ISSN 2515-8260 Volume 10, Issue 04, 2023

ASSOCIATION OF GST *T1/M1* GENE POLYMORPHISMS WITH RISK OF DIABETIC DYSLIPIDEMIA IN NORTH INDIAN POPULATION

Suman Gautam[1], Annu Chandra[2], Zeba Siddiqi[3], Tasleem Raza[4] Saumya Chandra *

[1] Department of Biochemistry, Era's Lucknow Medical University, Lucknow, Uttar Pradesh, India.

[2,4]Department of Biochemistry, Era's Lucknow Medical University, Lucknow, Uttar Pradesh, India

[3]Department of Medicine, Era's Lucknow Medical University, Lucknow, Uttar Pradesh, India.

***Corresponding Author**: Saumya Chandra, Assistant Professor, Department of Biochemistry, Autonomous State Medical College, Firozabad, Uttar Pradesh, India**.** Email id saumya.kgmu@gmail.com, contact no. 9889333606

Abstract

Introduction: Diabetes has emerged as one of the most serious and common chronic diseases of our times, causing life threatening, disabling and costly complications, and reducing life expectancy. Type 2 diabetes (T2D) is a multifactorial disease affecting mostly adults older than 40 years. The aim of the study was to examine *GST* gene polymorphism influence on the risk of T2D, especially in young adults. **Research design and methods**: 50 diabetic patients and 50 healthy controls participated in this study. *GST* gene polymorphism has been analyzed using TaqMan real-time quantitative PCR. **Results** The distribution of examined polymorphisms was similar in patient group and control group. Statistically no significant differences were demonstrated for *GSTT1/M1* genotypes. We found a significant association with GSTT1 gene with lipid profile and blood pressure. **Conclusion:** The results of our study suggest that *GST* polymorphism may be one of the risk factors for developing T2D Dyslipidemia and patients are at the high risk of coronary artery disease.

Keywords: GSTM1/GSTT1 gene, Genomic DNA, Blood Pressure, Genotypes

Introduction

Type 2 diabetes (T2D) is a global public health problem that is evolving with the increasing prevalence of obesity, unhealthy lifestyle and the population's aging problem. The disease affects mostly adults, but also children and adolescents, especially in high-income countries. [1] According to the International Diabetes Federation (IDF), T2D is recognized in over 90% of cases with diabetes mellitus[2] It is characterized by chronic hyperglycemia and other metabolic alterations resulting from a lack of insulin in the body and insulin resistance of tissues.[1-5] Pathogenesis of the disease is complex and involves not only environmental factors but also genetic vulnerability. Most of T2D cases are diagnosed after 40 years of age, especially in subjects with obesity or overweight following a western diet lifestyle [4,6] but there is also a

group of younger patients. The development of molecular technologies in the twenty-first century has allowed researchers to focus on individual genetic predispositions to T2D. It is considered that antioxidant and detoxification gene polymorphism play an important role in the risk of T2D [7,8] Some of them are *GST* genes coding glutathione S-transferases which are phase II key detoxifying enzymes. These enzymes are involved in the glutathione-coupling reactions of a broad range of electrophilic substances, thus facilitating their detoxification, metabolism and excretion. That is why they play an important role in cell protection against environmental pollutants, carcinogens, chemotherapeutics, oxidative stress products and a wide spectrum of xenobiotics. *GSTP1, GSTM1* and *GSTT1* gene polymorphisms result in low enzyme activity as reported for the *GST* gene family. Loss or reduction of enzyme activity leads to the reduction of the ability to neutralize toxins.[7,9] There have been many studies focused on *GST* gene polymorphism as a risk factor of T2D in last few years, but the results are ambiguous[1,2,6,12]

AIM:

The aim of present study was to study GSTM1/GSTT1 gene polymorphism in Diabetes Dyslipidemia cases as well as in controls.

MATERIALSANDMETHODS:

PLACE OF STUDY: The study was conducted in Central Research Lab, Department of Biochemistry and Medicine, Era's Lucknow Medical College and Hospital. **Ethical clearance** approved by Institutional Ethical Committee.

STUDY DESIGN: Case-control **CLINICAL SETTING**: OPD of Department of Medicine, Era's Lucknow Medical College and Hospital. A diagnosed and confirmed type 2 diabetes mellitus dyslipidemia patient was recruited from OPD of Department of Medicine.

INCLUSION CRITERIA

- *Patients willing and able to provide written consent.*
- *T2DM cases with Dyslipidemia.*
- *Healthy controls without family history of diabetes.*

EXCLUSION CRITERIA:

- *History of blood disorders, endocrine disorders, malignancies.*
- *Kidney failure, liver failure, alcoholism.*
- *T1DM and pregnant or lactating women.*

SAMPLE SIZE CALCULATION

- *Sample size is calculated on the basis of proportion of* GSTM1 *null genotype in case and control group using the formula*
- $n = \frac{(Z\alpha + Z\beta)2}{(1-e)2} \left[\frac{1-p1}{p1} + \frac{1-p2}{p2}\right]$
- *Where p1= 0.136, p2 = 0.097*
- *Type I error α=5%, type II error β=10%*
- *Power of study =90%*
- *Sample size is n=50 each groups.*
- *Total sample size 100 cases were enrolled for the study.*

LABORATORY INVESTIGATION

Blood sample collection: 2 ml venous blood was collected from all participants under aseptic condition in EDTA vial. Genomic DNA was extracted from peripheral blood samples by a standard phenol-chloroform extraction method and the quality/quantity of DNA was assessed by using a spectrophotometer and gel electrophoresis checked on 1% agarose gel and quantified in a biophotometer (Eppen dorf). GSTM1 and GSTT1 genetic polymorphismswas evaluated by using multiplex polymerase chain reaction (PCR) technique. GSTM1 and GSTT1 null genotypes were detected by using multiplex polymerase chain reaction (mPCR) using specific primers: F-5′ GAACTCCCTGAAAAGCTAAAGC-3′ and R-5′GTTGGGCTCAAATATA CGGTGG-3′; F-5′TCCTTACTGGTCCTCACATCTC-3′ and R-5′TCACCG GATCATGGCCAGCA-3′ respectively .

PCR was performed in a 25 µl reaction mixture of 150–200 ng genomic DNA, 5 pmol of each primer, 2× master mix (Takara), and 0.5 U of Taq DNA polymerase (G-Biosciences) per tube using a gradient Master Cycler (Bio-Rad). The PCR products were visualized by 1% agarose gels in a Gel Documentation System (EZ, Bio-Rad).

STATISTICAL ANALYSIS:

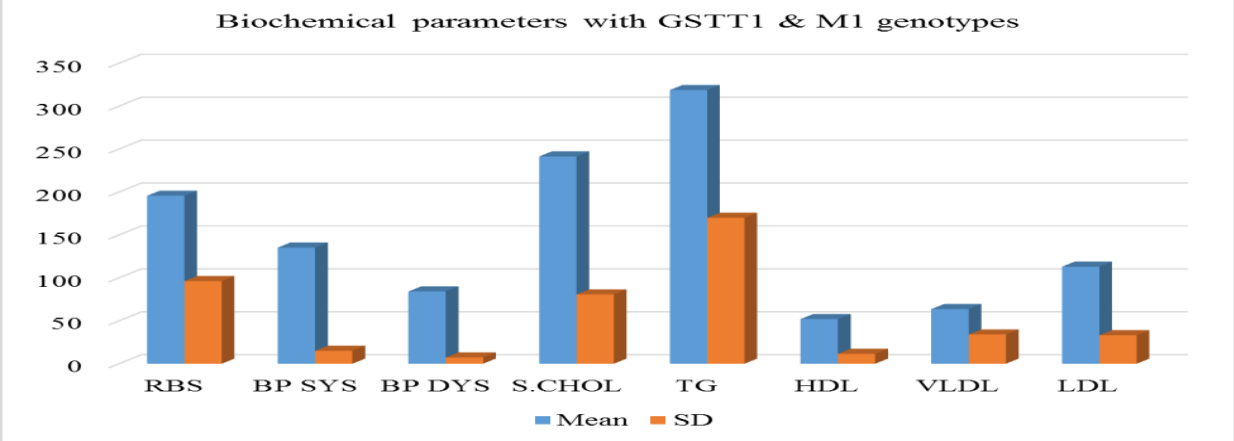
The Chi-square test with a Monte Carlo estimate of exact p-values as well as Fisher's exact test were used depending on the expected frequencies Chi-square test for goodness of fit was used to compare the observed frequencies of different GSTP1 genotypes among all subjects to expected frequencies according to Hardy–Weinberg equilibrium equation . A p-value less than 0.05 were considered statistically significant.

RESULT:

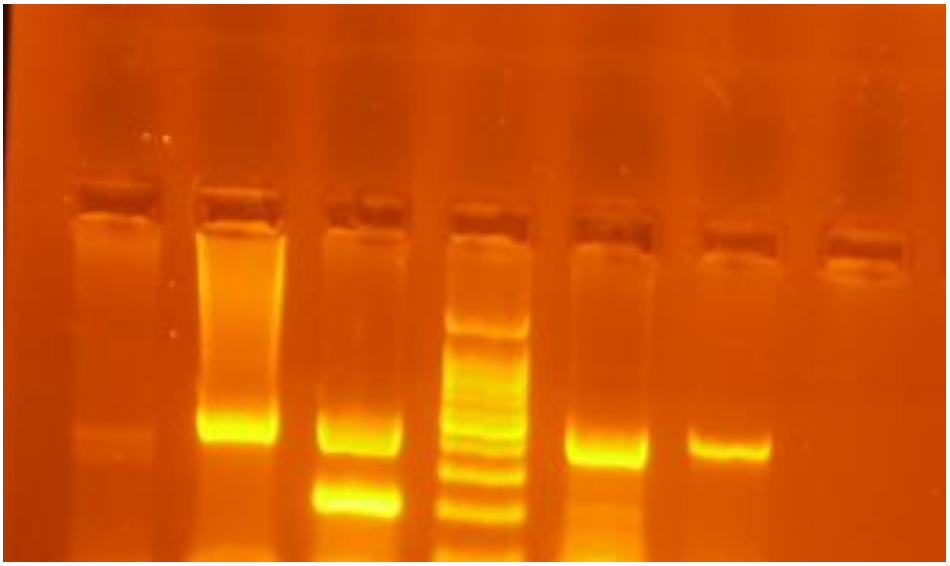
Table 1:Correlation of GSTT1 & GSTM1 genotypes with Biochemical parameters

ISSN 2515-8260 Volume 10, Issue 04, 2023

	RBS	BP SYS	BP DYS	S.CHOL	TG	HDL	VLDL	LDL
Mean	195.62	135.26	84.18	241.42	318.76	52.0232	63.568	113.008
SD	96.20745	15.03711	7.249743	80.64766	169.9176	11.489632	34.03574	33.11893



PCR PRODUCTS ANALYZED ON 1% AGAROSEGEL.



Bands at 459bp and 219bp represent GSTT1 and GSTM1 genes, respectively.

Absence of these bands indicates null genotype.

ISSN 2515-8260 Volume 10, Issue 04, 2023

Based on the electrophoresis method of genotyping (Figure 1), GSTT1 and GSTM1 genotypes were determined as numbers and percentages for healthy individuals (control) and patients. OR and 95% CI were calculated to find the significance differences among groups (Table 1).

ISSN 2515-8260 Volume 10, Issue 04, 2023

Table 1: Distribution of GSTT1 and GSTM1genotypes in case and controls

	Cases (50)		**Controls (50)**		**Analysis**			
Genotype	N	%	N	%	OR	95% CI	X2	p-value
GSTM1 (+)	31	62	33	66	1.00 (Ref)			
GSTM1 (-)	19	38	17	34	1.19	0.53-2.69	0.17	0.67
GSTT1 (+)	47	94	45	90	1.00 (Ref)			
GSTT1 (-)	3	6	5	10	0.57	0.13-2.55	0.54	0.46
GSTT1 (+)/ GSTM1 (+)	28	56	28	56	1.00 (Ref)			
GSTT1 (+)/ GSTM1 (-)	19	38	17	34	1.12	0.48-2.58	0.06	0.79
GSTT1 (-)/ GSTT1 (+)	3	6	5	10	0.6	0.13-2.75	0.43	0.5

ISSN 2515-8260 Volume 10, Issue 04, 2023

Table 2: The Associations Between GSTT1 Genotype And Glucose, Lipid Profile And Blood Pressure In Patients.			
Parameter	**Present N=47**	**Null N=3**	**P-Value**
Age	46.2 ± 10.9	48.7 ± 1.1	0.7
Age at onset	41.6 ± 10.24	43 ± 0	0.81
BMI	26.4 ± 3.8	24.02 ± 3.4	0.29
Random Blood Glucose (mg/dl)	190.6 ± 92.6	273 ± 140.3	0.15
Cholesterol (mg/dl)	241.7 ± 83.1	235.7 ± 18.5	0.9
Triglycerides (mg/dl)	332.3 ± 166	105.3 ± 23.1	**0.02**
HDL (mg/dl)	50.6 ± 10.2	74.1 ± 5.8	**0.0002**
VLDL (mg/dl)	66.3 ± 33.3	21 ± 4.6	**0.02**
LDL (mg/dl)	111.4 ± 33.5	137.7 ± 3.4	0.18
SBP (mm Hg)	134.4 ± 14.5	148.6 ± 19.6	0.11
DBP (mm Hg)	83.4 ± 6.6	96 ± 6.9	**0.002**

T**able 2: The Association Between GST*M1* Genotype And Glucose, Lipid Profile And Blood Pressure in Patients.**			
Parameter	**Present N=31**	**Null N=19**	**P-Value**
Age	45.9 ± 10.5	46.9 ±11.2	0.75
Age at onset	42 ± 10.5	41.2 ± 9.1	0.78
BMI	26.5± 4.1	25.8 ± 3.2	0.54

Random Blood Glucose (mg/dl)	181.8 ± 94.8	218 ± 96.7	0.21
Cholesterol (mg/dl)	234 ± 23.9	253.4 ± 128.5	0.25
Triglycerides (mg/dl)	273.5 ± 175.6	395.6 ± 133.9	**0.001**
HDL (mg/dl)	54.7 ± 11.5	47.5 ± 10.2	**0.006**
VLDL (mg/dl)	54.7 ± 35.1	78.03 ± 27.2	**0.001**
LDL (mg/dl)	121.9 ± 27.9	98.4 ± 36.3	**0.01**
SBP (mm Hg)	134.2 ± 15.1	136.9 ± 15.1	**0.01**
DBP (mm Hg)	83.3 ± 7.7	85.6 ± 6.3	**0.014**

The evaluation of clinical variables association with GST polymorphism in diabetic patients showed that the null GSTT1 and GSTM1 genotypes was significantly higher levels of triglycerides , LDL and blood pressure levels in both systolic and diastolic when compared to the present genotype.

Discussion: Most current studies concerning genetic factors of T2D are conducted in Asia, because it is a major area of the rapidly emerging T2D global epidemic. Some of these studies confirm a relationship between particular *GST* gene polymorphism and the risk of the disease[7,10,15,18] For example, according to Banerjee *et al*, the null/null allele combination of *GSTM1* and *GSTT1* increases the disease risk up to 1.7-fold. 15 Other studies, both conducted in the north India, showed the combined effect of *GSTM1*, *GSTT1* and *GSTP1* polymorphism on T2D risk.[17,18] A systematic review of 19 studies has shown that individually or a combination of *GSTT1null/null* and *GSTM1null/ null* genotypes are associated with T2D.[16] Another study, conducted in Iran, revealed that *GSTP1Ile105Val* polymorphism is associated with an increased risk of new-onset diabetes mellitus after liver transplantation,[10] On the other hand, a meta-analysis of 18 studies has shown no significant association between *GSTP1* polymorphism and the risk of T2D,[19] Our study finds also there was no significance differences found in GSTT1 and GST M1 genotypes .But we found there was significantly higher levels of triglycerides , LDL and blood pressure levels in both systolic and diastolic when compared to the present genotype. In our study we found a significant difference on comparing the GST T1 and M1 gene with respect to biochemical parameters.

Conclusion:

GSTT1 and GSTM1 gene polymorphism involved in T2DM dyslipidemia pathogenesis and can be considered as a marker to determine the possible susceptibility to diabetes. Null GSTT1 and

ISSN 2515-8260 Volume 10, Issue 04, 2023

null GSTM1 genotypes have an effect on blood lipids, blood glucose level and blood pressure. Large scale studies are required for further intensive and accurate results.

Conflict of the study: There was no conflict of interest in study.

References:

1 Stoian A, Bănescu C, Bălaşa RI, *et al*. Influence of GSTM1, GSTT1, and GSTP1 polymorphisms on type 2 diabetes mellitus and diabetic sensorimotor peripheral neuropathy risk. *Dis Markers* 2015;2015:1–10.
2 Ortega Ángeles, Berná G, Rojas A, *et al*. Gene-Diet interactions in type 2 diabetes: the chicken and egg debate. *Int J Mol Sci* 2017;18:1188.
3 International Diabetes Federation. IDF Europe members. Available: www. idf. org/ our-network/ regions- members/ europe/ members/ 152- poland. html
4 Polakowska M, Piotrowski W. Incidence of diabetes in the Polish population: results of the Multicenter Polish Population Health Status Study--WOBASZ. *Pol Arch Med Wewn* 2011;121:156–63.
5 Kozieł A, Pierzak M, Wychowaniec M, *et al*. Analysis of cognitive disorders in older people with diabetes – preliminary study. *Medical Studies* 2016;1:23–8.
6 Selph S, Dana T, Blazina I, *et al*. Screening for type 2 diabetes mellitus: a systematic review for the U.S. preventive services Task force. *Ann Intern Med* 2015;162:765–76.
7 Raza S, Abbas S, Ahmad A, *et al*. Association of glutathione-S- transferase (*GSTM1* and *GSTT1*) and *FTO* gene polymorphisms with type 2 diabetes mellitus cases in northern India. *Balkan J Med Genet* 2014;17:47–54. 8 Pahwa S, Sharma R, Singh B. Role of glutathione S-transferase in coronary artery disease patients with and without type 2 diabetes mellitus. *J Clin Diagn Res* 2017;11:BC05–8.
9 Moasser E, Azarpira N, Shirazi B, *et al*. Genetic polymorphisms of glutathione-S-Transferase M1 and T1 genes with risk of diabetic retinopathy in Iranian population. *Iran J Basic Med Sci* 2014;17:351–6.
10 Musavi Z, Moasser E, Zareei N, *et al*. Glutathione S-transferase gene polymorphisms and the development of new-onset diabetes after liver transplant. *Exp Clin Transplant* 2019;17:375–80.
11 Gönül N, Kadioglu E, Kocabaş NA, *et al*. The role of GSTM1, GSTT1, GSTP1, and OGG1 polymorphisms in type 2 diabetes mellitus risk: a case-control study in a Turkish population. *Gene* 2012;505:121–7.
12 Grubisa I, Otasevic P, Despotovic N, *et al*. Genetic polymorphism of glutathion S-transferase P1 (*GSTP1*) Ile105Val and susceptibility to atherogenesis in patients with type 2 diabetes mellitus. *Genetika* 2013;45:227–36.
13 American Diabetes Association. Diagnosis and classification of diabetes mellitus. *Diabetes Care* 2010;33 Suppl 1:S62–9.

ISSN 2515-8260 Volume 10, Issue 04, 2023

14 Klusek J, Nasierowska-Guttmejer A, Kowalik A, *et al*. *GSTM1*, *GSTT1*, and *GSTP1* polymorphisms and colorectal cancer risk in Polish nonsmokers. *Oncotarget* 2018;9:21224–30.
15 Banerjee M, Vats P, Kushwah AS, *et al*. Interaction of antioxidant gene variants and susceptibility to type 2 diabetes mellitus. *Br J Biomed Sci* 2019;76:166–71.