Association of the Glutathione s-transferase (GST) gene polymorphisms with type 2 diabetes mellitus risk factors in north Indian population

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Diabetes mellitus is associated with an increased production of reactive oxygen species (ROS) and a reduction in antioxidant defence. The oxidative stress becomes evident as a result of accumulation of ROS in conditions of inflammation and 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 and P1 genes result in decreased or loss of enzyme activity. Glutathione s-transferase theta 1 (GSTT-1) and glutathione s-transferase mu-1 (GSTM-1) enzymes of the glutathione detoxification pathway protect the embryo from oxidative stress. This study investigated GSTT1 and GSTM1 in relation to their role in conferring genetic susceptibility to pregnancy loss. The women carriers of GSTT1 and GSTM1 null genotypes are more often at genetic risk of pregnancy loss (Bid et al., 2010). In the present study we have investigated GSTT1 and GSTM1 in relation to their role in conferring genetic susceptibility for early pregnancy loss (EPL) and recurrent pregnancy loss (RPL). Meta-analysis on the polymorphisms was conducted to support our findings that the presence of mutant genotypes at this site increases the risk of pregnancy loss. The GSTT1 null genotype was significantly associated with both EPL and RPL. In the meta-analysis, the overall result showed that the association between GSTM1 null genotype and risk for RPL was statistically significant. The significantly increased risk with the GSTT1 null genotype in the Indian population, but no risk was found in the pooled population (Nair et al., 2013). The present study clearly suggests that GSTT1 and GSTM1 polymorphisms are genetic risk factors for pregnancy loss.). Glutathione s-transferases (GSTs) belong to a group of multigene and multifunctional detoxification enzymes, which defend cells against a wide variety of toxic insults.

Key words: Type 2 diabetes mellitus, glutathione s-transferase, and genetic polymorphism. GSTT1, GSTM1.

INTRODUCTION

Diabetes mellitus (DM) is a progressive and chronic endocrine disorder which results primarily in a hyperglycemic (excess glucose in the blood) condition. DM affects the body's ability to metabolize fat, carbohydrates and proteins and often leads to a serious micro and macrovascular complications, including cardiovascular diseases. The primary hormone, insulin, which maintains homeostasis of body glucose levels, is either insufficient or ineffective in individuals with DM.

Types of diabetes

1. Type 1 diabetes mellitus (T1DM) results from auto
immunological destruction of the insulin- producing cells of the pancreas and accounts for 5–10% of all cases of diabetes, with the major susceptibility gene mapping to the human leukocyte antigen (HLA) region of chromosome 6.

2. Type 2 diabetes mellitus (T2DM) is the most common form of diabetes, counting for approximately 90% of cases and affecting 10–20% of those over 45 years of age in many developed countries. T2DM indicates an individual who has a physiological resistance to the effects of insulin within the peripheral tissues. Basically, the insulin, which the body is still capable of producing, is not physiologically effective.

**Type 1 diabetes**

Type 1 diabetes known as ‘Juvenile diabetes’ because of its tendency to strike a person in their childhood up to their early adulthood. It is also sometimes known as ‘insulin dependent diabetes’, as a person with this condition is reliant upon insulin injections to survive. It is the most serious form of diabetes and the least common. The cause for type 1 diabetes is usually pancreatic failure due ‘Autoimmune’ malfunction.

**Type 2 diabetes**

Anyone can get type 2 diabetes. However, those at highest risk for the disease are those who,

- Are over 45
- Are obese or overweight,
- Have had gestational diabetes,
- Have family members who have type 2 diabetes.
- Have prediabetes.
- Are inactive
- Have low hdl cholesterol or high triglycerides levels.
- Have high blood pressure.
- Are members of certain racial or ethnic groups.

Type 2 diabetes known as ‘adult onset diabetes’, as when the term was given, it was thought only adults developed this form of diabetes. This has proven to be false as a soaring number of children throughout the world now develop this type of diabetes each year. Modern day living where too much bad food is consumed, not enough exercise is taken and childhood obesity is largely to blame for this. The prime cause for type 2 diabetes isn’t the failure of the pancreas, but more due to obesity and poor diets and unhealthy lifestyles. When a person overloads their body with sugar, as many obese people have done for years, this means persistent blood sugar levels, and the insulin and pancreas can struggle to deal with the sugar. The insulin usher’s the sugar into the muscles, but the muscles don’t burn the glucose off because no exercise is taken. The result is an accumulation of blood sugar for prolonged periods of time, this can lead to a condition called ‘insulin resistance’, unlike people with type 1 diabetes, people with type 2 diabetes produce insulin; however, either their pancreas does not produce enough insulin or the body cannot use the insulin adequately. T2DM also called as “non-insulin dependent diabetes” when a person becomes insulin resistant, their muscles and other would-be outlets for the glucose begin to resist.

Entry to the insulin, therefore the glucose isn’t delivered. This, over a period of time, results in a person experiencing symptoms of hyperglycaemia. Hyperglycaemia is an excess of blood sugar, and the immediate symptoms can be increased thirst, hunger and tiredness as well as increased urination, blurred vision, nausea and possibly even vomiting. Other symptoms of hyperglycaemia which can develop are irritation of the genitals and yeast infections. A person with type 2 diabetes usually doesn’t have to take medication, but is advised to take more exercise and stick to a healthy diet to help to keep their blood sugar levels under control. Most people who adhere to this advice can live their lives normally without medication or symptoms affecting them. In some cases of type 2 diabetes, a medication may be prescribed. Type 2 diabetes has strong hereditary tendencies.

**REVIEW OF LITERATURE**

Recent studies suggest that the common variant in the glutathione s-transferase m1 (GSTM1) and t1 (GSTT1) gene is associated with the risk of smoking-related coronary artery disease (CAD). Intra-ethnic as well as inter-ethnic differences are known to impact the frequencies of GST gene polymorphisms, thus influencing its interactive effect with tobacco smoking on CAD risk (Wang et al., 2008). An important condition to assess whether the GSTT1 and GSTM1 genotypes are associated with T2DM and to ascertain whether the levels of blood lipids given exposure to diabetes are modified by the specific genetic polymorphisms of GSTT1 and GSTM1. GSTT1 gene polymorphisms may play an important role in type 2 diabetes mellitus pathogenesis. The glutathione s-transferases (GST), a super family of phase ii metabolic enzymes play an important role in the cellular mechanism of detoxification by conjugating reactive electrophilic compounds with soluble glutathione. In addition, these enzymes are also believed to play a crucial role in the protection of DNA from oxidative damage. The genes encoding the mu class of enzymes are organized in a gene cluster on chromosome 1p13.3 and are known to be highly polymorphic. These genetic variations can change an individual’s susceptibility to carcinogens and toxins as well as affect the toxicity and efficacy of certain drugs. GSTM1 products catalyze the conjugation of glutathione to oxide derivatives of polycyclic aromatic hydrocarbons, the main carcinogens.
Oxidative stress, arising as a result of an imbalance between free radicals and anti-oxidant defences, is associated with damage to lipids, proteins and nucleic acids, which could contribute to cellular dysfunctions leading to the pathophysiology of various diseases including atherosclerosis, cancer, and diabetes mellitus (Zhong et al., 1991; Bruhn et al., 1998). Beta cells are very sensitive to cytotoxic stress because they express very little of the antioxidant enzymes. Hence, beta-cell is at greater risk of oxidative damage than other tissues with higher levels of antioxidant protection (West J.C, 2000). During pathogenesis of diabetes mellitus, oxidative and nitrosative stresses contribute to the destruction of insulin-producing beta cells. Moreover, it is believed that increased oxidative stress is one of the main factors in the etiology and complications of diabetes mellitus (Robertson et al., 2003). Glutathione (GSH) is the major cellular antioxidant that protects against environmental toxicants as well as reactive oxygen species (ROS) mediated cell injury. GSH detoxifies ROS, reduces peroxides and detoxifies multiple compounds through glutathione s-transferase (GST) conjunction (Bekris et al., 2005). Glutathione s-transferases (GSTs) belong to a group of multigene and multifunctional detoxification enzymes, which defend cells against a wide variety of toxic insults from chemical, metabolites, and oxidative stress (Robertson et al., 2003). An important condition affecting GST expression is oxidative stress, usually observed in diabetes (Kang et al., 2001). Saito et al., 1993). Decreased GST activity in the liver of streptozotocin induced diabetic rats as compared with normal rat livers. These data point to the fact that GSTs may offer protection against diabetes mellitus. The gene expressing GST enzymes is polymorphic and therefore, it is possible that individual variations in metabolic activities of each enzyme may regulate the clearance of toxic DNA intermediates and may be partially responsible for individual host susceptibility to oxidative stress damage of beta-cells (Yildirim, et al., 2005). The GST isoenzymes expressed in human tissues comprise alpha, mu, pi, theta, kappa, sigma, zeta and omega gene families. Because many GST genes are polymorphic, there has been considerable interest in determining whether particular allelic variants are associated with altered risk of a variety of pathologies including cancers and cardiovascular and respiratory diseases (Habdous et al., 2004). 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, pi and 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 null gene polymorphism (GSTM1) has been associated with chronic pancreatitis, leukemia and other cancers, rheumatoid arthritis and asthma. The GST theta null gene polymorphism (GSTT1) has been reported to be associated with breast cancer and colorectal cancer. Some studies indicated that genetic variations of GSTT enzyme are associated with the development of end-stage renal disease in diabetes mellitus patients (G Yang et al., 2004). GSTM1 and GSTT1 polymorphisms are the most common polymorphisms of GST enzymes in the human population with major ethnic differences and have been studied most extensively in many studies. Five mu class genes are situated (GSTM1 – GSTM5) on chromosome 1. Polymorphism has been identified in the GSTM1 are GSTM1*0, GSTM1*a and GSTM1*b. GSTM1*0 is deleted and homozygote (GSTM1-null genotype) express no protein and leads to absence of phenotypic activity, GSTM1-positive genotype, namely GSTM1*a and GSTM1*b, differ by one base, and the catalytic effectiveness of the enzymes encoded by these alleles is similar. The homozygous GSTM1-null genotype has attracted interest because its frequency varies from 45% to 50% in different populations (Capoluongo et al., 2006). There are two theta class genes, GSTT1 and GSTT2, located on chromosome 22. GSTT1 is represented by two alleles: a functional or wild allele (GSTT1*1) and a nonfunctional or null allele (GSTT1*0). The homozygous genotype for the null allele has been defined as GSTT1*0, and the genotype with at least one functional allele has been denoted as GSTT1*1. the GSTT1*0 frequency ranges from 16% to 38% of the overall population (Güven et al., 2007). To facilitate understanding the multifactorial causes of diabetes mellitus, it is reasonable to study whether genetic polymorphism of antioxidant enzymes contribute to the pathogenesis of diabetes mellitus. We hypothesized that if environmental toxicants and oxidative stress are associated with type 2 diabetes mellitus then GSTs, may be a modifying factor that contributes to type 2 diabetes progression and thus the GSTM1 and GSTT1 null genotypes will be associated with type 2 diabetes mellitus.

**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 (Wild et al., 2004). It is estimated that Egypt will be listed in the top 10 countries with the highest numbers of people with diabetes in 2030, reflecting anticipated changes in the population size and structure in Egypt (Stephens et al., 2009). 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. Glutathione s-transferase (GST) modulates the effects of various cytotoxic and genotoxic agents. GST, along with other antioxidant enzymes, such as selenium-dependent GPx1, provides the cell with protection against a range of harmful electrophiles produced during oxidative damage to membranes (Hayes et al., 1999).

GSTs constitute one of the major components of the phase II drug metabolizing enzyme and antioxidant systems (Wang et al., 2006). Therefore, there is 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. 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. Genetics type 2 diabetes mellitus is characterized by being a polygenic disorder and generally thought of as a syndrome, rather than a single specific entity. This suggests that a common adverse force is exerted on beta-cells in all patients. One such force is the oxidative stress. Oxidative phosphorylation during anaerobic glycolysis generates reactive oxygen species (ROS). The islet is unusually at risk for damage by pro-oxidant forces, because it expresses very low levels of antioxidant mRNA, protein, and activity. GSTs can work as endogenous antioxidants to protect cells from oxidative stress. The GSTs catalyze the conjugation of glutathione to a wide range of electrophiles and represent a protective mechanism against oxidative stress. The GST family of genes is critical in the protection of cells from ROS because they utilize as substrates a wide variety of products of oxidative stress. Most genetic polymorphisms do not cause an identifiable change in the organism in which they occur. However, some either cause disease outright or alter disease susceptibility.

The proportion of the GSTT1- and GSTM1-null genotypes was significantly greater in diabetic patients when compared to controls. Patients carrying both null polymorphisms had a 3.17-fold increased risk of having type-2 diabetes mellitus compared to those with normal genotypes of these two genes (P = 0.009). Additionally, patients with the GSTT1-null genotype had higher levels of triglycerides and very low-density lipoprotein cholesterol compared to those with the GSTT1-present genotype (table 1). On the other hand, patients with the GSTM1- null genotype had significantly higher levels of HbA1c and significantly higher diastolic blood pressure compared to those with the GSTM1- present genotype. The interaction between these genotypes and smoking status was not significant. These results give evidence that the GSTT1- and GSTM1- null genotypes, alone or combined, are associated with increased risk of type-2 diabetes mellitus, regardless of smoking status. Only the GSTM1-null genotype had an effect on glycemic control.

### Table 1: Association between glutathione s transferase (GST) genotypes and risk of type 2 diabetes mellitus development among the studied population:

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Control Group (N = 16)</th>
<th>Type 2 DM(N= 29)</th>
<th>OR</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSTTIa</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive N (%)</td>
<td>6 (37.5 %)</td>
<td>2 (41.38 %)</td>
<td>1.0</td>
<td>Reference</td>
<td>NS</td>
</tr>
<tr>
<td>Null N (%)</td>
<td>10 (62.5 %)</td>
<td>17 (58.62 %)</td>
<td>1.18</td>
<td>0.34 – 4.16</td>
<td>NS</td>
</tr>
<tr>
<td>GSTTIa</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive N (%)</td>
<td>15 (93.75 %)</td>
<td>24 (82.76 %)</td>
<td>1.0</td>
<td>Reference</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>Null N (%)</td>
<td>1 ( 6.25 %)</td>
<td>5 (17.24 %)</td>
<td>0.39</td>
<td>0.16 – 0.80</td>
<td>NS</td>
</tr>
</tbody>
</table>

OR: odds ratio  CI: Confidence interval from binary logistic regression  : Carriers of at least one intact allele are used as reference.

### CONCLUSION

The combined effect of GSTM1, T1 and P1 genotypes in Indian patients with T2DM. Since significant association was seen in GSTM1 null and GSTP1 (I/V) and multiple association in GSTM1 null, T1 present and P1 (I/I), these polymorphisms can be screened in the population to determine the diabetic risk. GSTM1, GSTT1 and GSTP1 variants might contribute to the development of T2DM and GSTT1 variant alone is involved in the development of T2DM associated CAD complications. In conclusion, the GSTT1 and GSTM1 genes, alone or combined, have an influence on the risk of having type 2 diabetes mellitus. All together, these results suggest that GSTT1 and GSTM1 cooperatively play a protective role against the development of type 2 diabetes mellitus regardless of smoking status. There is an association of GSTM1...
(present, null) and GSTT1 (present, null) genotypes with different clinical and biochemical parameters.

REFERENCES


procedure for extracting DNA from human cells, nucleic acids research, 16(3):1215.