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GSTT1 POLYMORPHISM IN SUDANESE PATIENTS WITH POLYCYTHAEMIA VERA

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Abstract

Background: Glutathione S-transferase (GST) enzymes that play a key role in detoxification of activated carcinogens are shown to be one of the potential modifiers of individualized risk for several cancer types.
Objective: This purpose of this study was to investigate the frequency of the GSTT1 null genotype in Polycythaemia vera patients in sudan.
Materials and Methods: Fifty polycythaemia vera patients and fifty controls were evaluated to determine the frequency of GSTT1 null genotype. Red cell parameters were performed by an automated cell analyzer. The GSTT1 null genotype was determined using polymerase chain reaction (PCR) method.
Results: The GSTT1 null mutation was detected in 23% of cases (17% males and 5% females) and in 22% of control subjects. But the difference was not statistically significant (OR=1.2, 95% CI= 1.06-1.41, P= 0.64).
Conclusion: We have observed that GSTT1 null genotype was similar among patients as well as control group and GSTT1 genotype was not related to laboratory findings in polycythaemia vera patients.

Keywords: Polycythaemia Vera, Polymorphism, GSTT1

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INTRODUCTION

Polycythaemia (erythrocytosis) is defined as an increase in the haemoglobin concentration above the upper limit of normal value for the patient's age and sex (Hoff brand *et al*., 2006). It mainly affects older people between the ages of 40 and 60 years with annual population incidence of about 14 per million (Zhao *et al*., 2005). Polycythemia vera (PV) is a clonal myeloproliferative disorder that leads to triliniage hyperplasia in the bone marrow with a principle clinical manifestation of erythrocytosis and plethora. (Sui *et al*., 1997). The fact that these measurements are ratios of the number of red blood cells to volume of plasma must be kept in mind. An increase in hemoglobin can result from a reduction in plasma volume without a true increase in red blood cell mass (Hillman, 2005).

Polycythaemia is classified according to its pathophysiology but the major subdivision is into absolute polycythaemia, in which the red cell mass (volume) is raised, and relative or pseudopolycythaemia in which the red cell volume is normal but the plasma volume is reduced (Hoff brand *et al*., 2006).

Phlebotomy is the mainstay of treatment for this disease, and hydroxyurea, interferon-alpha, anagrelide drug therapies, 32P radiation therapy have been commonly used. The mortality is high if the disease is untreated or is associated with leukemia. (Zhao *et al*., 2005). Structural variation in the human genome is increasingly recognized as being highly prevalent and having relevance to common human diseases (Copy-Number, ?). In everyday life, the human body is exposed to a number of xenobiotics including drugs, dietary compounds, or environmental carcinogens, which are metabolized by a variety of enzymes through phase I and phase II reactions. These enzymes mainly participate in the conversion of xenobiotics to more polar and water soluble metabolites which are readily excreted from the body. During metabolism of certain xenobiotics, a variety of unstable and reactive intermediates can be formed, which could attack DNA, causing cell toxicity and transformation (Bozina *et al*., 2009). Glutathione S-transferase (GST) enzymes that play a key role in detoxification of activated carcinogens are shown to be one of the potential modifiers of individualized risk for several cancer types (Ntais *et al*., 2005). Polymorphisms in GST gene family casue a decrease or loss in activity of the corresponding enzymes and lead to the accumulation of intracellular genotoxic metabolites, which resulted in impairment of the cancer prevention mechanisms (Guengerich *et al*., 1995). Three members of the GST enzymes; GSTM1, GSTP1, and GSTT1 catalyze the reactions with common carcinogens Inherited absence of two alleles (null genotype) in GSTM1 and GSTT1

genes result in lack of enzymatic activity (Turesky and Marchand , 2011). Two widespread genetic polymorphisms that involve deletions in the GSTT1 and GSTM1 genes, namely del (GSTT1) and del (GSTM1), have been reported to lead to abrogation of enzyme activity (Bolufer *et al*., 2007).

Inherited absence of alleles (null genotype) in GSTT1 genes result in lack of enzymatic activity (Srivastava *et al*., 2005). The frequencies of GSTs polymorphic alleles, especially GSTT1 and GSTM1, have been reported in various cancers. (Taspinar *et al*., 2008; San *et al*., 2010; Northwood *et al*., 2010). Several studies have been published on the relationship between GSTT1 and various types of cancers. In this study we evaluate the association of GSTT1 polymorphism and the suscepility of polycythemia among sudanese patient and To correlate the presence of this polymorphism with patients gender and age.

MATERIALS AND METHODS

This study is a case-control study, conducted in Khartoum state, Sudan, in the period from April to June 2015. It is included 50 patients with polycythemia vera (diagnosed according to the WHO criteria) and 50 healthy volunteers as control group.

Blood samples were collected from all subjects in ethylene diamine tetra acetic acid (EDTA) for measurement of red cell parameters using automated haematology analyzer "Sysmex KX-2IN, Japan". The control group consisted of healthy volunteers without a medical history of cancer or other diseases. This study was approved by ethical committee of the faculty of medical laboratory sciences, Al Neelain University, and informed consent was obtained from each participant before sample collection.

Molecular analysis

DNA extraction

Genomic DNA was extracted by using salting out method. DNA samples were stored below -20°C until analysis.

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-TTCCTTACTGGTCCTCACATCTC-3, Antisense primer: 5-TCACGGGATCATGGCCAGCA-3. PCR was carried out in a total volume of 20 µl. It consist of 2 µl of genomic DNA , 1 µl from each primer ,4 µl of "5X FIREPoL" ready to load master mix (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 at94°C for 45 seconds, annealing temperatures ranged between 63 °C for 1 minutes 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 489 bp.

RESULTS

This case control study includes 96partispants, 46 of them were Sudanese patients with polycythaemia and 50 apparently healthy volunteers were included in the study as control group. The patients' ages were ranged from 21-74 years (Mean 46). The ages of control group subjects were ranged from 25 to 55 years (Mean 40 years). 44 (96%) of patients were males and 2 (4 %) of them were females where as 34 (68 %) of controls were males and 16 (32%) were females. The GSTT1 null mutation was detected in 23% of cases (17% males and 5% females) and in 22% of control subjects (Table 1). But the difference was not statistically significant (OR=1.2, 95% CI= 1.06-1.41, P= 0.64). Therefore GSTT null genotype may not be a risk factor for polycythaemia vera. There is no significant association between gender and the presence of the mutation (P.Value: 0.6).

Table 1. GSTT1 Polymorphism and Red cell parameters correlation

	Patient with GSTT1 Null Genotype	Patients without with GSTT1 Null Genotype	P.Value
Haemoglobin(g/dl) Mean± SD	16.81	16.88	0.81
HCT L/L Mean± SD	0.49	0.50	0.35

The study also observe that GSTT1 null genotype was not related to red cell parameters in polycythaemia Vera patients (Table 1).

DISCUSSION AND CONCLUSION

Homozygotes for the null alleles (deletion) of GSTM1 and GSTT1 lack activity of the respective enzymes (Strange and Fryer, 1999) this decrease the reactivity of electrophilic substrates, which may affect the functions within cellular macromolecules, such as nucleonic acid, lipid and protein. So, the genetically determined differences in metabolism, related to GST enzymes, have been reported to be associated with various cancer susceptibilities. (Kim *et al*., 2000). Positive associations were found in certain types of cancers while not found in others. In our study we conclude that the GSTT1 null genotype was found to be non significance association for increasing PV risk (OR=1.2, 95% CI= 1.06-1.41, P= 0.64) as showen in Table 2, and without relation in ages and gender. This finding is in agreement with other studies, in which other cancers were studied. A study in China on leukemic patients showed no significance association of GSTT1 with acute myeloid leukemia (ALL), acute nonlymphoblastic leukemia (ANLL) and chronic myelogenous leukemia (CML) (Chen *et al*., 2008). Another study in China also showed there were no interaction in Oral cancer, smoking and GSTT1 null Polymorphisms (Zhang *et al*., 2011). In contrast also many studies showed positive results in the association between GSTT1 null genotype and various types of diseases and cancers. A case control study,in India found that GSTT1 was significantly associated with male infertility (Jaiswal *et al*.,

2012). All these xenobiotic-metabolizing enzymes and other related enzymes should be studied in different populations and in larger numbers. Also, other environmental factors such as smoking, drug treatments and exposure to radiation as a risk factor for increasing susceptibility to PV should also be studied this can help in disease prevention. The limitation of our study was the small sample size so, we recommended that further study with increased sample size should be conducted in the future.

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