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Association of Glutathione–S-Transferase (GSTP1) Genetic Polymorphism in Iraqi Patients with Diabetes Mellitus Type2

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Abstract:

Glutathione S-transferases (GSTs) are enzymes that included, in a more range of detoxifying reactions by conjugation of glutathione, to electrophilic material. Polymorphisms n the genes that responsible of 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 variation considerably among the studies; therefore, these control study was designed to leading to detecting know, as there are no studies on this performed in the people of Iraq. The polymerase chain reaction-restriction fragment length polymorphism was used to study *GSTP1*genetic polymorphism in 60 T2DM patients and 50 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 (40.00% and 32.00%, respectively; $p = 0.01$), as well as the found of the homozygous mutant of *GSTP1* allele Val/Val was common in T2DM patient and not found in the control group (3.33% and 0.00%, respectively; $p = 0.001$).*GSTP1* genotypes do not have an effect on blood lipids after infection with diabetes mellitus. Agarose gels used to determined genotypes according to the bands were that appeared in electrophoresis of gel.

Key words: Type2 Diabetes Mellitus, Glutathione-S-Transferase P1, Polymerase Chain Reaction-Restriction Fragment Length Polymorphism.

Introduction:

Diabetes mellitus represents a group of metabolic diseases caused by hyperglycemia resulting from defects in pancreatic insulin secretion and insulin action. The chronic hyperglycemia of diabetes is associated with large damage, loss of function, and destroyed of variation organs like us the eyes, kidneys, heart, and blood vessels [1].

Oxidative stress is mechanism that leading to infection with T2DM and their vascular complications and like a state of imbalance between pro-oxidants and antioxidant of defense system. The hyperglycemia caused increase production 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 [2, 3].The abnormalities of metabolic in diabetes lead to elevate mitochondrial superoxide more production in cells of endothelial of small and big vessels like the muscles of the heart .This due to the activate of several methods which elevate intracellular ROS [4, 5]. The antioxidant enzymes found in very little in beta cells because it's very sensitive to oxidative stress. Therefore beta cell is at risk of oxidative toxic damage than other member with large of antioxidant protection levels. In the pathogenesis of diabetes mellitus, oxidative and nitrosamine stresses play role to the destroyed of insulin that produce by beta cells in pancreas [6].

Several families of antioxidant enzyme have been known in detoxification and decreasing of product of ROS. Glutathione S-transferases (GSTs) are the active family of phase II of antioxidant enzymes is used to detoxify a different electrophilic material, like toxins of environmental, cancer material, chemotherapeutic material and products of DNA composed by ROS cause damage to internal compound [7] .Glutathione responsible in the defense system of the cell by destroyed free radicals and ROS compound [8]. Thus a decrease level of glutathione in patient of T2DM elevated the sensitivity of cells to toxic stresses [9].The (GST) genes enzymes species are classified into 8 classes like alpha, mu, pi, sigma, theta, kappa, omega and zeta [8,10]. GSTs used to eliminate toxic material like, carcinogens pharmaceutical drugs and others material catalyzing the reaction between organic compounds and glutathione leading to form thioethers. This mechanism used to eliminate damage that caused by free radical to cell components such as lipids, proteins and nucleic acids [6].

GSTP1 gene are found in three types involve the wild type allele (*GSTP1* *A) and two different alleles (*GSTP1* *B, *GSTP1* *C). GSTP1 single nucleotide polymorphism (SNP) lie on 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. Such replacement due to an appearance of a new allele with alteration in specific activity for substrate compared to wild-type allele [11]. Polymorphism of GSTP1 gene decrease the ability to conjugate electrophiles material with glutathione and thus sensitize cells to damage that caused by free radical .The GSTP1 variant has been correlated with susceptibility to different cancers [12] and heart disease [13,14].

There are several studies which have been investigated on the correlation between polymorphism of *GSTP1* and susceptibility to type2 diabetes and there are a big different about the results of study. These results are either from significant correlation by the polymorphisms to diabetes, or not significant relation [8]. This first case and control study on the Iraqi people was designed to provide more information about the effects of the polymorphisms *of GSTP1* on T2DM risk and the complications related with T2DM. We have found important results regarding the association of GST polymorphisms and patient of T2DM.

Material and Methods:

The study consisted of 60 clinically diagnosed diabetes mellitus type2 patients (30 male, 30 female) and 50

healthy control (22 male, 28 female) . Their age rang was (32-83) years, from National – Diabetes Center, Al-Mustansiriya University. The following detailed informations were obtained: Age, sex, weight, height, diastolic blood pressure and systolic blood pressure (DBP, SBP), Body Mass Index (BMI), Fasting Blood sugar (FBS), Blood Urea (BU), Total Cholesterol (TC), Triglycerides (TG), High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL), and Very Low Density Lipoprotein (VLDL).

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 tubes of EDTA using purification kit of the Wizard genomic DNA (promega , USA). All samples showed bands which represent the genomic DNA when the electrophoresis of the gel .The polymorphism of the GSTP1 gene was detecting using a PCR – RFLP according to the method detailed by Harries *et al*. [15].

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 follow GSTP1 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 (30 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 staining by ethidium bromide and visualized.

Statistical Analysis

The Statistical Analysis System-SAS (2012) was used to effect of different factors in 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 significant compare between means in this study.

Results:

A total of 110 subjects were enrolled in this study (60 T2DM patients and 50 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).

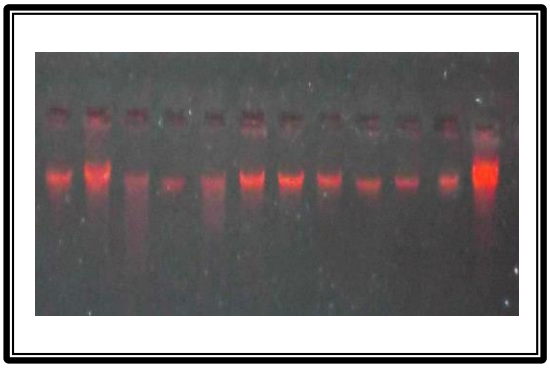

Fig.(1): Agarose gel electrophoresis of DNA extracted from blood sample. The extracted DNA was run on 0.8% agarose at 70 voltage for one hour, 1X Tris-borate buffer and stained with ethidium bromide before visualized by UV. transilluminator.

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

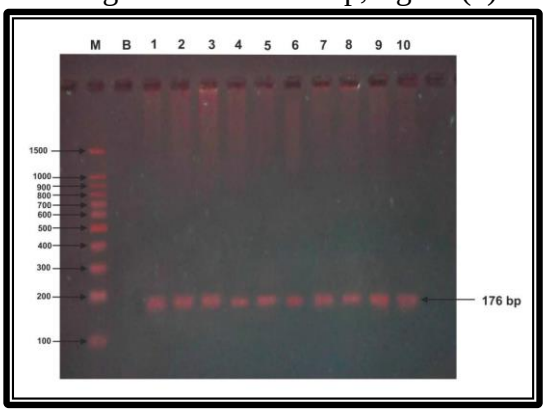

Fig. (2): *Agarose gel electrophoresis of* PCR product of the GSTP1 gene. The PCR product resolved by 2% agarose gel electrophoresis (70 volt/ 75 min). lane M, DNA molecular weight marker. Lane B, negative control. Lanes (1-10) are samples from patients. A176 bp DNA fragment corresponding to the GSTP1 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 AA allele, the presence of a restriction site resulting in two fragments (91 and 85 base pairs) referring to the presence of a GG homozygote mutant allele, and three bands (176, 91 and 85 base pairs) referring to the presence of a heterozygote mutant allele, Figure (3).

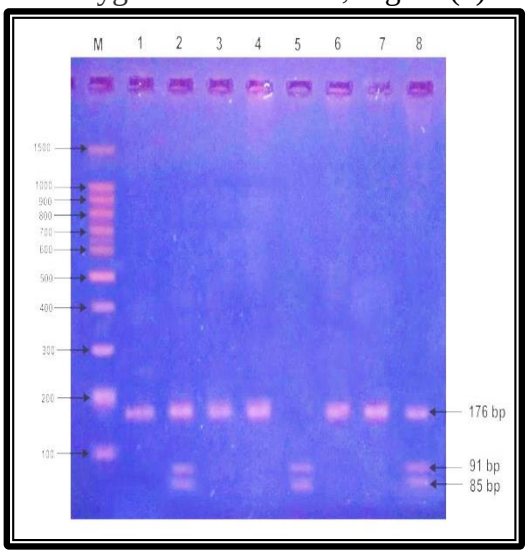

Fig. (3): Photograph of the PCR products of the GSTP1 gene after BsmAI enzyme digestion and on a 3% agarose gel. Lane M shows the 100 bp DNA ladder marker; lanes1, 3, 4, 6 and 7 show individuals with the Ile/ Ile genotype (176 bp). Lane 5 shows the Val/Val genotype (91bp, 85bp); and lanes 2 and 8 show the Ile/Val genotype (176bp, 91 bp, 85bp).

GSTP1 and the association of risk classification:

The results of polymorphism of GSTP1 represent by comparing the T2DM patient with controls are listed in Table 1. The GSTP1 allelic distributions among cases and controls were analyzed. Among the cases, 60% were homozygous for wild type (Ile/Ile), 40% were heterozygous (Ile/Val) and 3.33% were homozygous for the variant (Val/Val). Among the controls, 68 %were homozygous for the wild type (Ile/Ile), 32% were heterozygous (Ile/Val) and 0% were homozygous for the variant (Val/Val). We found that significant risk was correlated with GSTP1 to the type2 DM development.

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

GSTP1 polymorphism	Healthy control (No. = 50)		Cases (No. = 60)		P-value
	No.	%	No.	%	
Ile/ Ile	34	68.00	36	60.00	0.013**
Ile/Val	16	32.00	22	36.67	0.013**
Val /Val	0	0.00	2	3.33	0.001**

Ile : Isoleucine , *Val :* Valine

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

The correlation between different genotypes of exon 5 of the *GSTP1* gene with clinical and functional parameters is presented in Table2. We found no significant influence of *GSTP1* genotypes on lipid profile.

Table 2. The relationship between *GSTP1* genotypes with lipids parameters in type 2 diabetic patients

Lipids Profile	*Ile/ Ile*	*Ile/ Val*	p-value
TC	183.97 ± 8.35	177.81 ± 6.05	0.57
TG	178.59 ± 14.46	140.19 ± 13.43	0.063
HDL	45.17 ± 2.07	47.69 ± 3.65	0.52
LDL	107.53 ± 7.34	104.00 ± 6.57	0.73
VLDL	34.97 ± 3.01	27.15 ± 2.65	0.065

TC**:** Total Cholesterol, TG: Triglycerides, HDL: High Density Lipoprotein, LDL: Low Density Lipoprotein, VLDL: Very Low Density Lipoprotein.

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 in lipids and the toxic stress [8].

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 have 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 [8]. A defect in detoxifying reactive oxygen species that is detecting genetically may influence the development and pathogenesis of diabetes mellitus [16].

There were many studies applied with polymorphism of GSTP1gene 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. Our results determined that the significant differences in the frequencies of the Ile/Val genotype between patients and the control group were observed (40% vs. 32% respectively) also that the significant appearance in the frequencies of the Val/Val genotype between patients and the control group were found (3.33% vs. 0% respectively). We thus suggest that the G allele (Val) of GSTP1 Ile105Val 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 [17] 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 appearance when compared to allele of Ile. The presence of the heterozygous mutant allele of *GSTP1* was found in subjects with patient than in the healthy control .The *GSTP1* homozygous mutant allele was not found in T2DM patient and control. In the Indian study [18] showed that the *GSTP1* heterozygous genotype is significantly (*P*=0.001) related with T2DM in compared in control .In contrast, Yalin *et al*. [8] and Oniki *et al*. [19] 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 [20].

Some of groups of the GST family showed activity of selenium independent glutathione peroxidase that plays an active role in protecting cells against lipid and nucleic acids [6].The investigators have found a different of relation between asthma, cancer and GST gene polymorphisms. 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 [21]. 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 sure with the previous study that found that there was no correlation between polymorphism of GSTP1 and blood lipids in T2DM patients [18, 22].

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 were significant appearance, 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.

Conclusion:

This is the first study to determine the correlation of type 2 diabetes with GSTP1 genetic polymorphism in the population of Iraq. Our results suggest that GSTP1 gene polymorphisms may play an active role in the pathogenesis of type 2 diabetes mellitus.

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العلاقه بين تعدد النمط الوراثي للجين (GSTP1) ومرض السكري النوع الثاني لمرضى عراقيين

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الخلاصة:

الكلوتاثيون أس- ترانفيراز(GSTs):- هي الانزيمات التي تشترك في تفاعلات ازالة السمية بواسطة ارتباط المركبات ذات الالفة مع الكلوتاثيون. تعدد الاشكال الجينية في هذه الجينات ربما تؤثر في وظيفة هذه الانزيمات التي تلعب دورا مهما في الحماية الخلوية ضد الاكسدة . التعدد الشكلي لجين GSTP1 في الحامض الاميني رقم 105 تشكل موقع فعال لهذا الجين للارتباط مع المركبات الخارجية . استبدال الحامض الاميني الآيزوليوسين بالحامض الاميني فالين اثر في نشاط هذه الانزيمات والذي يرتبط بسهولة التأثر بالإصابة بمختلف الامراض مثل مرض السكري النوع الثاني . تم التحقق من وجود علاقة بين هذه الأشكال والتغيرات في المعايير السريرية لمرضى السكري. نتيجة للاختلاف الكبير في النتائج للدراسات فقد تم تصميم هذه الدراسة لمعرفة ارتباط جينات إزالة السمية النوع الثاني المتعدد النمط الوراثي GSTP1 في ظهور مرض السكري . هذه الدراسة من أول الدراسات حول هذا الموضوع أجريت على مرضى السكري في المجتمع العراقي. أجريت تحاليل التضاعف التسلسلي (polymerase chain reaction) وتباين اطوال قطع الدنا المقيدة (Restricton Fragment Length Polymorphism) في 110 عينة دم [60 عينة لأشخاص مصابين بالسكري النوع الثاني و 50عينة لأشخاص طبيعيين كمجموعة سيطرة].

أظهرت النتائج بان معدل تكرار التركيب الوراثي ايزوليوسين/فالين كان اعلى في المرضى بالمقارنة مع السيطرة (40% مقابل 32%). كذلك معدل تكرار التركيب الوراثي فالين/فالين كان موجود في المرضى وغير موجود في السيطرة (3.33% مقابل 0.00%) .علاقة التعدد الوراثي ل GSTP1 مع الدهون كانت غير معنوية. التراكيب الوراثية للجين تم تحديدها وفقا للحزم التي ظهرت في الهلام بعد الترحيل الكهربائي .

الكلمات المفتاحية : مرض السكري من النوع الثاني، كلوتاثايون S- ترانسفريز P1، التضاعف التسلسلي وتباين اطوال قطع الدنا المقيدة.