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RESEARCH ARTICLE

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

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Abstract

Introduction:-Essential thrombocythemia (ET) is a chronic myeloproliferative disorder (MPNs) that affectsprimarily the megakaryocytic cell line.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 ofessential thrombocythemia (ET) among Sudanese patient.

Materials and methods:-A Total of 53 Sudanese patients from both gender diagnosed by ET according to World Health Organization (WHO) diagnostic criteria were recruited in this study after informed consent and ethical approval from Alneelain ethnical committee. On the other hand, 50 healthy volunteers were recruited as control group. EDTA blood sample was collected to determine the GSTT1 mutation, leukocytes count, platelets count and hemoglobin concentration for each patient.

Result and conclusion:-In our study and for the first time, GSTT1-null genotype was found tobe more frequent in the controls than the cases, with OR 1.9 suggesting that, GSTT1-null genotype is a genetic risk factor ET.

Introduction:-

Essential thrombocythemia (ET) is a chronic myeloproliferative disorder (MPNs) that affectsprimarily the megakaryocytic cell line. In this condition there is a sustained increase in platelet count, because of megakaryocyte proliferation and overproduction of platelets[1]. 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[2].

The families of genes that has been long studied and linked to increased cancer and MPNsriskcontinue to be the CYP, GST, and NAT2 genes. The National Institute of EnvironmentalHealth Sciences Environmental Genome Project (NIEHS EGP) has already categorizedenvironmentally sensitive genes (ESG) in the human genome. A total of 647 genes areincluded 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 tobe used as a toxicological marker for susceptibility, including Cytochrome P450superfamily (CYP genes), Polymorphic N-acetyltransferase (NAT2), and Glutathione Stransferase(GST) polymorphisms. The benefits of using Mendelian randomization areapparent when the genetic variants are functional SNPs and the biological function of thevariant is known[3, 4].

Glutathione S-transferase (GSTs) are a class ofphase II enzymes present in many tissues, and are involvedin xenobiotic detoxification, and thus contributeto the protection from broad range of compounds including carcinogens, chemotherapeutic agents, and environmental pollutants[5,6] , the GSTM1 and GSTT1 null genotype is associatedwith differential susceptibility to various formsof cancer [7], resistance to chemotherapy treatment,drug

response and disease susceptibility and outcome[8]. Several studies have been published on the relationshipbetween GSTT1 and various types of cancers. In this study and for the first time ,weevaluated the association of GSTT1 polymorphism and thesusceptibility ofessential thrombocythemia (ET) amongSudanese patient.

Materials and Methods:-

A cross sectional study conducted at Alneelain University and Radiation Isotope Center, Khartoum, Sudan during Oct. 2015to May. 2016. A Total of 53 Sudanese patients from both gender diagnosed by ET according to World Health Organization (WHO) diagnostic criteria were recruited in this study after informed consent and ethical approval from Alneelain ethnical 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 thenthe 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 theGSTT1. 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 atotal 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 µldistilled water. PCR was initiated by denaturation step at 94°Cfor 5 minutes followed by 40 cycles of denaturation at94°C for45 seconds, annealing temperatures ranged between 63 °C for1 minutes and 55 °C for 30 second, extension at 72°C for 1 minute, and final extension at 72°C for 5 minutes. Afteramplification, PCR products were electrophoresed on 2%agarose gel containing ethidium bromide, and visualized bygel documentation system. 100 bp DNA ladder was run witheach batch of patients' samples.GSTT1 genotypes weredetermined by the presence and absence (null) of bands of 489bp.

Results:-

Fifty threepatients with Essential thrombocythemiaand 50 apparentlyhealthy controls were enrolled in thisstudy. The patientsmean age was 43years, while the control was 40 years.The means of platelets, total white blood cells and hemoglobin were 1.692.113 cu/mm, 14.185cu/mm and 12.3 g/dl respectively with no significant difference.

The GSTT1-nullmutation was detected in 18 (34%) of cases and in 35 (66%) of controls. But thedifference 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 significantassociation 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 andsome key metabolites of toxicants, there are potentiallinks between genetic polymorphisms of these enzymesand the pathogenesis of a number of chronic diseases in particular, important insights into the effects of theGSTM1 and GSTT1 gene deletions on the pathogenesisof human diseases have been derived from molecularepidemiological studies [10]. In our study and for the first time, the GSTT1-null genotype was found tobe 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 concluded that GSTT1-null individuals are at risk ofET.

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