The higher allele frequency of C allele (0.16) was observed in T2D patients compared to control (0.04).

Table 2: Genotype distribution and allele frequencies of *CRP* (+1056G/C) gene among T2D patients and controls

Variables	GG n(%)	GC n(%)	CC n(%)	p value	Allele frequency	
					G allele	C allele
Patients (n = 100)	76 (76)	16 (16)	8 (8)	0.001	0.84	0.16
Controls (n = 100)	92 (92)	8 (8)	0 (0)		0.96	0.04

Odds ratio with 95 % confidence intervals was calculated for each group to estimate the degree of association between the *CRP* genotype and risk of T2D in Indian patients depicted in Table 3. Compared to the GG genotype, the OR 2.72 (1.12-6.61) and 20.56 (1.16-362.1) for the heterozygous GC and homozygous CC genotypes were estimated,

suggesting a possible dominant effect of *CRP* polymorphism on T2D risk.

Table3: Risk of T2D associated with *CRP* (+1056G/C) genotype

Genotype	Healthy controls (n = 100)	T2D patients (n = 100)	OR (95% CI)
GG	92 (92%)	76 (76%)	**(ref)**
GC	8 (8%)	16 (16%)	2.42 (0.98-5.96)
CC	0 (0%)	8 (8%)	20.56 (1.16-362.1)
GC+CC	8 (8%)	24 (24%)	3.63 (1.54-8.54)

The risk associated with Hypertension (HTN), Obesity, smoking and alcoholism was calculated in T2D patients and depicted in (Table 4). In HTN, compared to the GG genotype, the OR 1.52 (0.47-4.79), 4.82 (0.56-41.20) for the heterozygous GC and homozygous CC genotypes. In obesity, compared to the GG genotype, the OR 1.61 (0.44-5.80) and 2.90 (0.61-13.72) for the heterozygous GA and homozygous AA genotypes. Patients with smoking, alcoholism status compared to the GG genotype, the OR for GC 1.87 (0.59-5.92), 3.02 (0.95-9.56) and for CC 2.56 (0.48-13.51), 4.12 (0.78-21.79) respectively.

Table 4: Risk of *CRP* genotype in T2D patients associated with different variables

Genotype	HTN No (n = 37)	HTN Yes (63)	OR* (95% CI)
GG	**31**	**45**	**(ref)**
GC	**5**	**11**	1.51 (0.47-4.79)
CC	**1**	**7**	4.82 (0.56-41.20)
Genotype	**Obesity No (n = 80)**	**Obesity Yes (n = 20)**	**OR* (95% CI)**
GG	**63**	**13**	**(ref)**
GC	**12**	**4**	1.61 (0.44-5.80)
CC	**5**	**3**	2.90 (0.61-13.72)
Genotype	**Smoker No (n=42)**	**Smoker Yes (n=58)**	**OR* (95% CI)**
GG	35	41	**(ref)**
GC	5	11	1.87 (0.59-5.92)
CC	2	6	2.56 (0.48-13.51)
Genotype	**Alcohol No (n=49)**	**Alcohol Yes (n=51)**	**OR* (95% CI)**
GG	32	44	**(ref)**
GC	11	5	3.02 (0.95-9.56)
CC	6	2	4.12 (0.78-21.79)

Other serum markers such as cholesterol, TG, LDL, VLDL, HDL, serum uric acid, bilirubin total, fasting blood glucose and postprandial glucose were compared between T2D patients, and healthy controls were shown in table 6. High lipid parameter such as TG, LDL, VLDL and were observed higher in T2D patients compared to controls and the differences was found to be significant ($p < 0.0001$, $p < 0.0001$, $p < 0.0001$), however no significant difference was observed in cholesterol level among T2D cases and controls ($p = 0.73$). Serum uric acid and total bilirubin were observed to be high compared to healthy control group ($p < 0.0001$, $p < 0.0001$). High fasting and postprandial blood glucose were observed in T2D cases compared to healthy controls, and the difference was found to be significant ($p < 0.0001$, $p < 0.0001$).

Table 5: Serum markers level of T2D patients and healthy controls

Serum markers	T2D patients (Mean ± SD)	Healthy controls (Mean ± SD)	p-value
Cholesterol	206.8 ± 43.02	205.2 ± 19.05	0.73
TG	183.3 ± 42.62	139.5 ± 24.92	<0.0001
LDL	147.4 ± 28.77	113.3 ± 14.10	<0.0001
VLDL	34.16 ± 5.62	25.32 ± 4.81	<0.0001
Serum uric acid	5.44 ± 1.85	4.20 ± 0.74	<0.0001
Bilirubin total	0.86 ± 0.44	0.64 ± 0.20	0.0003
Fasting blood sugar	171.1 ± 39.55	92.75 ± 10.88	<0.0001
Postprandial blood sugar	257.7 ± 51.94	126.7 ± 9.58	<0.0001

Patients and controls with different risk factor were analysed to see the effect on biochemical parameters, and it was observed that the patients and controls had hypertension history had a significant impact on biochemical parameters. T2D patients with HTN history had elevated level of TG, LDL, VLDL, Serum uric acid, bilirubin, fasting blood sugar and postprandial blood sugar compared to healthy control with HTN history. In the same way, others risk factors were found to affect most of the biochemical parameters and found to be significant.

Table 6: Effect of different risk factor positive on serum markers level among T2D patients and healthy controls

HTN history yes	In T2D	Healthy control	p-value
Cholesterol	208.3 ± 43.49	210.2 ± 17.95	0.82
TG	182.8 ± 43.10	138 ± 23.67	< 0.0001
LDL	138.9 ± 27.19	118.4 ± 15.54	0.0003
VLDL	34.62 ± 5.74	26.32 ± 3.78	< 0.0001
Serum uric acid	5.51 ± 1.73	4.26 ± 0.59	< 0.0001
Bilirubin total	0.83 ± 0.43	0.67 ± 0.20	0.23
Fasting blood sugar	174.8 ± 42.39	92.54 ± 11.66	< 0.0001
Postprandial blood sugar	256.2 ± 54.99	127.1 ± 1.60	< 0.0001
Obesity history yes	**In T2D**	**Healthy control**	**p-value**
Cholesterol	207.6 ± 45.59	208 ± 27.16	0.98
TG	194.4 ± 47.36	174.8 ± 49.06	0.42
LDL	140 ± 27.69	109.2 ± 12.66	0.02
VLDL	33.80 ± 5.55	23.60 ± 4.27	0.0009
Serum uric acid	5.41 ± 1.51	4.02 ± 0.73	0.009
Bilirubin total	0.68 ± 0.33	0.78 ± 0.16	0.16
Fasting blood sugar	167.1 ± 31.61	93.0 ± 11.90	< 0.0001
Postprandial blood sugar	251.5 ± 56.60	128.8 ± 10.03	< 0.0001
Alcoholism history yes	**In T2D**	**Healthy control**	**p-value**
Cholesterol	210.7 ± 45.08	201.6 ± 18.35	0.33
TG	191.1 ± 44.13	136.0 ± 14.46	< 0.0001
LDL	139.1 ± 28	117.2 ± 14.20	0.0004
VLDL	34.22 ± 5.46	24.81 ± 4.36	< 0.0001
Serum uric acid	5.75 ± 2.05	4.19 ± 0.87	0.0004
Bilirubin total	0.87 ± 0.44	0.68 ± 0.20	0.08
Fasting blood sugar	180.4 ± 46.93	93.42 ± 11.73	< 0.0001
Postprandial blood sugar	262.6 ± 55.92	123.3 ± 9.04	< 0.0001
Smoking history yes	**In T2D**	**Healthy control**	**p-value**
Cholesterol	208.2 ± 42.54	203.5 ± 20.04	0.56
TG	185.8 ± 48.01	136.3 ± 24.74	< 0.0001
LDL	139.4 ± 29.59	113.1 ± 12.85	< 0.0001
VLDL	35.09 ± 6.06	25.0 ± 4.08	< 0.0001
Serum uric acid	5.44 ± 1.73	4.23 ± 0.92	0.0002
Bilirubin total	0.82 ± 0.39	0.66 ± 0.21	0.03
Fasting blood sugar	173.7 ± 42.02	92.0 ± 10.86	< 0.0001
Postprandial blood sugar	261.1 ± 57.51	128.3 ± 8.92	< 0.0001

Discussion

Identification of the genetic determinants could facilitate the risk prediction of disease development and the implementation of individualised treatment for therapy. Up to now, genome-wide association studies (GWAS) for diabetes have identified more than 80 susceptibility loci, but only a small part of the heritability of diabetes can be explained by those findings [12]. In the present study we observed a significant difference in the distribution of *CRP* genotype among T2D case and healthy controls, higher mutant allele distribution was observed among T2D cases compared to healthy controls. An independent association of mutant CC genotype and GC heterozygous genotype of *CRP* gene were found to be associated with increased risk of T2D.

It was observed that the CC and GC genotype in patients showed more than 20 and 2 fold increase risk of T2D compared to healthy controls. Patients with different status like hypertension (HTN), doing alcoholism showed more than 4 fold increased the risk of T2D with mutant CC genotypes of the *CRP* gene. However, obesity, smoking had mutant CC genotypes of *CRP* gene showed more than 2 fold higher risk of T2D. Hypertension, alcoholism, obesity and smoking behaviour are suggestive to a possible risk factor for developing T2D. It has been observed that several lipid parameters such as TG, LDL, and VLDL were found to be elevated in T2D cases compared to healthy controls.

Serum uric acid and total bilirubin were found to elevated in T2D compared to healthy controls as the basal values of *CRP* appear to be significantly heritable [13]. D. Thalmaier et al. found that polymorphisms in the CRP gene and in genes controlling *CRP* expression influence *CRP* levels and *CRP* (+1059G/C) polymorphism involved in the same way [14]. It has been observed that +1059 G/C (rs1800947) nucleotide polymorphism (SNP) in exon 2 of the *CRP* gene. +1059G/C is a synonymous polymorphism, which has been reported to affect the protein levels of *CRP* and contribute to the progression of CAD (coronary artery disease) and T2D [15].

Hypertension is recognised globally as a major risk factor for CVD, stroke, diabetes, and renal diseases [16]. Several studies have shown that most of the hypertensive patients have significant instability of serum lipid parameters in hypertensive patients [17, 18]. Smoking may be linked to impaired glucose and lipid metabolism, and a wealth of evidence indicates that insulin resistance, abnormal glucose and lipid metabolism [19]. Parchwani DN et al. reported that glucose and altered lipid profile are directly found to be correlated with smoking and length of smoking years [20], and Haggard also reported that smoking even a single could be the cause of increased blood sugar [21].

It has been suggested moderate alcohol intake increases the risk of type 2 diabetes, especially in Japanese lean subjects [22]. Considering facts that East Asian subjects with normal BMI level (< 25 kg/m^2) can easily develop type 2 diabetes [23], and large differences in the polymorphic distribution of alcohol-metabolizing enzymes have been reported between East Asian and Caucasian, the effects of alcohol intake on type 2 diabetes might vary according to ethnicity [24].

The study concluded that *CRP* gene polymorphism was found to be associated with risk of Type 2 Diabetes and risk was linked with heterozygosity and mutant homozygosity.

Hypertension, Obesity, Smoking and alcoholism significantly altered biochemical parameters in Type 2 Diabetes patients. These interesting results of the study deserve further validation on a larger population.

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