International Journal of Sciences

Research Article Volume 6 – May 2017 (05)

Genetic Polymorphism of Glutathione-S-Transferase Gene (*GSTP1*) in Type 2 Diabetes Mellitus Patients in Basra province/Iraq

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Abstract: Glutathione S-transferases (GSTs) are enzymes that included, in a wide range of detoxifying reactions by conjugation of glutathione, to electrophilic material. Polymorphisms in the genes that are responsible for GSTs affect, the function of the GSTs. GSTs play an active role in protection of cell against oxidative stress mechanism. Polymorphisms of *GSTP1* at codon 105 amino acids forms *GSTP1* important site for bind of hydrophobic electrophiles and the substitution of Ile/Val affect substrate specially catalytic activity of the enzyme and may correlate with reach to different diseases in human like diabetes mellitus type2 disease. Correlation between these polymorphisms and changes in the parameters file of diabetic patients has also been found, therefore, the results vary considerably among the studies. The polymerase chain reaction-restriction fragment length polymorphism was used to study *GSTP1*genetic polymorphism in 60 T2DM patients and 40 healthy individuals. Our results showed that presence of the *GSTP1* heterozygous mutant allele Ile/Val was more common in subjects with T2DM than in the control group (35.00% and 17.50.00%, respectively. Among patients there is an association between *GSTP1*and the risk of T2MD, both genotypes *Ile/Val* and *Val/Val* were more prevalent which result in 2.90 and 2.58 respectively risk towards T2DM .According to Hardy–Weinberg principle there was no deviation appears in the distribution of *GSTP1* Alleles. *GSTP1* genotypes do not have an effect on blood lipids after infection with diabetes mellitus.

Keywords: *GSTP1*, Polymorphism,T2DM, Type2 Diabetes Mellitus

Introduction

Type 2 mellitus (T2DM) represents a group of metabolic diseases characterized by hyperglycemia resulting from defects in pancreatic insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes associates with long term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels (ADA,2012).Oxidative stress is one of several mechanisms that contribute in the pathogenesis of T2DM and its vascular complications. It represents a state of imbalance between prooxidants and antioxidant defense system. The hyperglycemia induced overproduction of reactive oxygen species (ROS) like superoxide, peroxide of hydrogen and others. Also reactive nitrogen species (RNS) like nitric oxide leading to oxidase of DNA, proteins and other components of cell causes damage in members in the cell of body leading to cellular components damage (Pereira *et al*.,2008 ; Pitocco *et al*.,2010) .Glutathione S-Transferases (GSTs) are the active family of phase II of antioxidant enzymes is detoxify different electrophilic materials , like environmental toxins , cancer material, chemotherapeutic material and products of DNA composed by ROS cause damage to internal compound. GSTs thus plays a major role as cellular antioxidant defense mechanism (Hayes *et al*.,2005).The glutathione S-transferase P1 (*GSTP1*) gene spanning approximately 2.8 kb is located at 11q13 and contains seven exons(Cowell *et al*.,1988 ; Kano *et al*.,1987).Two polymorphic sites in the coding DNA sequence of the *GSTP1* gene have been identified, which are characterized by an A→G transition at nucleotide 313translating an isoleucine → valine substitution at codon 105 (Ile105 → Val105) in exon 5 and in second, a C→T transition at nucleotide 341 resulting in replacement of alanine → valine at the amino acid position 114 (Ala114 → Val114) in exon 6. Hence, the human *GSTP1* locus comprises of four different alleles : *GSTP1* *A (wild type Ile 105 → Ala114), *GSTP1**B (Val105 → Ala114) , *GSTP1* *C (Val 105 → Val 114) and *GSTP1**D (Ile105 → Val114) (Board *et al*.,1989 ; Harries *et al*.,1997 ; Watson *et al*.,1998). *GSTPI* plays a central role in the inactivation of toxic and carcinogenic electrophiles (Hengstler *et al*.,1998) .*GSTP1* single nucleotide polymorphism (SNP) lie on

Published at: http://www.ijsciences.com/pub/issue/2017-05/
DOI: 10.18483/ijSci.1265; Online ISSN: 2305-3925; Print ISSN: 2410-4477

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exon 5 is caused by guanine base replacing adenine at position 313 in the nucleotides of gene and this due to valine to isoleucine amino acid substitution at 105 positions of amino acids in the *GSTP1* enzyme. (Zimniak *et al.*,1994). Several studies have been conducted to investigate the association between *GSTP1*polymorphisms and T2DM (Bid *et al.* 2010, Amer *et al.* 2011). This study was designed to provide more information about the effects of the polymorphisms of *GSTP1* on T2DM risk and the complications related with T2DM in Basra -Iraq patients

Material and Methods

The study consisted of 60 clinically diagnosed diabetes mellitus type2 patients (30 male, 30 female) and 40 healthy control (20 male, 20 female) . Their age range was 35-75 years, from Almoanaa Hospital, Diabetes Center. The following detailed information were obtained: Age, sex, weight, height, Body Mass Index (BMI), Fasting Blood sugar (FBS), Total Cholesterol (TC), Triglycerides (TG), High Density Lipoprotein (HDL) , Low Density Lipoprotein (LDL) , and Very Low Density Lipoprotein (VLDL).The study was approved by the ethical committee of the study hospital.

Collection of Blood Samples:

Five milliliters of blood of each patient and healthy human were obtained by vein puncture using 5 ml disposable syringes after 12 -14 hours fasting. The blood sample was divided in to two aliquots: 3 ml and 2 ml. The first aliquot 3 ml is dispensed in a plain test tube and left for around an hour to clot at room temperature , and then separated by centrifugation at 3000 rpm for 10 min to collect serum. The separated serum used for assays of lipid profile and fasting blood sugar. The second aliquot 2ml was put into EDTA tube, this blood was mixed gently and put on shaker for 5 min then all blood samples were placed in a cool – Box under aseptic condition and this tube was stored in the freezer (-20C°) and then used for DNA extraction.

Genomic DNA extraction and genotyping:

DNA was isolated using 2 ml whole blood collected in EDTA tubes using purification kit for genomic DNA (Genaed Taiwan,). All samples showed bands which represent the genomic DNA when gel electrophoresis was applied. The polymorphism of the *GSTPI* gene was detecting using RFLP - PCR according to the method detailed by (Harries *et al.* 1997). PCR amplifications were detecting in a total volume of 30 µL consisted of 5 µL genomic DNA, 8 µl D.W., 15 µl master mix and 1 µL of each primer as follows *GSTPI* forward were 5′- ACC CCA GGG CTC TAT GGG AA-3′;and reverse were 5′ TGA GGG CAC AAG AAG CCC CT-3′ . The conditions were as follows : 95° C for 5 min of an initial denaturation step , 94°C for 30 sec. 35 cycles , 55°C for 30 sec and 72°C for 30 sec, and 72°C for 5 min of a final extension step .The fragment 176 bp that consisted by PCR was separated on a 2% agarose gel and using ethidium bromide staining to confirm the presence of these fragment. After amplification, 15 µL of PCR products was digested with 4U of *BsmAI* restriction enzyme (New England Biolabs) in a total volume of 30 µl. The mixture was incubated at 37 °C for 24 hour using an incubator .The digestion products separated on a 3% agarose gel was stained by ethidium bromide and visualized by UV transilluminator.

Statistical Analysis

The Statistical Analysis System SAS (2012) was used to study the effect of different factors on study parameters. Chi-square test was applied to compare differences in clinical parameters between patients and controls. *GSTP1* was classified as homozygous wild type Ile/Ile, heterozygous mutant Ile/ Val, mutant Val/Val. P-values were a value of ≤ 0.01, 0.05 was considered statistically significant. Least significant difference LSD test was used to compare the significance level between means in this study.

Results:

A total of 100 subjects were enrolled in this study 60 T2DM patients and 40 sex- and age matched controls. Genomic DNA extracted from all blood samples of individuals included in the study was of a good quality and integrity as seen in figure (1)

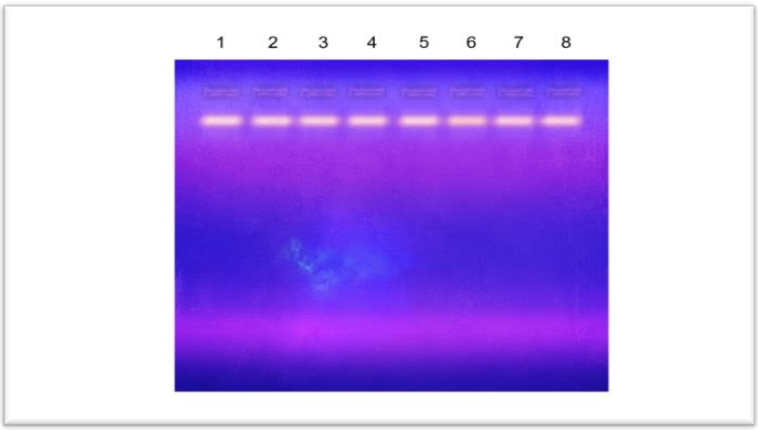

Fig.(1): Agarose gel electrophoresis of DNA extracted from blood sample. The extracted DNA was run on 0.8% agarose at 70 voltage for one hour, 1X Tris-borate buffer and stained with ethidium bromide before visualized by UV. transilluminator.

Gel electrophoresis of amplified DNA products showed the band of *GSTP1* gene at level 176 bp, figure (2).

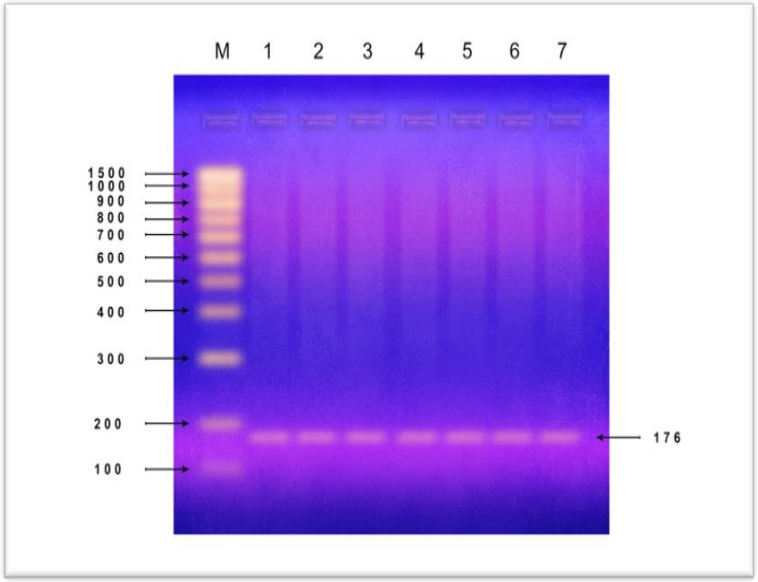

Fig. (2): Agarose gel electrophoresis of *PCR* product of the *GSTPI* gene. The PCR product resolved by 2% agarose gel electrophoresis (70 volt/ 75 min). Lane M, DNA molecular weight marker. Lane B, negative control. Lanes (1-7) are samples from patients. A176 bp DNA fragment corresponding to the *GSTP1* gene.

Products of amplified DNA were digested with *BsmA1* enzyme due to one of three possibilities; a single undigested band at 176 base pairs referring to the presence of a homozygote *ILe/Ile* allele, the presence of a restriction site resulting in two fragments (91 and 85 base pairs) referring to the presence of a *Val/Val* homozygote mutant allele, and three bands (176, 91 and 85 base pairs) referring to the presence of a heterozygote mutant allele *ILe/Val* , Figure (3).

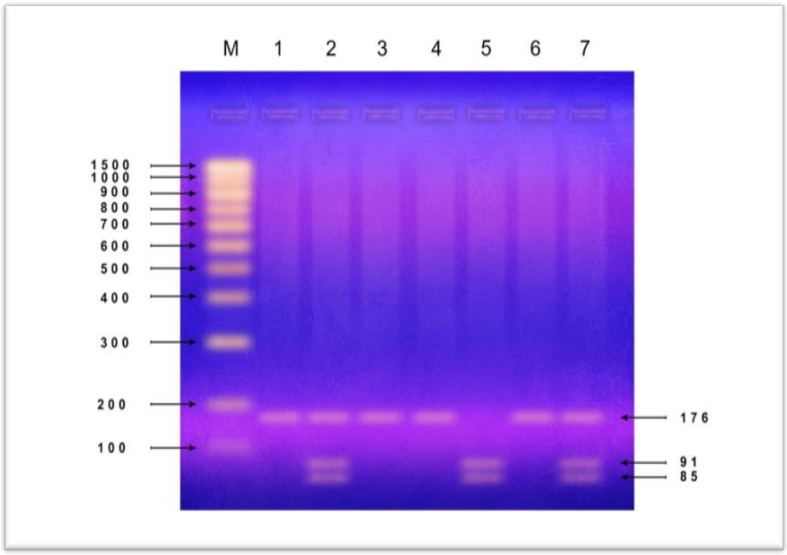

Fig. (3): Photograph of the PCR products of the *GSTPI* gene after BsmAI enzyme digestion and on a 3% agarose gel. Lane M shows the 100 bp DNA marker; lanes1, 3, 4, 6 and 7 show individuals with the Ile/ Ile genotype (176 bp). Lane 5 shows the Val/Val genotype (91bp, 85bp); and lanes 2 and 8 show the Ile/Val genotype (176bp, 91 bp, 85bp).

GSTP1 allelic distributions among cases and controls

Table 1 summarizes the *GSTP1*gene polymorphisms distribution in cases and control. Type II diabetic patients had higher frequency of heterozygous *Ile/Val* Genotype (35%) in comparison to the control group (17.50%) with an (OR =2.90; 95% CI 1.077-7.827) there was 3-fold increased type 2 diabetes mellitus risk with *Ile/Val* While homozygous for the variant (*Val/Val*) Among the cases , (13.33%) in comparison to the control group (2.50%) with an (OR = 2.58 CI; 95% CI 0.625-10.662) there was more than two and half fold increased type 2 diabetes mellitus.

Table 1 .Genotype distribution of *GSTP1* gene *Ile/Val* polymorphism in control and type 2 diabetic patients

GSTP1 Polymorphism	Case (n=60)	Control (n=40)	OR	95%CI	P-Value
Ile/Ile	31(51.67)	30(75%)	0.1	-	-
Ile/Val	21(35%)	7(17.50%)	2.90	1.077-7.827	0.031
Val/Val	8(13.33%)	3(2.50%)	2.58	0.625-10.662	0.171
P-Value	0.0001**	0.0001**			
(P< 0.05) ,(P< 0.01**)*					

Ile : Isoleucine , *Val :* Valine ; OR : Odds Ratio
95%CI: Confidence interval

According to Hardy–Weinberg principle we found there was no deviation appears in the distribution of *GSTP1*Alleles .and Ile Frequency was 0.69 in patients lower than Control 0.84 and Val frequency was in patients 0.31 higher than Control 0.16 , and Zygotic Distribution 2pq(Ile/Val) for patients was 0.428 and control 0.269 .

Table 2: Allelic and Zygotic Distribution for *GSTPI* gen According to Hardy–Weinberg principle

Allele Frequency	Patients			Control		
Ile	0.69			0.84		
Val	0.31			0.16		
Zygotic Frequency	p^2	2pq	q^2	p^2	2pq	q^2
	0. 476	0.428	0.069	0. 476	0.428	0.069
H & W law	NO			NO		

Ile : Isoleucine , *Val :* Valine
H&W**:** Hardy–Weinberg

Clinical and functional characteristic in relation to *GSTP1* genotypes:

The correlation between different genotypes of exon 5 of the *GSTPI* gene with clinical and functional parameters is presented in Table 3. We found no significant influence of *GSTPI* genotypes on lipid profile

Table 3. The relationship between *GSTPI* genotypes with lipids parameters in type 2 diabetic patients

Lipid Profile	*Ile/Ile n=31*	*Ile/Val n=21*	*Val/Val n=8*	P-value	OR	95%CI	LSD
TC	4.99±0.21	5.46± 0.26	4.78± 0.34	0.275	0.074	0.85-1.60	0.868 NS
TG	2.80 ±0.33	3.84± 0.64	2.40± 0.55	0.174	0.083	0.87-1.62	1.697 NS
HDL	1.100 ±0.14	1.090 ± 0.1	1.125 ± 0.31	0.992	0.158	0.86-1.59	0.541 NS
LDL	3.525 ±0.25	3.309 ± 0.3	3.775 ±0.78	0.761	0.64	0.88-1.61	1.173 NS
VLDL	1.552 ±0.27	1.663 ± 0.34	1.187 ± 0.23	0.556	0.072	0.86-1.60	1.086 NS
* (P<0.05)., ** (P<0.01) NS: Non Significant							

TC: Total Cholesterol, TG: Triglycerides, HDL: High Density Lipoprotein, LDL: Low Density Lipoprotein, VLDL: Very Low Density
Lipoprotein; LSD : Least Significant Difference
Ile : Isoleucine , *Val :* Valine ; OR : Oddo Ratio
95%CI: Confidence interval

Discussion:

Diabetes Mellitus Type 2 is disease that develops through an exposure to risk factors in environment and genetic susceptibility There are a common variation force is exerted on beta cells in all patients such force are the abnormal lipids and the toxic stress (Yalin *et al*., 2007). Oxidative phosphorylation during anaerobic glycolysis lead to development of (ROS) .The cell in pancreases is unusually at risk for damage by pro-oxidant because it has low levels of antioxidant system .The family of GST genes have an active role in protecting cells from reactive oxygen speciese.*GSTP1* causes the detoxification of products arising from oxidation of DNA (Yalin *et al*.,2007). A defect in detoxifying reactive oxygen species that is detecting genetically may influence the development and pathogenesis of diabetes mellitus (West,2000).

There were many studies applied with polymorphism of *GSTP1* gene in different diseases but only some studies have detected the role of polymorphism of *GSTP1* gene in diabetes mellitus. Thus, the present study was designed to detecting the role of the polymorphism of *GSTP1* gene in T2DM patients and controls groups at Basra province. Our results determined that there were significant differences in the frequencies of the *Ile/Val* genotype between patients and the control group also that the significant appearance in the frequencies of the *Val/Val* genotype between patients and the control group . We thus suggest that the allele *Val* of *GSTP1 Ile*105*Val* plays an active role in predisposition to T2DM.

There have been some results determined the relation between *GSTP1* gene polymorphism and development of diabetes mellitus disease. In an Egyptian study (Amer *et al*.,2011) it has been found that the presence of the allele of valine in the

GSTP1 gene in T2DM patients was higher than that found in controls groups, the difference was considered significant when compared to *Ile* allele. The presence of the heterozygous mutant allele of *GSTP1* was found in patient subjects more than in the healthy control .The *GSTP1* homozygous mutant allele was not found in T2DM patient and control. In the Indian study (Bid *et al*., 2010). Showed that the *GSTP1* heterozygous genotype is significantly (*P=0.001*) related with T2DM in compared in control .In contrast, Yalin *et al*. (2007) and Oniki *et al*.(2008) found that the polymorphism in *GSTP1* may not play an active role in the pathogenesis of disease in the Turkish people and Japanese people respectively. These data could be determent by differences in ethnic groups in the selected groups of study (Delles *et al*.,2008).

Some groups of the GSTs family showed activity of selenium independent glutathione peroxidase that plays an active role in protecting cells against lipid and nucleic acids (Wang *et al*.,2006) .The investigators have found an association between *GSTP1* polymorphism and cancer (Hengstler *et al*. 1998) and. But little is known about the effect of GST gene polymorphisms on blood lipids.

Increased in the amount of lipids that found in T2DM is one of the some factors responsible to vascular risk (Turner *et al.*,1998) .In the present study, we further investigated the effect of the genotypes on the lipid profile . There was no correlation between the genotypes and lipid profile in patients of diabetes . This data are in accordance with the previous study that found there were no correlation between polymorphism of *GSTP1* and blood lipids in T2DM patients (Bid *et al.*,2010 ; Ramprasath *et al*.,2011).

The mechanisms detecting the results of relation obtained in this study and works still need to be detecting with other research. Although some of our data significant effects. We acknowledge that the findings presented here are preliminary because of the small number of subjects and that the study requires confirmation in separate larger groups.

This study did not showed Hardy- Weinberg equilibrium and could be due to the small size number of the studied group.

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