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Association of Glutathione S Transferase (*GSTM1* and *GSTT1*) Genetic Polymorphism and Type 2 Diabetes Mellitus

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Abstract

The occurrence of abnormal balance between both anti-oxidant defenses and free radicals raises the oxidative stress, which is associated with damage to macromolecules and cell dysfunction which leads to variety of diseases such as atherosclerosis, cancer and diabetes mellitus. Glutathione S-transferases (GSTs) are multifunctional detoxification enzymes. An important condition influencing GSTs gene expression is oxidative stress, often noticed in diabetes.

Multiplex PCR was conducted to detect the polymorphisms of *GSTT1* and *GSTM1* genes. Observations on a 100 type 2 diabetes mellitus (T2DM) patients and 40 non diabetic as control were used. The associations of various null and present genotypes of both *GSTT1* and *GSTM1* genes with fast blood glucose, lipids profile and blood pressure were compared between the two groups.

Null *GSTT1* genotype (66%) was 5.11 folds more than control (27.5). Null *GSTM1* genotype (65%) was 4.33 folds more than control (30%). Null*GSTT1* and null *GSTM1* genotype (60%) was 5.16 folds more than control (22.5%).

Both present *GSTT1* and present *GSTM1* genotypes conferred statistical significance decrease in the risk to T2DM comparing to null genotypes.

Systolic and diastolic blood pressure (SBP & DBP) was significantly higher in individuals of null *GSTT1* and *GSTM1* than in those with present *GSTT1* and *GSTM1* genotypes respectively. Fasting blood glucose and triglycerides were significantly higher in individuals of null *GSTT1* than in those with present genotype, whereas, HDL was significantly lower than in those with present *GSTT1* genotype. Fasting blood glucose and triglycerides of null *GSTM1* genotype were significantly higher than in those with present *GSTM1* genotype, whereas HDL was significantly lower than in those with present *GSTM1* genotype ($p \leq 0.05$).

Key Words: Type 2 diabetes mellitus, Glutathione S-transferase, Genetic polymorphism.

INTRODUCTION:

Defects in insulin secretion and insulin receptors result in group of metabolic diseases represent diabetes mellitus described by high blood sugar, this leads to injury and affect normal work of different body system[1-3].

Distribution of diabetes increased in the world in late years because of urbanization, growth in population and high in obesity especially in developing countries, for instance, it was estimated by International Diabetes Federation (IDF), that a dramatic increase in the incidence of diabetes in India by 2030 [4].

Reactive oxygen species (ROS) resulting from excess in blood glucose which in turn leads to cells injury resulted in more complications in diabetes mellitus **[**5, 6**]**. Enzymes of antioxidant like manganese superoxide dismutase (MnSOD), catalase (CAT) **[**7-9**]** and two classes of multi-functional GST enzymes such as GSTM1 (GST-mu) and GSTT1 (GST-theta) are important in detoxification of many toxic and carcinogenic compounds **[**10-12**]**.

GSTs involved in the defense mechanisms against the raising of free radicals, formed during oxidative stress in different tissues of diabetic **[**13**]**. GSTs group are candidate genes whose polymorphisms is known to be associated with chronic diseases development such as T2DM and malignancies **[**14-17**]**. Null genotypes of *GSTM1* and *GSTT1* polymorphism have been shown to be associated with lacking enzyme activity **[**18, 19**]**. In homozygous subjects, null *GSTM1* or *GSTT1* have no functional enzyme activity.

There were many assessment studies on correlation between GSTs polymorphism and diabetes severity. Previous articles on Caucasian population have indicated possible association of GSTs polymorphism with T2DM as microvascular complications.

The aim of our study was to evaluate the frequency of GSTs genotypes in T2DM patients and to find out the possible effects of GSTs gene polymorphism on some biochemical parameters and blood pressure**.**

MATERIALS AND METHODS

Subjects

A number of 140 persons were involved in this investigation, 100 patients of T2DM from AL-Saddir Hospital, in AL- Najaf province and 40 healthy individuals, with no history of diabetes, were used as control. The American Diabetes Association Guidelines **[**20**]** were followed to identify the T2DM patients. To be more accurate in differentiation between healthy and diabetic individuals, the essential standard criterion we adopted in sampling was the blood glucose in mg/dl, in which less than 120 for healthy and more than 140 for diabetic individuals.

Standard laboratory tests were performed on blood samples and an accurate blood pressure reading were recorded for each subject. The blood parameters under investigation were; fasting blood glucose (mg/dl), cholesterol (mg/dl), triglycerides (mg/dl), SBP (mm Hg), DBP (mm Hg), LDL (mg/dl), and HDL (mg/dl). The observations were subjected to statistical analysis. We excluded subjects' age less than 20 years, blood diseases history, liver disorders and cancer, and those with severe diabetes such as chronic pancreatitis, Cushing's disease, polycystic ovary disease, and drug induced diabetes.

Multiplex PCR and electrophoresis were used to determine the presence and absence of *GSTT1* and *GSTM1* genotypes.

GST genotyping

The null *GSTM1*and null *GSTT1* genotypes were detected using a multiplex PCR method of a total 20 µl multiplex PCR mixture containing 5 µl genomic DNA, 10pmol of primer for each gene as follows; *GSTM1* (F-5 - GAACTCCCTGAAAAGCTAAAGC-3)/ (R-5-CTTGGG CTCAAATATACGGTGG-3) and *GSTT1* (F-5-TTCCTTACT GGTCCTCACATCTC-3)/ (R-5 - TCACCGGATCATGGCCAGC A-3) and *ALBUMIN* gene primers as internal control, (F-5'-GCCCTCTGCTAACAAGTCCTAC-3') / (R-5'-GCCCTAAAAAG AAAATCGCC AATC-3') in 5 µl of AccuPower PCR PreMix (Intron Bio, Korea) was used. Initial melting temperature of 95°C for 3 minutes was adopted and followed by 30 cycles of amplification (1 minute at 95°C, 1 minute at 58°C and 1minute at 72°C). A final 7 minutes extension step (72°C) terminated the process.

PCR products were analyzed on 2% agarose gel electrophorwsis. A fragment of 219 bp represent the presence of *GSTM1*; a fragment of 459 bp represent the presence of *GSTT1*; and a fragment of 350 bp represent the presence of internal control *ALBUMIN*. The subjects were classified as (+) when the genotype was detected (present) or (-) when they are (null).

Statistical analysis

Comparisons between population means were conducted using *t*-test with statistical significance $p \leq 0.05$. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated for both groups using MEGASTAT software.

RESULT:

GSTM1 and GSTT1 polymorphism frequency analysis

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).

Deletion in *GSTT1* gene was 30% and 55% in control and patients respectively. The occurrence of deleted gene in patients was 2.85 folds more than in control with 95% CI between 0.35- 0.49.

Deletion in *GSTM1* gene was 35% and 51% in control and patients respectively. The occurrence of deleted gene in patients was 2.9 folds more than in control with 95% CI between 0.36-0.49.

Deletion in both *GSTT1* and *GSTM1* genes together was 10% and 29% in control and patients respectively. The occurrence of deleted genes together in patients was 3.67 folds more than in control with 95% CI between 0.14-0.24.

The null *GSTT1*genotype significantly increasesfasting blood glucose (mg/dl), triglycerides (mg/dl), SBP and DBP (mm/Hg), while significantly lowers HDL (mg/dl). There are no significant differences in all other studied parameters (Table 2).

The null *GSTM1* genotype significantly increases the fasting blood glucose (mg/dl), triglyceride (mg/dl), SBP and DBP (mm Hg), while significantly lowers HDL (mg/dl). There are no significant differences in all other studied parameters (Table 3).

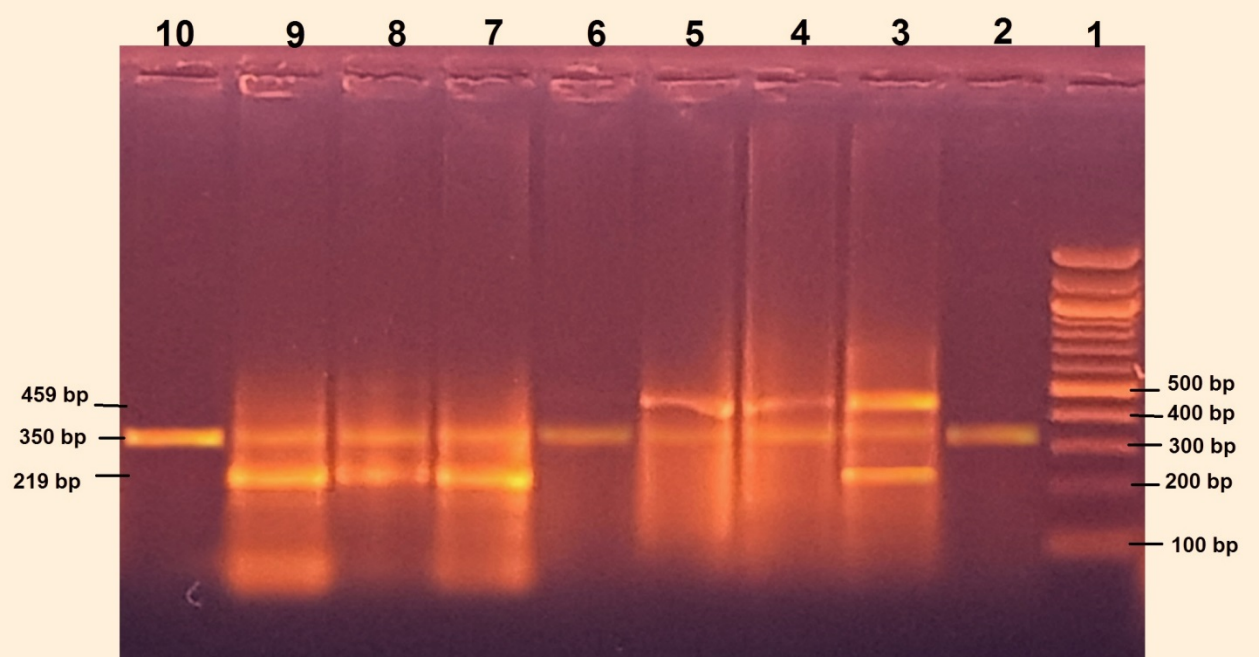

Figure (1): PCR products analyzed on 2% agarosegel. Bands at 459bp and 219bp represent *GSTT1* and *GSTM1* genes, respectively. Absence of these bands indicates null genotype. ALBUM1N was considered an internal control (350bp). Lane 1: 100 bp ladder, lane 2 and 6: null *GSTT1*&null *GSTM1*, Lanes 3: both present *GSTT1*& present *GSTM1* (wild type), lane 4 and 5: present *GSTT1*, lane 7, 8 and 9 present *GSTM1*, and lane 10: negative control.

Table (1): Distribution of *GSTT1* and *GSTM1*genotypes in control and T2DM patients

Genotype	Control N=40 (%)	T2DM patients N=100 (%)	OR	95 % CI
GSTT1 (+)	28 (70)	45 (45)	0.35	0.50-0.64
GSTT1 (-)	12 (30)	55 (55)	**2.85**	0.35-0.49
GSTM1 (+)	26 (65)	49 (49)	0.51	0.50-0.63
GSTM1 (-)	14 (35)	51 (51)	**2.9**	0.36-0.49
GSTT1 & GSTM1 (+,+)	18 (45)	23 (23)	0.36	0.27-0.40
GSTT1 & GSTM1 (-,-)	4 (10)	29 (29)	**3.67**	0.14-0.24

Table (2): The relationship between *GSTT1* genotype and Glucose, Lipid profile and blood pressure in diabetic patients.

Parameter	Null N=66	Present N=34	P-Value
Fasting Blood Glucose (mg/dl)	269.3 ± 12.07	227.3 ± 13.29	***0.03**
Cholesterol (mg/dl)	210.5 ± 3.29	202.1 ± 3.31	0.11
Triglycerides (mg/dl)	217.5 ± 4.11	195.9 ± 8.61	***0.01**
HDL (mg/dl)	48.11 ± 0.71	51.09 ± 0.99	***0.01**
LDL (mg/dl)	153.6 ± 0.95	150.5 ± 1.32	0.06
SBP (mm Hg)	157.9 ± 1.34	151.0 ± 1.63	***0.002**
DBP (mm Hg)	101.1 ± 1.15	95.38 ± 1.52	***0.004**

Table (3): The relationship between *GSTM1*genotype and Glucose, Lipid profile and blood pressure in diabetic patients.

Parameter	Null N=65	Present N=35	P-Value
Fasting Blood Glucose (mg/dl)	271.7 ± 12.14	230.7 ± 14.40	***0.04**
Cholesterol (mg/dl)	205.0 ± 3.69	198.1 ± 3.31	0.22
Triglycerides (mg/dl)	213.1 ± 4.45	191.9 ± 8.61	***0.01**
HDL (mg/dl)	44.81 ± 1.06	48.09 ± 0.99	***0.04**
LDL (mg/dl)	154.7 ± 1.31	152.5 ± 1.32	0.29
SBP (mm Hg)	154.7 ± 1.34	149.9 ± 1.58	***0.03**
DBP (mm Hg)	100.1 ± 1.15	95.38 ± 1.52	***0.01**

DISCUSSION

T2DM thought to be a polygenic disorder syndrome and it is a harmful force which exerted on beta-cells. The average of fasting blood glucose of all patients (256.1±6.60 mg/dl) was significantly higher than the average of control (94.43±2.22 mg/dl), (p-value ≤ 0.0001). High blood glucose level can spur free radical production. Weak defense system of the body becomes unable to face the motivated ROS production, and this lead to disequilibrium between ROS and their protection which increases of oxidative stress [21, 22]. Gene signals which encode transcription, differentiation, and growth factors, in addition to stimulating cell-cell adhesion and cell signaling are involved in vasoregulation, fibroblast proliferation, and increased expression of antioxidant enzymes [23, 24, 25] However, over and/or uncontrolled production of ROS is deleterious. Due to oxidative stress, metabolic disorders causes mitochondrial superoxide overproduction in endothelial cells of large and small vessels, as well as in myocardium [26, 27, 28]. For this reason, GSTs family is critical in equilibrate between ROS and cells' protection. The products of oxidative stress are utilized as substrates for GSTs.

Our results indicated that null *GSTT1* and null *GSTM1* genotypes were associated with T2DM comparing with control. It was clear from higher OR, that lacking either gene was associated with the rise in susceptibility to T2DM. Null genotypes of both genes would further increase the risk of induction and development of the disease. The most dangerous factor is the impact of the oxidative stress which induces ROS. GSTs play a vital role in metabolites detoxification of oxidative stress that leads to inflammation and susceptibility to T2DM. This finding was supported by the results of Al-Badran and Al- Meyah 2014.[29] and agreed with Yalin et al., (2007) [8] who suggested that null *GSTM1* genotype may significantly influencing etiopathogeneses of T2DM and considered as an important marker in T2DM prediction susceptibility in Turkish population. Present results were agreed with the report by Banerjee and Vats (2014) [30] in which GSTT1, GSTM1 and GSTP1 have been involved in T2DM development and various diabetic related complications.

The frequency of the null *GSTTI* genotype was 55 % in T2DM patients, which is lowest comparing to Dubai (60.0%) and highest comparing to Egyptian (35.0%) populations [31, 32]. The genotypic frequencies of present *GSTT1* and present *GSTM1* genotypes were 45 % and 49%, respectively, which is higher than in Dubai (40%) and (42.5%), respectively [32]**.** The variation in genotypic frequencies in different populations may be due togenetic constituent of a populations and variable environmental factors. There is a genetic predisposition in Iraqi population due to deletions in antioxidant *GSTT1* and *GSTM1* genes which are important factors in pathogenicity. In addition, the high environmental pollution may induce genetic alteration; moreover, the heavy metals have been recorded as a factor induced genetic alteration and induce oxidative stress in cells.

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. This allows us to infer that the absence of *GSTM1* and *GSTT1* genes may contribute to T2DM related complications, such as dyslipidemia (*GSTT1*), glycemic decompensating and hypertension (*GSTM1*). These results are consistent with studies conducted on the Chinese population [33], population of Egyptian [34] and population of Indian [35], where a null *GSTT1* was associated with lipid alterations and the *GSTM1* on glycemic.

Increase triglycerol and cholesterol in blood are factors affecting normal oxidative modification of LDL, auto oxidation of glucose and protein glycation. These cause a rise in production of lipid peroxidation products which increase oxidative stress in higher lipids. Oxidative stress plays a major role in the pathogenesis of T2DM causes several unfavorable effects on the cellular physiology particularly β-cells in islet tissue which has the lowest levels of intrinsic antioxidant defenses. Chronic hyperglycemia and oxidative stress have deleterious effects on the function of vascular, retinal, and renal tissues [36]

CONCLUSION:

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

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