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Elevated genetic deletion of GSTT1 in Pakistani population

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Abstract

Mutations in single nucleotide are common but genetic mutation as deletion of whole gene is rare. Gene GSTT1, selected in this study is polymorphically deleted and have importance not only in xenobiotic metabolism but also in chemotherapy of certain drugs. Genetic deletion of GSTM1 and GSTT1 was carried out by using PCR and gel electrophoresis in local population. The results depicted 71% deletion of GSTT1. The percent deletion in female and male subjects was 72% and 70% respectively. An increased deletion of GSTT1 seen in local population predict impaired response to xenobiotics and some chemotherapeutic drugs.

Key words: Polymorphism, GSTT1, Deletion mutation, Genetic predisposition

Introduction

Polymorphism of drug-metabolizing enzymes, such as glutathione S-transferase (GST) has been reviewed in relation to susceptibility and individual to individual differences in biological monitoring. Genetic factors may influence the vulnerability of a person affected by chemicals, called genetic predisposition. There may exist some association between polymorphism and possibility of disease. Role of drug metabolizing enzymes in relation to chemicals is crucial. Investigations are insufficient to evaluate the relationship between genetic polymorphism and acquired risk. Data is generated implicating GSTT1 polymorphisms in biological monitoring of some chemicals (Nakajima and Aoyama, 2000).

Glutathione S transferases are family of enzymes that use, glutathione to conjugate xenobiotics, adds hydrophilic property, detoxify chemicals and facilitate its excretion thus protect body from probable exogenous carcinogens. It also metabolizes chemotherapeutic drugs. Human body has varying capacity for detoxification of different toxicants may be related to the risk of different types of cancers. GSTT1 is found to be polymorphic. Most of the polymorphic genes vary in single nucleotide but total deletion of gene is rare therefore deletion polymorphism supposed to highlight the risk factor for different diseases including cancers.

On the other hand response to chemotherapy is observed variant across populations. Inherited differences in inter-individual drug response is emerging tool to predict drug response by an individual. GST expression has importance in determining efficacy and cytotoxicity of drugs for example anthracyclines and Alkylating chemotherapeutic agents that are frequently used in treatment of cancer. (Mossallam *et al.*, 2006).

Materials and Methods

The project was designed and conducted according to the good clinical practice (GCP) and the ethical principles laid down in declaration of Helsinki (WHO, 2008). Written consent signed by all subjects were collected. The study was approved by Higher Education commission Pakistan and Ethics Committee of the University.

A total of one hundred healthy subjects took part in this study in which fifty female and fifty male volunteers were selected. The physical examination and laboratory tests were conducted for each subject to check their health status. The subjects who had any of abnormality in physical examination or clinical investigation were excluded from the study. All subjects were adult (above 18 years), non-smokers, non alcoholics, no one was on any medication.

The blood sample collected from each volunteer was used for isolation of DNA and was collected in sterilized EDTA containing centrifuge tubes for DNA extraction. Whole blood was stored at -20°C till further processing.

Primers were synthesized from Gene Link, USA. DNA blood isolation kits (Vivantis GF-1 Blood DNA Extraction Kit), *Taq* polymerase, dNTPs, 6x loading dye, DNA ladder (1 Kb) from Fermentas USA. Gel Electrophoresis (BioRad), Gel Documentation System (Syngene, UK) PCR (Perkin Elmer USA) and Gene Quant from USA was used during research.

Vivantis blood DNA extraction kits were used to isolate DNA from blood. Isolated DNA was quantified on Nano Quant, run on 1% w/v agarose gel electrophoresis stained with ethidium bromide. DNA was used for polymerase chain reaction (PCR) to determine the wild type and deleted gene GSTT1 in local human healthy subjects (Sheikhha *et al.*, 2005).

Primer sequences for amplification of GSTT1 gene.

GSTT1 F-TTC CTT ACT GGT CCT CAC ATC TC

R-TCA CCG AT CAT GGC CAG CA

F: forward primer, R: reverse primer

Results and Discussion

Genetic polymorphism was measured for the deletion of GSTT1 from whole blood sample of all healthy female and male volunteers.

The overall deletion rate in healthy volunteers was 71% (71/100) for GSTT1 in present study. Individually healthy females showed 72% (36/50) and male volunteers showed 70% (35/50) deletion of GSTT1.

Very high frequency for deletion of GSTT1 gene is observed for Pakistani healthy population compared to other population for example Sweden and United Kingdom has only 10 and 14

percent population with deleted GSTT1 gene (Rollinson *et al.*, 2000). The deletion for GSTT1 ranges from 15 to 25 percent in African, 10-20 percent in European and 16-63 percent in some areas of Asian population (Cotton *et al.*, 2000). In healthy volunteers GSTT1 deletion was found more than 50 percent which is distinguishably different than tested in different ethnic groups. There may be a great risk of developing cancer in Pakistani population as some DNA lesions are peroxides and GSTT has higher activity towards DNA hydroperoxides. It has a protective role but it depends on the nature of agents involved. In some cases GSTT1 deletion has positive role as it metabolize a less toxic chemical to more toxic one for example methylene chloride is converted to formaldehyde that is more toxic than methylene (Schulte-Frohlinde and Sonntag 1985; Mannervik and Widersten, 1995).

The prevalence of more percentage deletion compared to other populations may be due to our specific regional environmental conditions and our dietary differences. On the other hand close family marriages make genetic deletions persistent generation after generation.

Prediction of risk is possible by chemical of toxic substances and polymorphism of enzymes involved in metabolism of these substances (Belitsky and Yakubovskaya, 2008). Several drug metabolizing enzymes have been described for functional genetic polymorphisms. Ethnic variability increase the understanding of populations differences in drugs effect having known metabolic pathways that leads to individualize therapy (Goh *et al.*, 2002) and use of pharmacogenetically-guided therapies can give better outcome of therapy and reduce the risk of treatment-related diseases like cancers (Perentesis *et al.*, 2001).

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