Association of GSTT1 and GSTM1 polymorphisms in South Indian Epilepsy Patients

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Received 15 April 2016; Revised 05 May 2016

Experimental studies suggest that oxidative stress is one of the contributing factors in the onset of epileptic seizures. Glutathione S-transferases (GSTs) are able to conjugate electrophilic compounds, and thus possess neuroprotective role by removing exogenous and endogenous oxidants, detoxifying therapeutic drugs, environmental toxins through conjugation with glutathione (GSH). Several studies from different ethnic groups showed that polymorphisms of the GST gene have been associated with Epilepsy. In the present study, we investigated the association of GST polymorphism in the South Indian epilepsy patients population. A total 371 samples (110 cases and 261 controls) were genotyped for the GST1 and GSTM1 polymorphism by multiplex PCR method. We observed a significant association of GSTT1 null polymorphism in patients with epilepsy. The frequency of the GSTT1 null genotype was found to be significantly higher in cases (35.45 %) than the controls (18.39 %) (OR: 2.44, 95%CI: 1.4-4.02, \( P <0.0001 \)). In contrast, the frequency of the GSTM1 null variant was significantly lower in cases (11.81%) than controls (32.95%) (OR: 0.27, 95%CI: 0.14-0.51, \( P <0.001 \)) indicating a protective role. These results indicated that individuals who have GSTT1 null variant are at higher risk for developing seizure than those of GSTT1 wild genotype. On the other hand, individuals carrying GSTM1 null variant showed protective role against seizure. Further, these two null variants did not show any significant association with antiepileptic drug-induced skin rash.

Keywords: Antiepileptic drugs, CNS, GST polymorphism, Seizures, Skin rash

Epilepsy is a common serious neurological disorder, prevalent in humans from neonates to elderly, and is commonly associated with symptomatic or structural etiologies that includes central nervous system (CNS) tumors, neurodevelopmental abnormalities, CNS trauma and inflammation. Several studies have linked the role of oxygen free radicals in the pathogenesis of neurodegenerative diseases some of which may subsequently develop characteristics of epilepsy over time. The role of oxidative and nitrosative stress in the pathogenesis of epilepsy was also demonstrated. An increase in mitochondrial oxygen derived free radicals and subsequent neuronal cell damage after persistent seizures was also reported in several studies.

The glutathione-S-transferases (GST) present in several isoforms, are involved in the conjugation of a wide range of electrophilic substances and oxidants with glutathione, thus facilitating detoxification and excretion. Additionally, GST plays a major role in antiepileptic drug (AED) metabolism, and thus preventing adverse events, particularly liver damage associated with these AEDs. Studies have shown that genetic polymorphisms reported for GSTM1, GSTP1 and GSTT1, resulting in either decreased or altered enzyme activity. In a study in Tunisian population, it was demonstrated that GSTM1 null genotype were at an increased risk of developing epilepsy, whereas no significant effects were observed between the individuals with GSTT1 null genotype and epilepsy risk, suggesting that the absence of GSTM1 activity could be contributing factor for the development of epilepsy. In contrast, in a case-control study in Serbian population, GSTT1-null genotype was found to be associated with the increased risk and enhanced susceptibility to...
oxidative stress in progressive myoclonus epilepsy patients\textsuperscript{19}.

In view of contrasting findings and the presence of wide inter-ethnic differences in the occurrence of GST polymorphism, here, we studied the association of GSTT1 and GSTM1 polymorphism with epilepsy patients from South India.

Materials and Methods

The current study was carried out on 110 patients with epilepsy visiting the Neurology department of the Nizam’s Institute of Medical Sciences, Hyderabad, India, as well as 261 age- and sex matched healthy controls. The consecutive patients who attended the epilepsy clinic were taken into study. Their demographic data including age of onset, duration of illness, frequency of seizures, diurnal/nocturnal, type of seizures, family history of seizures, history of birth asphyxia, antiepileptic drugs were taken. Syndromic approach was done according International League Against Epilepsy (ILAE) classification, depending on age of onset, semiology of seizures, frequency of seizures, EEG findings and MRI Brain (either 1.5 T or 3.0 T). All the patients were on antiepileptic drugs either carbamazepine, phenytoin, valproate or oxycarbamazepine. Exclusion criteria included were history of pseudo seizures, alcohol or drug abuse, or any other malignant diseases such as brain tumor, secondary metastasis, hepatic or renal failure. Male/female ratio of the patients was 52/58, respectively with a mean age of 28.2±15.6 years (Table 1). Eligible controls were subjects with no history of epilepsy were enrolled as controls from a group of 261 healthy volunteers (132 males and 129 females) with a mean age of 32.6±10.9 years. The volunteers comprised mostly of hospital staff who had no history of epilepsy or any other diseases. Their recruitment was done at Nizam’s Institute of Medical Sciences, Hyderabad, India after careful evaluation of their personal health and family history. Blood samples were collected from each subject in 5 ml EDTA tube. The informed consent was obtained from all the subjects. The study was approved by the ethics committee (EC/NIMS/1284/2012) of Nizam’s Institute of Medical Sciences (NIMS), Hyderabad, India.

Table 1—Demographic characteristics of cases and controls

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<thead>
<tr>
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<th>Cases (n=110)</th>
<th>Controls (n=261)</th>
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<tr>
<td>Age (yrs) (mean±SD)</td>
<td>28.2±15.6</td>
<td>32.6±10.9</td>
</tr>
<tr>
<td>Male: N (%)</td>
<td>52 (47.2)</td>
<td>132 (50.5)</td>
</tr>
<tr>
<td>Female: N (%)</td>
<td>58 (52.7)</td>
<td>129 (49.4)</td>
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DNA Extraction and Genotyping

Genomic DNA was isolated from all the samples using the standard phenol-chloroform extraction protocol. Deletion status of GSTM1 and GSTT1 was simultaneously determined by the multiplex polymerase chain reaction method. GSTM1 and GSTT1 genes were amplified using the following primers: 5’GAA CTC CCT GAA AAG CTA AAG C 3’ and 5’GTT GGG CTC AAA TAT ACG GTG G 3’ for GSTM1 and 5’ TTC CTT ACT GGT CCT CAC ATC TC 3’ and 5’ TCA CCG GAT CAT GCC CAG CA 3’ for GSTT1. As an internal control, exon 4 of MTHFR was amplified using primers 5’-TTT GAG GCT GAC CTG AAG CAC TTG AAG GAG-3 and 5’-GAG TGG TAG CCC TGG ATG GGA AAG ATC CCG-3. Components of the PCR reaction mix of 25 µl included 10 pM of each primer, 300 µM dNTPs, 2 mM of MgCl\textsubscript{2}, 1.5 units of Taq DNA polymerase and 50 ng of genomic DNA as template. Agarose gel electrophoresis (2%) resolved amplified DNA fragments of 480, 215 and 178 bp for GSTT1, GSTM1 and MTHFR, respectively (Fig. 1). Presence of respective bands indicates the presence of wild allele whereas absence of bands indicates deletion (Null).

Quality control for genotyping

Cases and controls were analyzed in the same set of PCR. For all genetic analyses, each PCR set was accompanied by a negative control without genomic DNA in order to check the contamination of components.

Fig 1—GSTT1 and GSTM1 multiplex PCR products with 100bp DNA marker. [480 bp band correspond to GSTT1 wild allele, 215 bp band correspond to GSTM1 wild allele and band at 173 bp correspond to MTHFR (internal control). Lane 1, GSTT1 null; Lane 2, GSTM1 null; Lane 3, GSTT1 null; Lane 4, Wild genotype GSTT1/GSTM1; Lane 5, Wild genotype GSTT1/GSTM1; and Lane 6, 100 bp ladder]
Statistical analysis
The frequencies of the GSTT1 and GSTM1 genotypes were determined in patient and control groups using the $\chi^2$ test. The same test was used to evaluate significant associations between the epilepsy patients and control. The differences between different groups were considered significant if the $P < 0.05$. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated by unconditional logistic regression.

Results
The genotype frequencies of GSTT1 polymorphisms among the epilepsy patients and healthy controls are shown in Table 2. The frequency of GSTT1 null genotype was found to be higher in cases (35.45%) than controls (18.39%) and observed to be significantly associated with the risk of epilepsy (OR: 2.4375; 95% CI: 1.477-4.021, $P < 0.0001$). On the other hand, the frequency of GSTM1 null genotype was low in cases (11.81%) than controls (32.95%) and found to be protective against epilepsy (OR: 0.2727, 95% CI: 0.144-0.514, $P < 0.0001$) (Table 2).

We observed 12 out of 110 cases (10.9%) and 27 out of 261 controls (10.34%) for GSTT1-GSTM1 double null, which is not statistically insignificant. Further, we did not find any significant association either with GSTT1 or GSTM1 polymorphism with epilepsy when the data was stratified into male and female or with different age groups (data not shown). Further, there was no significant association of AED-induced skin rash with GSTT1 null and GSTM1 null genotypes (Table 3).

Discussion
The increased level of reactive oxygen species (ROS) in brain tissue is presumably due low antioxidant defenses and due to high rates of metabolism in the brain. It has been shown that brain tissue utilizes approximately 20% of the total $O_2$ consumption for the ATP production and during this process it also releases of ROS. The brain tissue is regarded as highly susceptible to oxidative stress because of the high-energy demand and the lower antioxidant activities (SOD, catalase and glutathione peroxidase) in neuronal cells. The low level activities of antioxidants expressed in neurons make them highly sensitive to oxidative damage.

Several studies have shown the increased oxidative stress markers in Parkinson's disease (PD). In addition, the levels of antioxidant, glutathione (GSH) was found to be decreased in substantia nigra of PD patients. The post-mortem analysis of the brain of PD patients revealed that altered oxidative stress would in turn mediate degeneration of nigrostriatal neurons. Studies have also suggested that neurodegenerative diseases, over the time, might develop characteristics of epilepsy. Increased oxidative and nitrosative stress are also regarded as possible mechanisms in the pathogenesis of epilepsy.

The GST enzymes are antioxidant enzymes, participate in the metabolism of a wide range of chemicals and the known substrates for GST enzyme
which include reactive peroxide intermediates generated from the activation of polycyclic aromatic hydrocarbons produced by exogenous and endogenous pathways by CYP450 enzymes. The loss of function or reduced activity of GST enzyme due to polymorphisms could be due to defective detoxification activities associated with increased susceptibility to human diseases including epilepsy. There are several studies reporting large inter-individual variability for GSTM1 and T1 due to the presence of the null (zero activity) genotype. The frequency of GSTT1 null genotype in Caucasians, Asians, and in African Americans were 18, 40-60 and 22%, respectively, while the frequency of GSTM1 null genotype is 40–60% in Caucasians and Asians, and 20–25% in African Americans. The meta-analysis by Dey et al. reported that the frequency of null genotypes of both GSTT1 and GSTM1 genes are considerably lower among South Indian population compared to the rest of Indian population.

In the present study, we observed the frequency of GSTT1 null genotype is significantly higher in patients with epilepsy that controls, suggesting that the absence of GSTT1 activity could be contributing factor for the development of epilepsy disease. Recently, Ercegovac et al. showed that GSTT1-null genotype is associated with the increased risk and enhanced susceptibility to oxidative stress in progressive myoclonus epilepsy (PME) patients. In the current study, we observed the protective role of GSTM1 null variant against epilepsy. However, in a recent study in Tunisian population, it was demonstrated that individuals with the GSTM1 null genotype were at an increased risk of developing epilepsy whereas no significant effects were observed between the individuals with GSTT1 null genotype and epilepsy risk. In the same study, they concluded the defective detoxification of the neurotoxic metabolite of dopamine i.e., O-quinones due to GSTM1 null phenotype might contribute to the development of epilepsy.

Apart from CYP2C9 and CYP2C19 polymorphisms, GST polymorphisms also play a crucial role in the metabolism of antiepileptic drugs (AED). The presence of GSTT1 and GSTM1 null phenotype, adversely affects the metabolism and excretion of AEDs leading toxicity to various tissues. In the present study, we did not observe any significant association of antiepileptic drug-induced skin rash with either GSTT1 or GSTM1. A retrospective study in Japanese patients with epilepsy implicated the GSTM1 null genotype as a risk factor for carbamazepine-induced mild hepatotoxicity. An association between the common polymorphisms in the GSTM1 and GSTT1 null genes and the increased levels of γ-glutamyltransferase, a marker for hepatotoxicity, was reported in valproic acid-treated patients with epilepsy. Further studies on the association of GSTT1, GSTM1 polymorphism with different types of epileptic seizures, antiepileptic drug(s)-induced thrombocytopenia and hepatotoxicity with increased sample size in different ethnic groups are warranted.

**Conclusion**

Results of our current study indicated that individuals who have GSTT1 null phenotype are at higher risk for oxidative stress and possibly developing seizure than those with GSTT1 wild genotype. On the other hand, individuals carrying GSTM1 null variant showed the protective role against seizure. Further, these two null variants did not show any significant association with anti-epileptic drug-induced skin rash.

**References**


