

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

Hakim Bahlok Jebur<sup>1&</sup>, Mirza Masroor<sup>2&</sup>, Hafiz Ahmad<sup>3</sup>, Naushad Ahmad Khan<sup>4</sup>, Juheb Akther<sup>1</sup>, Dipu Bharali<sup>5</sup>, Vijay Kumar Singh<sup>6</sup>, Amit Verma<sup>7</sup>, Shahbaz Khan<sup>7</sup>, Vasiuddin Khan<sup>7</sup>, Rameez Hasan<sup>7</sup>, Deepti Bhatt<sup>7</sup>, Yamini Goyal<sup>7</sup>, Kapil Dev<sup>7</sup>

<sup>1</sup>Department of Pharmacology, Faculty of Medicine, WAIST University, Iraq; <sup>2</sup>Department of Medical Elementology and Toxicology, Jamia Hamdard, New Delhi, India; <sup>3</sup>Department of Medical Microbiology and Immunology, RAK Medical & Health Sciences University, Ras Al Khaimah, UAE; <sup>4</sup>Department of Environmental Medicine, University of Rochester, New York, USA; <sup>5</sup>Department of Medicine, Maulana Azad Medical College, New Delhi, India; <sup>6</sup>Department of Biochemistry, Maulana Azad Medical College, New Delhi, India; <sup>7</sup>Department of Biotechnology, Jamia Millia Islamia, University, New Delhi, India

## Abstract

**Citation:** Jebur HB, Masroor M, Ahmad H, Khan NA, Akther J, Bharali D, Singh VK, Verma A, Khan S, Khan V, Hasan R, Bhatt D, Goyal Y, Dev K. CRP Gene Polymorphism and Their Risk Association With Type 2 Diabetes Mellitus. Open Access Maced J Med Sci. 2019 Jan 15; 7(1):33-37.  
<https://doi.org/10.3889/oamjms.2019.014>

**Keywords:** Type 2 Diabetes; CRP gene polymorphism; Biochemical parameters

**\*Correspondence:** Dr Kapil Dev, Department of Biotechnology, Jamia Millia Islamia, University, New Delhi, India. E-mail: kapil1814db@gmail.com

**Received:** 10-Aug-2018; **Revised:** 18-Oct-2018; **Accepted:** 20-Oct-2018; **Online first:** 11-Dec-2019

**Copyright:** © 2019 Hakim Bahlok Jebur, Mirza Masroor, Hafiz Ahmad, Naushad Ahmad Khan, Juheb Akther, Dipu Bharali, Vijay Kumar Singh, Amit Verma, Shahbaz Khan, Vasiuddin Khan, Rameez Hasan, Deepti Bhatt, Yamini Goyal, Kapil Dev. This is an open-access article distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International License (CC BY-NC 4.0)

**Funding:** This research did not receive any financial support

**Competing Interests:** The authors have declared that no competing interests exist

**&:** Equal contribution of authors

**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 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' CATTGTACAAGCTGGGAGT 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 MgCl<sub>2</sub>, 0.3 µl of 5 U/µl Taq polymerase with 2.5 µl of 10X Taq Buffer (Fermentas) and 14.7 µl of nuclease-free ddH<sub>2</sub>O. 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%)
<b>Gender</b>		
Males	62 (62%)	64 (64%)
Females	38 (38%)	36 (36%)
<b>Age at diagnosis (Years)</b>		
≤ 50	61 (%)	66 (%)
> 50	39 (%)	34 (%)
Mean age (years)	49.82	47.89
<b>HTN</b>		
Yes	63 (63%)	28 (28%)
No	37 (37%)	72 (72%)
<b>Obesity</b>		
Yes	20 (20%)	5 (5%)
No	80 (80%)	95 (95%)
<b>Smoking status</b>		
Yes	58 (58%)	30 (30%)
No	42 (42%)	70 (70%)
<b>Alcoholism</b>		
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 ± 11.60	< 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
Alcohol 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<sup>2</sup>) 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.

## Acknowledgements

We gratefully acknowledge the study subjects who were part of this study.

## References

1. Wild S, Rolic C, Green A. Global prevalence of diabetes: Estimates for the year 2000 and projection for 2030. *Diabetes Care*. 2004; 37:1047-1053. <https://doi.org/10.2337/diacare.27.5.1047>
2. MarreM, Hadjadj S, Bouhanick B. Hereditary factors in the development of the diabetic renal disease. *Diabetes Metab*. 2000; 26:30–6. PMID:10922971
3. Schulze M, Rifai N, Rimm E, Stampfer M, Li T, Hu F. C-reactive protein and incident cardiovascular events among men with diabetes. *Diabetes Care*. 2004; 27:889–894. <https://doi.org/10.2337/diacare.27.4.889> PMID:15047644
4. International Diabetes Federation, "IDF diabetes atlas," in International Diabetes Federation, pp. 1–144, Brussels, Belgium, 7th edition, 2015. <http://www.diabetesatlas.org>.
5. American Diabetes Association. Standards of medical care in diabetes—2013. *Diabetes care*. 2013; 36(Suppl 1):S11. <https://doi.org/10.2337/dc13-S011> PMID:23264422 PMID:PMC3537269
6. Thorand B, Löwel H, Schneider A, Kolb H, Meisinger C, Fröhlich M, Koenig W. C-reactive protein as a predictor for incident diabetes mellitus among middle-aged men: results from the MONICA Augsburg cohort study, 1984-1998. *Archives of internal medicine*. 2003; 163(1):93-9. <https://doi.org/10.1001/archinte.163.1.93> PMID:12523922
7. Tousoulis D, Hatzis G, Papageorgiou N, Androulakis E, Bouras G, Giolis A, Bakogiannis C, Siasos G, Latsios G, Antoniadis C, Stefanadis C. Assessment of acute coronary syndromes: focus on novel biomarkers. *Current medicinal chemistry*. 2012; 19(16):2572-87. <https://doi.org/10.2174/092986712800493011> PMID:22489714
8. Carlson CS, Aldred SF, Lee PK, Tracy RP, Schwartz SM, Rieder M, Liu K, Williams OD, Iribarren C, Lewis EC, Fornage M. Polymorphisms within the C-reactive protein (CRP) promoter region are associated with plasma CRP levels. *The American Journal of Human Genetics*. 2005; 77(1):64-77. <https://doi.org/10.1086/431366> PMID:15897982 PMID:PMC1226195
9. Miller DT, Zee RY, Suk Danik J, Kozlowski P, Chasman DI, Lazarus R, Cook NR, Ridker PM, Kwiatkowski DJ. Association of common CRP gene variants with CRP levels and cardiovascular events. *Annals of human genetics*. 2005; 69(6):623-38. <https://doi.org/10.1111/j.1529-8817.2005.00210.x> PMID:16266402
10. Brull DJ, Serrano N, Zito F, Jones L, Montgomery HE, Rumley A, Sharma P, Lowe GD, World MJ, Humphries SE, Hingorani AD. Human CRP gene polymorphism influences CRP levels: implications for the prediction and pathogenesis of coronary heart disease. *Arteriosclerosis, Thrombosis, and Vascular Biology*. 2003; 23(11):2063-9. <https://doi.org/10.1161/01.ATV.0000084640.21712.9C> PMID:12842840
11. Wang JR, Zhang YL. Association between ATPbinding cassette transporter A1 R219K gene polymorphisms and acute myocardial infarction. *Academic Journal of Chinese PLA Medical*

School. 2013:777–78.

12. Fuchsberger C, Flannick J, Teslovich TM, Mahajan A, Agarwala V, Gaulton KJ, Ma C, Fontanillas P, Moutsianas L, McCarthy DJ, Rivas MA. The genetic architecture of type 2 diabetes. *Nature*. 2016; 536(7614):41.

<https://doi.org/10.1038/nature18642> PMID:27398621  
PMCID:PMC5034897

13. MacGregor AJ, Gallimore JR, Spector TD, Pepys MB. Genetic effects on baseline values of C-reactive protein and serum amyloid a protein: a comparison of monozygotic and dizygotic twins. *Clinical chemistry*. 2004; 50(1):130-4.

<https://doi.org/10.1373/clinchem.2003.028258> PMID:14633907

14. Thalmaier D, Dambacher J, Seiderer J, Konrad A, Schachinger V, Pfennig S, OTTE JM, Crispin A, Göke B, Ochsenkühn T, Lohse P. The +1059G/C polymorphism in the C-reactive protein (CRP) gene is associated with involvement of the terminal ileum and decreased serum CRP levels in patients with Crohn's disease. *Alimentary pharmacology & therapeutics*. 2006; 24(7):1105-15.

<https://doi.org/10.1111/j.1365-2036.2006.03093.x> PMID:16984505

15. Lange LA, Carlson CS, Hindorf LA, Lange EM, Walston J, Durda JP, Cushman M, Bis JC, Zeng D, Lin D, Kuller LH.

Association of polymorphisms in the CRP gene with circulating C-reactive protein levels and cardiovascular events. *Jama*. 2006; 296(22):2703-11.

<https://doi.org/10.1001/jama.296.22.2703>

PMid:17164456

16. Saha MS, Sana NK, Shaha RK. Serum lipid profile of hypertensive patients in the northern region of Bangladesh. *Journal of Bio-Science*. 2006; 14:93-8.

17. Bambara R, Mittal Y, Mathur A. Evaluation of Lipid Profile of North Indian Hypertensive Subjects. *Asian Journal of Biomedical*

and Pharmaceutical Sciences. 2013; 3:38–41.

18. Ijeh I, Ejike CE, Okorie U. Serum lipid profile and lipid pro-atherogenic indices of a cohort of Nigerian adults with varying glycemic and blood pressure phenotypes. *International Journal of Biological and Chemical Sciences*. 2010; 4(6):2102–2112.

19. Attvall S, Fowelin J, Lager I, von Schenck H, Smith U. Smoking induces insulin resistance: a potential link with the insulinresistance syndrome. *J Intern Med*. 1993; 233:327-332.

<https://doi.org/10.1111/j.1365-2796.1993.tb00680.x> PMID:8463765

20. Parchwani DN, Upadhyah AA, Chandan DH. Effect of active smoking on glucose tolerance and lipid profile. *Int J Med Sci Public Health*. 2013; 2:20-25.

<https://doi.org/10.5455/ijmsph.2013.2.20-25>

21. Haggard HW, Greenberg LA. The effects of cigarette smoking upon the blood sugar. *Science*. 1934; 79(2042):165-6.

<https://doi.org/10.1126/science.79.2042.165> PMID:17788147

22. Seike N, Noda M, Kadowaki T. Alcohol consumption and risk of type 2 diabetes mellitus in Japanese: a systematic review. *Asia Pacific Journal of Clinical Nutrition*. 2008; 17(4):545-51.

PMid:19114388

23. Chan JC, Malik V, Jia W, Kadowaki T, Yajnik CS, Yoon KH, Hu FB. Diabetes in Asia: epidemiology, risk factors, and pathophysiology. *Jama*. 2009; 301(20):2129-40.

<https://doi.org/10.1001/jama.2009.726> PMID:19470990

24. Druesne-Pecollo N, Tehard B, Mallet Y, Gerber M, Norat T, Hercberg S, Latino-Martel P. Alcohol and genetic polymorphisms: effect on risk of alcohol-related cancer. *The lancet oncology*. 2009; 10(2):173-80.

[https://doi.org/10.1016/S1470-2045\(09\)70019-1](https://doi.org/10.1016/S1470-2045(09)70019-1)