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Association of Glutathione S-Transferase (GSTM1, T1) Gene Polymorphisms with Type 2 Diabetes Mellitus (T2DM) in the Iraqi Patients

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Abstract: Diabetes mellitus is associated with an increased production of reactive oxygen species (ROS) and a reduction in antioxidant defense. The oxidative stress becomes evident as a result of accumulation of ROS in conditions of inflammation and Type 2 diabetes mellitus (T2DM). The genes involved in redox balance, which determines the susceptibility to T2DM remain unclear. In humans, the glutathione S-transferase (GST) family comprises several classes of GST isozymes, the polymorphic variants of GSTM1, T1 genes result in decreased or loss of enzyme activity. Aims: The present study evaluated the effect of genetic polymorphisms of the GST gene family on the risk of developing T2DM in the Iraqi patients. GSTM1, T1 polymorphisms were genotyped in 25 T2DM patients and 25 healthy controls from Iraq to analyze their association with T2DM susceptibility. Materials and Methods: Analysis of GSTM1 and GSTT1 gene polymorphisms was performed by multiplex polymerase chain reaction (PCR) .There was significant association in the GSTM1 gene polymorphism and Type-2 Diabetes Mellitus ($P<0.05$). Moreover, significant relationship was found between the polymorphism of GSTT1 genes and higher risk of Type-2 Diabetes Mellitus among Iraqi subjects ($P<0.05$).

Key words: Glutathione S-transferase, Type-2 Diabetes Mellitus, polymorphism, multiplex PCR.

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Introduction

Diabetes Mellitus Type 2 (T2DM) is a multifactorial disease that develops through an exposure to environmental risk factors, lifestyle habits and genetic susceptibility. This heterogeneous syndrome is characterized by chronic hyperglycemia and other alterations include dyslipidemia and hypertension, that leads to a development of macro and microvascular complications. The pathogenesis of disease involves a combination of beta-cell insufficiency and insulin resistance (1, 2).

Oxygen free radicals and lipid peroxides have been implicated in the pathogenesis of a large number of diseases such as diabetes, cancer, rheumatoid arthritis, infectious diseases, atherosclerosis and aging. (3), (4),(5) Growing evidence indicates that oxidative stress is increased in diabetes due to overproduction of reactive oxygen species (ROS) and decreased

efficiency of antioxidant defenses. (6) It has also been reported that defects in antioxidant defense against oxidative stress play an important role in the etiology of diabetic complications. (7), (8). This initiated us to explore the antioxidant enzyme gene polymorphisms and their association with T2DM. The proposed work will add to the existing knowledge of understanding the genetic basis of T2DM in Iraqi population. The genotyping might help in early diagnosis and help in the management of the disease.(9) Three of the GST genes, GSTM1, GSTT1 and GSTP1 have been found to have Functional polymorphisms that are frequently present in the general population. Expression of GST alpha, mu, piand theta in the pancreas, varies by cell type. For example expression of GST mu and GST alpha have been reported in human islet tissue. The GST mu nullgene polymorphism (GSTM1) has been associated with chronic pancreatitis, leukemia, rheumatoid arthritis and asthma. The GST theta nullgene polymorphism (GSTT1) has been reported to be associated with breast cancer and colorectal cancer (10).Some studies indicated that genetic variations of GSTTT enzyme are associated with the development of end-stage renal disease in diabetes mellitus patients (11). According to the reviewed literature, there is astudy have been published on the association between GSTT1/GSTM1 polymorphism and susceptibility to diabetes, and there are large divergences among the study results. These range from significant associations by only one of the polymorphisms to diabetes, by both of them, or by neither (8).

Materials and Methods

Specimen Collection

Blood samples (2-3ml) were collected in EDTA tubes for DNA isolation (Molecular genetic studies) from (25) apparently healthy people and (25) patients, all of them were diagnosed as (T2DM) attended the (AL-Yarmook) Hospital in Baghdad. Total genomic DNA isolated from the whole blood collected in EDTA anticoagulant tubes for molecular studies was applied using genomic DNA purification kits (geneaid) South Korea. The isolation of DNA was preseptated by salting out methods (12). Multiplex Polymerase Chain Reaction (PCR) for GSTM1 and GSTT1 genotyping was done by Using specific primer. Primer was custom synthesized at Bioneer / south korea Company as a lyophilized product. Lyophilized primer was dissolved in a free DNase / RNase water to give a final concentration of (100 pmol /µl) (as stock solution), to prepare 20µM concentration as work primer resuspended 20 pmol/µl in 80 µl of deionized water to reach a final concentration 20µM.Table (1) show the primer sequence and PCR product size.

Table (1): primer sequences used multiplex PCR amplification of GSTM1, GSTT1, CYTP1A1 genes

Primer	Primer sequences	TA	PCR Product size
CYP1A1 (used as control)	F 5- GAACTGCCACTTCAGCTGTCT -3	59°C	312bp
	R 5- CAGCTGCATTTGGAAGTGCTC -3	59°C	
GSTM1	F 5- GAA CTC CCT GAA AAG CTA AAGC -3	59°C	219bp
	R 5-GTTGGGCTCAAATATACGGTGG -3	59°C	
GSTT1	F 5- TTCCTTACTGGTCCTCACATCTC -3	59°C	459bp
	R 5- TCACCGGATCATGGCCAGCA -3	59°C	

***TA= Temperature of Annealing**

Multiplex PCR was performed in a 20µl total volume, Primer forward1µ l (20PM for each one), Primer reverse 1µl (20 PM for each one), Template DNA4 µl ,(4- 6µg/ml) . A total of 35 PCR cycles with denaturation at 94oc for 1 minute, annealing at 59oc for 1 minute and extension at 72oC for 1 minute ladder were conducted. An initial DNA denaturation at 95oc was carried out for 3 minutes and final extension at 72oc were carried out for 5 minutes each. The PCR product was then subjected to electrophoresis on a 2% agarose gel. The presence of bands of 459bp and 219bp was indicated of the GSTT1 and GSTM1 genotypes respectively, whereas the absence indicated the null genotype for that gene. CYP1A1indicated by a 312 bp product was used as an internal control Figure (1).

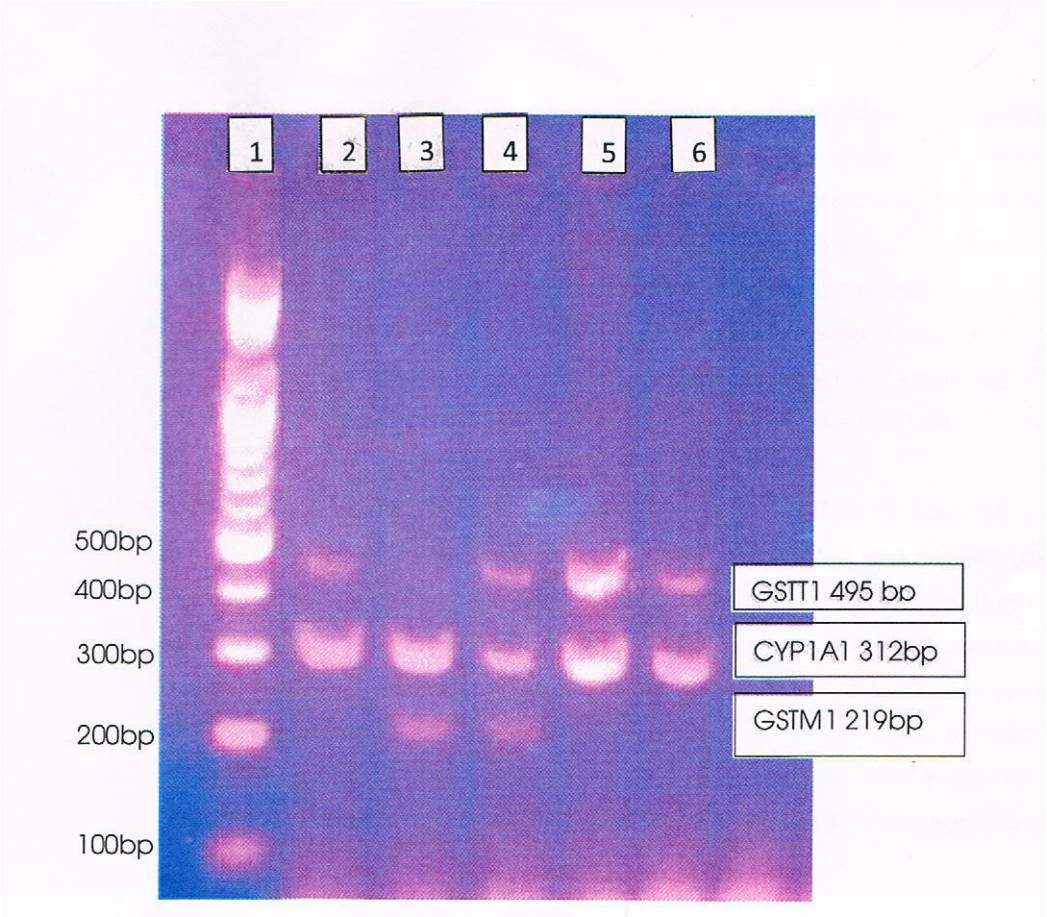

Figure1: PCR product for GSTT1and GSTM1 polymorphisms on 2% agarose gel. Lane 1: DNA. ladder. Lane 2, 5, 6: GSTM1 deletion. Lane 3: GST11 deletion. Lane 4: normal genotype

Results and Discussion

The results showed that 17 samples of patients have deletion in one genes or both (Null genotype). The chi-square test reflected significant association of the mutations and their combination, (deletion or presence), with the occurrence of T2DM as reflected from comparing the patients and control group. Table (2).

Table (2) Number & percentage of type 2 diabetes mellitus patients and control according to type of gene

Group	No. & Percentage	Type of gene			
		GSTM1 gene deleted	*GSTM1* gene present	*GSTT1* gene deleted	*GSTT1* gene present
Patients	No.	16	9	17	8
	%	64%	36%	68%	32%
Control (no. 25)	No.	5	20	3	22
	%	20.83%	55.56%	37.50%	42.31%
Chi-square–$\chi 2$ (P<0.05)					

In the present study, an analysis of the T2DM associated with the deletion polymorphism for GSTT1, it was found that the null genotypes related to an increased predisposition for T2DM conferring a 3.2-fold increased risk of developing the disease relative to the present genotype. It was also observed that there was association of the GSTM1 deletion with susceptibility to disease in the population studied.GSTM1 null genotype may play a significant role in the etiopathogenesis of T2DM like the previous report of GSTM1 used as a useful marker in the prediction of T2DM susceptibility (13).Recently, in Egyptian population, GSTT1 null and GSTM1 null genotypes, alone or combined, were found to be associated with increased risk of T2DM, like our study (14).

There are studies that reported significant association to T2DM for both null genotypes of GST (14,16) and others that verified no association between GSTT1 and GSTM1 polymorphisms and T2DM (15,17). In addition, others studies showed that only the GSTM1-null genotype may play a significant role in the etiopathogenesis of T2DM (18, 19). In the Turkish population study (18), the authors suggested that the GSTM1 gene may be a useful marker in the prediction of T2DM susceptibility. The GSTM1-null genotype was indicating an association between the incidence of diabetes and GSTM1 deletion polymorphism. In accordance, an Indian population study reported a significant association of GSTM1-null with T2DM and no significant association with GSTT1 (19).

The deletion of GSTT1 and GSTM1, which are associated with abolished enzyme activity (20), have been associated with type 2 diabetes mellitus when compared to control subjects (21). The presence of the GSTM1-null genotype seems to increase of havingT2DM by 0.54-fold (22).

Wang and colleagues (2006) performed a study on Chinese type 2 diabetic patients investigating the polymorphism of GSTT1. They found that the GSTT1-null genotype was observed in 61% of

patients versus 51% of controls. Their study suggests that The GSTT1-null genotype important in development of type 2 diabetes mellitus (21). Our study showed a significant difference in the frequencies of the GSTT1-null mutations between the patients and the control group (Table 2).

Pinheiro *et al*., (2013) performed a study on Brazilian type 2 diabetic patients they found that the GSTT1 polymorphism may has an critical role in the pathogenesis of T2DM in the Brazilian patients. So this gene could be added to a panel of genetic markers to identify individuals with an increased rate of developing T2DM and blood pressure levels were verified. The both null gene (GSTM1and GSTT1) may participate to the clinical course of T2DM patients.

Amer *et al*.,(2011) found the proportion of the GSTT1- and GSTM1-null genotypes was significantly greater in Egyptian diabetic patients when compared to controls so Patients carrying both null polymorphisms had a 3.17-fold increased risk of having type-2 diabetes mellitus compared to those with normal genotypes .(14)

References

1- Robertson, R. P. ; Harmon J.; Tran, P. O. and Poitout, V. (2004). Beta-cell glucose toxicity, lipotoxicity, and chronic oxidative stress in type 2 diabetes. Diabetes, 53: S119–24.

2- Andreassi, M. G. (2009). Metabolic syndrome, diabetes and atherosclerosis: influence of gene-environment interaction. Mutat. Res., 667: 35–43.

3- Zhang, J.; Liu, H.; Yan, H.; Huang, G. and Wang, B. (2013). Null genotypes of GSTM1and GSTT1 contribute to increased risk of diabetes mellitus: a meta-analysis. Gene., 518: 405–11.

4- Taysi, S.; Polat, F., Gul, M.; Sari, R. A. and Bakan, E. (2002). Lipid peroxidation, some extracellular antioxidants and antioxidant enzymes in serum of patients with rheumatoid arthritis. Rheumatol. Int., 21:200.

5- Yagi, K. (1984). Increased serum lipid peroxides initiate atherogenesis. Bio. Essays, 1:58-60.

6- Griesmacher, A.; Kindhauser, M.; Andert, S.E.; Schreiner, W.; Toma, C.; Knoebl, P.; *et al*. (1995). Enhanced serum levels of thiobarbituric acid-reactive substances in diabetes mellitus. Am. J. Med., 98:469-75.

7- Giron, M. D.; Salto, R.; Gonzalez, Y.; Giron, J. A.; Nieto, N.; Periago, J. L., *et al*. (1999). Modulation of hepatic and intestinal glutathione S-transferases and other antioxidant enzymes by dietary lipids in streptozotocin diabetic rats. Chemosphere, 38:3003-13.

8- Opara, E. C. (2002). Oxidative stress, micronutrients, diabetes mellitus and its complications. J. R. SocPromot Health, 122:28-34.

9- Friedlyand, L. E.; Philipson, L. H. (2005). Oxidative reactive species in cell injury: Mechanism in diabetes mellitus and therapeutic approaches. Ann. N. Y. Acad. Sci., 1066:136-51.

10- Bekris, L.; Shephard, M.; Peterson, J.; Hoehna, B.; Van Yserloo, E.; Rutledge, F.; Farin, T. J. and Kavanagh, A., (2005). Glutathione-stransferaseM1 and T1 polymorphisms and associations with type 1 diabetes age-at-onset. Autoimmunity, 38(8): 567-75.

11- Yang, Y.; Kao, C.; Chang, S. : Chung, C.; Chen, J. and Tsai, J. (2004). Glutathione S-transferase T1 deletion is a riskfactor for developing end-stage renal disease in diabetic patients. Int. J. Mol. Med., 14(5):855-9.

12- Sambrook, J.; Fritsch, E. F.; and Maniatis, T. (1989). Molecular cloning: A laboratory manual. 2nd. Cold spring harbor laboratory press, cold spring Harbor, New York.

13- Yalin, S.; Hatungil, R., Tamer, L.; Ates N. A.; Dogruer, N.; Yildirim, H.; *et al*. (2007) Glutathione S-transferase gene polymorphisms in Turkish patients with diabetes mellitus. Cell Biochem. Funct., 25:509-13.

14- Amer, M. A.; Ghattas, M. H.; Abo-Elmatty, D. M. and Abou-El-Ela, S. H. (2011). Influence of glutathione S-

transferase polymorphisms on type-2 diabetes mellitus risk. Genet. Mol. Res., 10:3722-30.

15- Hori, M.; Oniki, K.; Ueda, K.; Goto, S.; Mihara, S. , *et al.* (2007) Combined glutathione S-transferase T1 and M1 positive genotypes afford protection against type 2 diabetes in Japanese. Pharmacogenomics, 8: 1307–1314.

16- Ramprasath, T.; SenthilMurugan, P.; Prabakaran, A. D.; Gomathi, P.; Rathinavel, A., *et al*. (2011). Potential risk modifications of GSTT1, GSTM1 and GSTP1 (glutathione-S transferase) variants and their association to CAD in patients withtype-2 diabetes. BiochemBiophys Res. Commun., 407: 49–53.

17- Datta, S. K.; Kumar, V.; Pathak, R.; Tripathi, A. K.; Ahmed, R. S.; *et al.* (2010). Association of glutathione S–transferase M1 and T1 gene polymorphism with, oxidative stress in diabetic and nondiabetic chronic kidney disease. Ren. Fail., 32:1189–95.

18- Yalin, S.; Hatungil, R.; Tamer, L., Ates, N. A.; Dogruer, N.; *et al.* (2007). Glutathione S-transferase gene polymorphisms in Turkish patients with diabetes mellitus. Cell Biochem. Funct., 25: 509–13.

19- Bid, H. K.; Konwar, R.; Saxena, M.; Chaudhari, P.; Agrawal, C. G.; *et al.* (2010). Association of glutathione S-transferase (GSTM1, T1 and P1) gene polymorphisms with type 2 diabetes mellitus in north Indian population. J. Postgrad. Med., 56: 176–181.

20- London, S. J.; Yuan, J. M.; Chung, F. L.; Gao, Y. T., *et al.* (2000). Isothiocyanates, glutathione S-transferase M1 and T1polymorphisms, and lung-cancer risk: a prospective study of men in Shanghai, China. Lancet, 356: 724-729.

21- Wang, G.; Zhang, L. and Li, Q. (2006). Genetic polymorphisms of GSTT1, GSTM1, and NQO1 genes and diabetes mellitus risk in Chinese population. Biochem. Biophys. Res. Commun. 341: 310-313.

22- Bid, H. K., Konwar, R.; Saxena, M.; Chaudhari, P., *et al.* (2010). Association of glutathione S-transferase (GSTM1, T1 and P1) gene polymorphisms with type 2 diabetes mellitus in north Indian population. J. Postgrad. Med., 56: 176-181

23- Pinheiro, D. S.; Filho, C. R., Mundim ,C. A., Ju´ nior PdM; Ulhoa, C. J., *et. al.* (2013). Evaluation of Glutathione S-Transferase GSTM1 and GSTT1 Deletion polymorphisms on Type-2 Diabetes Mellitus Risk. Plos. ONE, 8(10):e76262.