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THE INFLUENCES OF GSTT1 AND GSTM1 NULL GENOTYPE AS THE RISK FACTOR IN DIABETES TYPE 2 COMPLICATIONS

Medical Science

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ABSTRACT

Diabetes is one of the multifactorial disorders with genetics and environmental factors playing important role in its cause. In diabetes there are defects in cellular metabolism that results in increasing free radicals. These radicals react with other vital cellular molecules which are responsible in diabetes side effects. Human glutathione-S transferases (GST) are a family of enzymes that catalyses conjugation of electrophilic substances with glutathione. This family divided to four classes named alpha (α), mu (μ), teta (θ) and pi (π). Two member of this four classes are GSTT1 and GSTM1.

Material and Methods: In this study we investigated deletion of GSTT1 and GSTM1 genes in 104 diabetics patients with side effect and 58 diabetic peoples without any coplication. DNA was extracted from peripheral blood and then multiplex PCR was performed following agarose gel electrophoresis to detect GSTT1 and GSTM1 null genotypes. Data were analyzed with SPSS software.

Results: The results indicated that there was no significant relationship between GSTT1 null genotypes (2=0.365, P=0.351, df=1), and GSTM1 null genotypes (2=1.207, P=0.177, df=1), with side effects of diabetes type 2.

Conclusion: No significant correlation between GSTM1 and GSTT1 null genotype with side effect of diabetes indicate this fact that impair cellular metabolism result in increase free radicals and oxidative pressure, GST null genotypes may result in decrease antioxidant capacity which cause side effects of diabetes but the performance of different classes of GST null genotypes can be tissues specific. Additional studies are required to confirm this study.

KEYWORDS

Glutathion-S transferase, Diabetes type 2, Diabetes complications

Introduction:

There are many genetics and environmental factors involve in multifactorial diseases such as heart diseases, diabetes, high blood pressure and cancer. Interaction of these factors and inheritance pattern is complex. Unlike monogenic disease the occurrence chance of these diseases can not be predicted, but we can predict the incidence rate of the disease (1).

Type 2 diabetes mellitus (diabetes) is recognized as a worldwide public health problem due to the high medical and socioeconomic costs that result from complications associated with the disease. In general, type 2 diabetes is the most common metabolic and multifactorial disease in which both genetic and environmental factors are involved (1-3). Diabetes is the latest step of a chronic and accelerating disorder which results from insulin resistance, decrease of functional pancreatic β cells and increase of glucose level. Approximately all of the diabetic peoples (DM2) are insulin resistance. Despite of numerous studies on insulin resistance, the main cause of it is still not known. It seems that post translation modification and mutations in the genes lead to defect in the cell signaling pathway which can result in insulin resistance (4). Several genes have been identified that are involved in the cellular pathway of glucose metabolism and storage. Defects in these genes can lead to diabetes or diabetes background. Among these genes are: *Adiponectin* (1,2), *PTPN1* (4), *GLUT4,2* (5,6), *PAX4* (7), *HNF1B* (8) and *PPARG* (9). People with type 2 diabetes are at risk for several complications, including damage to the vascular system that lead to increase mortality (10). Many side effect of DM2 are cardiovascular disease, nephropathy, retinopathy, and neuropathy. Diabetic retinopathy is the most severe complication that cause blindness in 20–30 years old patients. Blindness in diabetic patients is 25 times higher than non-diabetics (11). These complications could be due to the cellular metabolism leading to hyperglycemia and to the productionn of free radicals which combined with vital molecules result in various diseases. There are several enzymes such as glutathione S-transferase (GST) in body as the defense systems for neutralize free radicals. The human glutathione S-transferases (GSTs) are a family of enzymes known to play an important role in the detoxification of several carcinogens found in tobacco smoke. GSTs are dimeric proteins that catalyze conjugation reactions between glutathione and tobacco smoke substrates, such as aromatic heterocyclic radicals and epoxides (12-14).

Conjugation facilitates excretion and thus constitutes a detoxification step. In addition to their role in phase II detoxification, GSTs also modulate the induction of other enzymes and proteins important for cellular functions, such as DNA repair. This class of enzymes is therefore important for maintaining cellular genomic integrity and, as a result, may play an important role in cancer susceptibility (15). GST enzymes are coded for at five distinct loci, known as alpha, mu, theta, pi, and gamma. Two loci in particular, *GSTM1* and *GSTT1*, may be of relevance for susceptibility to squamous cell carcinoma of the head and neck (SCCHN). The *GSTM1* locus has been mapped on chromosome 1p13.3, while the *GSTT1* locus exists on chromosome 22q11.2. Persons with homozygous deletions of either the *GSTM1* or the *GSTT1* locus have no enzymatic functional activity of the respective enzyme. This has been confirmed by phenotype assays that have demonstrated 94 percent or greater concordance between phenotype and genotype. Deletion variants of *GSTM1* and *GSTT1* that result in no functional enzymatic activity for each locus have been characterized (3). Three alleles have been identified at the *GSTM1* locus: one deletion allele and two others (*GSTM1a* and *GSTM1b*) that differ by C→G substitution at base position 534. This C→G substitution at base position 534 results in the substitution Lys→Asn at amino acid 172. The Lys→Asn substitution results in no functional difference between the two alleles. As a result, *GSTM1a* and *GSTM1b* are categorized together as the positive conjugator phenotype. Two alleles have been identified at the *GSTT1* locus—one functional and the other nonfunctional. Persons who are of the homozygous deletion genotype are categorized into the negative conjugator phenotype, while those who carry either one or both of the functional alleles are grouped into the positive conjugator phenotype (13).

Diabetes is associated with genetic and environmental factors such as; weight, height, smoking, gender, age, lifestyle, and hormonal and nutritional status. In this study one of the genetics factors which may be related to the diabetes and its complications is investigated.

Materials and Methods

In this study, diabetic patients has been selected from individuals referred to Yazd Diabetes Research Center, Yazd, Iran. Other factor such as age, sex, response to treatment and changes in hematologic indexes were extracted from patient records. Among patients with diabetes, 115 patients were selected who were 35 to 65 years old. Among them, 58 patients had no complication of diabetes and 104 patients had diabetes with side effect. The criteria of side effect was based on examination by physician (based on the WHO index). The

patients were selected by physician after examination. To examine *GSTT1* and *GSTM1* gene deletion in patients, a sample of 10 ml peripheral blood was taken in tubes and DNA was extracted by salting out method. Molecular examination preformed by multiplex PCR using 3 sets of primer pairs for *GSTT1*, *GSTM1* and *β globin* gene for control.(**Figure 1)** The annealing temperature was 62^{O}C and the PCR was performed in 35 cycles.

The PCR products were visualized using 2% agarose gel electrophoresis. The data were analyzed by SPSS software and Chi-Square test.

Gene	Nucleotide sequences of primers	Ann. seq.	Acc. No.
GSTM1	F-GAA CTC CCT GAA AAG CTA AAG G	2401-2422	X68676
	R-GTT GGG CTC AAA TAT ACG GTG G	2598-2619	
GSTT1	F-TTC CTT ACT GGT CCT CAC ATC TC	469-491	X79389
	R-TCA CCG AT CAT GGC CAG CA	704-723	
β-globin	F-GAA GAG CCA AGG ACA GGT AC	61992-62011	U01317
	R-CCA CTT CAT CCA CGT TCA CC	62240-62259	

F: forward primer, R: reverse primer, Ann. seq.: nucleotide numbers for annealing with, Acc. No.: GeneBank accession no.

Figure 1. primer pairs for GSTT1, GSTM1 and β globin gene for control

Results:

From 162 diabetes patients studied, 58 had no side effect coplications. Genotyping of *GSTM1* revealed that among these 58 patients without side effect coplications, 38 patients (65.5%) showed null genotype while 20 patients (34.5%) were positive for *GSTM1* gene. Among 104 diabetic patients with side effecf, 59 patients (56.7%) had null genotypes and 45 patients (43.3%) were positive for *GSTM1* gene. The statistical analysis of *GSTM1* gene deletion in controls (diabetes without side effect coplications) (65.5%) and cases (diabetes with side effect coplications) (56.7%) group indicates a no significant relationship with *df=1, p-value= 0.177, 2= 1.207*.

Regarding *GSTT1* genotypes, in 104 diabetics patients with side effect coplications, 22 patients had null genotypes (21.2%) and 82 patients were *GSTT1* positive (78.8%). While among 58 diabetic peoples without side effect coplications, 10 patients had null genotypes (17.2%) and 48 patients were *GSTT1* positive (82.8%).

The statistical analysis of *GSTT1* gene deletion in control (diabetes without side effect coplications) (17.2%) and case (diabetes with side effect coplications) (21.2%) group indicates no significant relationship with *df=1 ,p-value=0.351*, ^{2}*=0.365*.(**Table 1.)**

Table 1. The statistical analysis of GSTT1 and GSTM1 genotype

		Group		Total
		Control	Case	
GSTM1	Null	38	59	97
		65.5%	56.7%	59.9%
	Positive	20	45	65
		34.5%	43.3%	40.1%
GSTT1	Null	10	22	32
		17.2%	21.2%	19.8%
	Positive	48	82	130
		82.8%	78.8%	80.2%

The statistical analysis of GSTT1 and GSTM1 interaction gene deletion in control (diabetes without side effect coplications) (77.59%) and case (diabetes with side effect coplications) (67.1%) indicates no significant relationship with df=1 , p-value=0.122, X^2 =1.854.

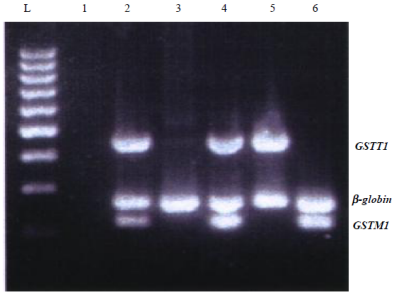

Figure 2. Multiplex PCR products of GSTT1, GSTM1 and β-globin. A 2% (w/v) agarose gel showing the PCR product. DNA from patients with positive GSTM1, GSTT1, and β -globin alleles yielded 219bp, 480bp, and 268bp products respectively. The absence of GSTM1 or GSTT1 (in the presence of β -globin PCR product) indicates the respective null genotype for each (3, double null; 5, GSTM1 null; 6, GSTT1 null). Samples positive for all three PCR products were considered 'wild-type' (2 & 4). Lane 1 is negative control and L is Molecular weight marker.

Discussion

Diabetes mellitus is one of the most common chronic diseases in nearly all countries; the number of people with diabetes is increasing due to population growth, aging, urbanization, and increasing prevalence of obesity and reduced physical activity.

Oxidative stress plays a major role in the pathogenesis of T2DM. β-cells are particularly sensitive to ROS because they are low in antioxidant factors such as glutathione peroxidase, catalase and SOD. Therefore, increased oxidative stress may not only result from hyperglycemia associated with diabetes, but may also have an important causal role in β-cell failure and the development of insulin resistance and T2DM.(25)

There are several complex mechanisms in human that protect the body against environmental agents including inappropriate dietary, UV radiation, smoking and free radicals which are produced from defective oxidation. The ability of human for metabolizing carcinogens (cancer causing substances) varies and people who have little ability to produce detoxification substance are at high risk of various diseases including diabetes and cancer. It seems that glutathione is important as a carcinogen neutralizing for free radicals (12,13). Glutathione S-transferase (GST) modulates the effects of various cytotoxic and genotoxic agents. GST genes encode a family of phase II enzymes (molecular mass 17-28 kD) that have major roles in catalyzing the conjugation of glutathione to a wide variety of hydrophobic and electrophilic substrates and carcinogens such as benzpyrene and reactive oxygen species (ROS). Therefore, there is an increasing interest in the role that polymorphisms in phase I and phase II detoxification enzymes may play in the etiology and progression of diseases. Polymorphisms reducing or eliminating these enzyme detoxification activities could increase a person's susceptibility to diseases including T2DM.(25) Glutathione-S-transferase (GSTs) are multifunctional proteins that can function as enzymes catalyzing the conjugation of glutathione thiolate anion with a multitude of second substrates or as non-covalent binding proteins for a range of hydrophobic ligands (12,13). Peoples act in different ways to detoxification, this theory can describe the risk differences for various disease include cancer and diabetes that cause by exogenous and endogenous agents. GSTT1 and GSTM1 genes expressed in many form in populations and people with null genotype have no active enzyme for detoxification (16, 17). GSTT1 and GSTM1 genes are absent in 10 – 60% of different populations (18-21). We thus determined the polymorphism frequency for each of these enzymes in our study populations and looked for relationships between them and the clinical parameters in type 2 diabetics.

There are many studies dealing with GST polymorphism in various diseases, but only a few studies have addressed the role of GST polymorphisms in diabetes and diabetes type 2 complications. In the current study, we attempted to move beyond single gene polymorphism to two-gene polymorphisms that may help predict the susceptibility to the incidence of T2DM and their effect on diabetes type 2 complications in the IRAN-Yazd province population.

Although these genes have significantly associated with type 2 diabetes., but in this study the statistical analysis between GSTT1 (p=0.351), GSTM1(p=0.171) and side effect coplications show no significant association that confirms the research of others (22-28) but Is inconsistent with others study that show GSTT1 null genotype is a risk factor for diabetic retinopathy in Caucasians with type 2 diabetes. (26).

Finally, the statistical analysis between GSTT1 and GSTM1 interaction in side effect coplications show no significant association (p=0.122).

To our knowledge there is no more research about the effect of GST genotype in side effects of diabetes (diabetes complication), therefore more researches with more cases is needed (22).

Conclusion

Therefore Although these genes have significant associated with type 2 diabetes, but no significant association with diabetic complications that could be due to the tissue-specific and variables effects of thes genes in different tissue.

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