THE RISK OF NULL-GLUTATHIONE S TRANSFERASE THETA 1 (GST-T1) POLYMORPHISM IN ESSENTIAL THROMBOCYTHEMIA.

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Manuscript Info

Abstract

Introduction: Essential thrombocythemia (ET) is a chronic myeloproliferative disorder (MPNs) that affects primarily the megakaryocytic cell line. In this condition there is a sustained increase in platelet count, because of megakaryocyte proliferation and overproduction of platelets. The haematocrit is normal and Philadelphia chromosome or BCR-ABL1 rearrangement is absent. The bone marrow shows no collagen fibrosis. A persisting platelet count of >450 × 10/L is the central diagnostic feature but other causes of raised platelet count (particularly iron deficiency, inflammatory or malignant disorder and myelodysplasia) need to fully exclude before the diagnosis can be made.

The families of genes that has been long studied and linked to increased cancer and MPN risk continue to be the CYP, GST, and NAT2 genes. The National Institute of Environmental Health Sciences Environmental Genome Project (NIEHS EGP) has already categorized environmentally sensitive genes (ESG) in the human genome. A total of 647 genes are included in the NIEHS EGP list in the following categories: cell structure, metabolism, DNA repair, cell cycle, cell division, cell signalling, gene expression, and homeostasis. This was the starting for our search for candidate genes, and specific polymorphisms to be used as a toxicological marker for susceptibility, including Cytochrome P450 superfamily (CYP genes), Polymorphic N-acetyltransferase (NAT2), and Glutathione S-transferase (GST) polymorphisms. The benefits of using Mendelian randomization are apparent when the genetic variants are functional SNPs and the biological function of the variant is known.

Glutathione S-transferase (GSTs) are a class of phase II enzymes present in many tissues, and are involved in xenobiotic detoxification, and thus contribute to the protection from broad range of compounds including carcinogens, chemotherapeutic agents, and environmental pollutants, the GSTM1 and GSTT1 null genotype is associated with differential susceptibility to various forms of cancer, resistance to chemotherapy treatment, drug...
response and disease susceptibility and outcome\textsuperscript{(8)} Several studies have been published on the relationship between GSTT1 and various types of cancers. In this study and for the first time, we evaluated the association of GSTT1 polymorphism and the susceptibility of essential thrombocythemia (ET) among Sudanese patients.

**Materials and Methods:**
A cross-sectional study conducted at Alneelain University and Radiation Isotope Center, Khartoum, Sudan during Oct. 2015 to May 2016. A total of 53 Sudanese patients from both genders diagnosed by ET according to World Health Organization (WHO) diagnostic criteria were recruited in this study after informed consent and ethical approval from Alneelain ethical committee. On the other hand, 50 healthy volunteers were recruited as control group. Three ml of EDTA blood sample was collected to determine the GSTT1 mutation, leukocytes count, platelets count and hemoglobin concentration for each patient.

**DNA Extraction and PCR testing:** DNA was extracted by using salting out protocol and then the mutant allele was assessed by using allele specific PCR reaction to detect the GSTT1 gene.

**Detection of GSTT1 polymorphism:**- Allele specific polymerase chain reaction (Techne, TC-412, UK) was used for detection of the polymorphic deletion of the GSTT1. The following pair of primers was used in the genotyping analysis:

**Sense primer:** - 5-TTCCTTACTGGTCTCCTCATCTC-3  
**Antisense primer:** - 5-TCACGGGATCATGGCCAGCA-3

PCR was carried out in a total volume of 20 μl. It consisted of 4μl of genomic DNA; 1μl from each primer and 4μl of “5X FIREPOL” ready to load mastermix (SOLIS BIODYNE, TARTU-ESTONIA) and 12 μl distilled water. PCR was initiated by denaturation step at 94°C for 5 minutes followed by 40 cycles of denaturation at 94°C for 45 seconds, annealing temperatures ranged between 63 °C for 1 minute and 55 °C for 30 second, extension at 72°C for 1 minute, and final extension at 72°C for 5 minutes. After amplification, PCR products were electrophoresed on 2% agarose gel containing ethidium bromide, and visualized by gel documentation system. 100 bp DNA ladder was run with each batch of patients' samples. GSTT1 genotypes were determined by the presence and absence (null) of bands of 489bp.

**Results:**
Fifty three patients with Essential thrombocythemia and 50 apparently healthy controls were enrolled in this study. The patients' mean age was 43 years, while the control was 40 years. The means of platelets, total white blood cells and hemoglobin were 1.692.113 cu/mm, 14.185 cu/mm and 12.3 g/dl respectively with no significant difference.

The GSTT1-null mutation was detected in 18 (34%) of cases and in 35 (66%) of controls. But the difference was not statistically significant (P.value < 0.05). The odd ratio was more than one (2) indicated risk for the exposure group (GSTT1-null cases). There is no significant association between gender and the presence of the mutation (P.value < 0.05).

**Discussion and conclusions:**
As GSTM1 and GSTT1 are involved in the processing of reactive oxygen, lipid peroxidation products and some key metabolites of toxicants, there are potential links between genetic polymorphisms of these enzymes and the pathogenesis of a number of chronic diseases in particular, important insights into the effects of the GSTM1 and GSTT1 gene deletions on the pathogenesis of human diseases have been derived from molecular epidemiological studies [10]. In our study and for the first time, the GSTT1-null genotype was found to be more frequent in the controls compared to the patients of ET, and that suggesting the GSTT1-null genotype to be as a genetic risk factor ET. We may conclude that GSTT1-null individuals are at risk of ET.
References:


