Genetic variants of glutathione S-transferase and the risk of acute myeloid leukemia in a Saudi population

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Objective: This study aims to investigate the genetic association of acute myeloid leukemia and glutathione S-transferase (GST) gene polymorphisms in a Saudi population.

Method: 100 AML cases and 100 healthy controls were recruited from the Riyadh regional hospital. In the GST gene, GSTM1 and GSTT1 variants were genotyped by multiplex PCR, and GSTP1 variants were genotyped by PCR-RFLP analysis. Statistical analysis between AML cases and controls included anthropometric measurements and evaluation of the genotypic and allelic frequencies.

Result: The null genotypes of GSTM1 and GSTT1 showed no association with AML [OR 0.56 (0.26–1.19); p = 0.31 and OR 0.65 (0.37–1.16); p = 0.14]. Similarly, the GSTP1 genotype and allele frequencies did not indicate any association with AML [GG + AG vs. AA: OR 0.75 (0.43–1.31) and p = 0.32; GG vs. AA: OR 1.73 (0.55–5.44) and p = 0.34; G vs. A: OR 0.95 (0.61–1.46) and p = 0.82]. Further, a haplotype analysis between AML cases and controls did not show any positive association (p < 0.05).

Conclusion: In conclusion, there was no statistical association of the genotypes and alleles in GSTM1, GSTT1, and GSTP1 with AML. Our results confirm the negative association of the investigated genetic markers with susceptibility to AML. Further association studies would be required in different ethnic populations to facilitate a meta-analysis in the future. Our findings suggest that the GST gene has no role in the pathogenesis of AML in patients from Saudi Arabia.

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1. Introduction

Leukemia is defined as hematological malignant clonal disorder caused due to excessive abnormal leucocytes in the bone marrow (Huang et al., 2018). Among leukemia’s, acute myeloid leukemia (AML) is a heterogeneous disease with multiple molecular pathways and characterized by uncontrolled proliferation without differentiation of myeloid progenitors (Talati and Sweet, 2018). Acute leukemia includes acute lymphoblastic leukemia (ALL) and AML, with their etiology unknown until now. For acute leukemia (ALL and AML), genome-wide association studies (GWAS) have identified two chromosomal loci, 7p12.2 and 10q21.2, harboring the risk variants in the study subjects from Caucasian, Asian, and African ethnicities (Cao et al., 2018). The disease AML is most common in adults and specifically diagnosed in the elder population (Czemerska et al., 2018). AML has been registered as the sixth leading cause of mortality among numerous malignancies (Lv et al., 2017). AML patients demonstrate 50–60% of chromosomal abnormalities during the diagnosis and karyotyping plays a major role in disease-related prognostic factors for the treatment (Ramzi et al., 2018). Still, poor prognosis was appearing in AML patients after the significant progress has been documented in the diagnostic and therapeutic process (Butrym et al., 2018). AML is associated with favorable, intermediate, and unfavorable risks as per the national comprehensive cancer networks (Xu et al., 2017). Genomic changes play a vital role in AML (Niu et al., 2018). The recent world health organisation (WHO) classifications defines AML with biallelic mutations of CEBPA is recognized as distinct category with favorable prognosis (Ng et al., 2017). So, apart from cytogenetic, the molecular analysis role has been implemented in the AML disease. Zou et al. (2017) studies have confirmed; AML has been associated with multiple genetic mutations. Many individuals inherited genetic mutations are related with cancer/carcinogen metabolism and Glutathione-S-transferase (GSTs) is one of the functional and genetic polymorphisms encodes to the susceptibility of AML.
(Weich et al., 2016). Earlier studies have suggested that there is a connection between GSTs and leukemogenesis and polymorphisms of these genes may also affect the treatment of leukemia, as GSTs have a role in detoxifying active metabolites of cytotoxic chemotherapeutic agents used in killing tumor cells (Guvven et al., 2015; Rollinson et al., 2000). The phase-II enzymes can be detoxified by xenobiotics such as GSTM1, GSTT1 and GSTP1 which were involved in detoxification of reactive oxygen species with several environment carcinogens, pollutants, drugs and other xenobiotics (Zhou et al., 2013). The functional polymorphisms of GST have been reported for a minimum of three genes encoding GSTM1 (μ), GSTT1 (τ), and GSTP1 (π). Combination of GSTM1 and GSTT1 polymorphisms leads to null genotypes of the specific gene, causing loss of the enzyme activity (Stoian et al., 2015). The GSTM1 polymorphism has functional effects in the metabolism of large hydrophobic electrophiles, whereas the GSTT1 polymorphism is involved in the metabolism of smaller compounds. The GSTP1 polymorphism plays a major role in the conjugation of both exogenous and endogenous hydrophobic electrophiles with reduced glutathione (Minina et al., 2017). The protein expression is absent in individuals carrying GSTM1 and GSTT1 null genotypes (Malik et al., 2017). GSTP1 polymorphism is a non-synonymous variation involving the Ile105Val change, which could affect the expression and activity of enzyme, leading to impaired detoxification and cancer (Chen et al., 2017). Genetic impairment in GSTs are associated with an increased risk of solid tumors caused due to malignant hematological diseases such as myelodysplastic syndrome and acute leukemia (Mossallam et al., 2006). Earlier meta-analysis-based studies revealed that the null genotype of GSTM1 and GSTT1 showed a significant association globally (He et al., 2014). At present, there is limited information about GSTT1, GSTM1, and GSTP1 polymorphisms and susceptibility to AML in Saudi population, therefore, this study was initiated on GSTT1, GSTM1, and GSTP1 gene polymorphisms and AML risk in Saudi Arabia.

2. Patients and methods

2.1. Sample collection

The ethical approval for this study was received from the Ministry of Health Affairs in Riyadh regional, and an informed consent was obtained from all participants involved in this study in accordance with the Declaration of Helsinki. The diagnosis of acute leukemia was confirmed through full blood count, bone marrow examination, and flow cytometry. Apart from these tests, chromosomal and fluorescent in situ hybridization was also carried out to confirm AML. All the samples were collected during the period of January 2016 to November 2017 from the Department of Hematology and Oncology. In this study, blood samples were collected from 200 patients, and 100 of them were diagnosed with adult acute myeloid leukemia. Further, 100 healthy control samples were collected from regional laboratory in the Riyadh city of Saudi Arabia. Ethylenediaminetetraacetic acid (EDTA) vacutainer was used to collect 2 ml of venous blood from 100 acute myeloid leukemia patients and 100 healthy controls. Genomic DNA was extracted from blood samples using the genomic DNA purification kit (Sigma-Aldrich) as per the manufacturer's instructions. Purified genomic DNA was checked through 1% agarose gel electrophoresis, and DNA samples were stored at −40 °C in the freezer.

2.2. Molecular analysis

Four polymorphisms (i) GSTT1 +/del (ii) GSTM1 +/del (iii) GSTP1 (A313G) and (iv) CYP1A1 (rs4646903) were selected for this study. Genotyping of GSTT1 and GSTM1 polymorphisms were carried out with multiplex polymerase chain reaction (PCR) using CYP1A1 gene as an internal control, as described in prior publication (Arand et al., 1996). PCR was carried out in final volume of 25 μL contains 15 μL of PCR master mix consists of following reagents: 0.075 units/μL of Taq DNA polymerase, 4 mM MgCl2, 10X buffer reaction mixture and 0.4 mM of each dNTP (dATP, dCTP, dGTP and dTTP) (BIO-RAD, California, USA). Forward and reverse primers (10 pmole or 1 μL) were added to the master mix followed by the addition of 2 μL of genomic DNA (60 ng/μL) and 6 μL of distilled water. The annealing temperatures for GSTT1 and GSTM1 were 66 °C (5 mins), respectively. Multiplex PCR was performed on an Integrated Gulf Biosystems (Thermo Fisher) as described previously (Minina et al., 2017). The null variants of GSTT1 and GSTM1 genes were formed by the absence of 459 bp and 219 bp respectively (Fig. 1). Internal control for this study was very helpful to avoid the false positive results.

The GSTP1(A313G; Ile105Val; rs1695 T > C) polymorphism was determined with the PCR-restriction fragment length polymorphism (PCR-RFLP) analysis. Amplification cycles includes denaturation and initial denaturation at 95 °C for 5 mins and 95 °C for 30 s for 35 cycles. The annealing temperature was found to be 56 °C, and extension and final extension ends at 72 °C for 45 s/ 72 °C for 5 min. The 176-base pair PCR product were digested with BsmAI (Fermentas, USA). The digested products when electrophoresed through 2.5% agarose gel indicated the normal homozygote (II) as 176 bp. Mutant homozygote (VV) showed two bands of 91 bp and 85 bp, whereas the heterozygous (IV) genotype was inferred from three bands of 176 bp, 91 bp, and 85 bp (Fig. 2). The primers used for GSTT1, GSTM1, and GSTP1 polymorphic regions were selected from earlier studies (Kumar et al., 2017; Mandegary et al., 2011) are documented in Table 1. All the undigested PCR products were run on 2% ethidium bromide stained agarose gel to perform the analysis.

2.3. Statistical analysis

Clinical data were statistically analysed using Openepi software (Khan et al., 2015). Hardy-Weinberg equilibrium (HWE) was investigated using the goodness-of-fit χ² to compare the observed allele and expected frequencies determined from control subjects. Differences in genotype frequencies between cases and controls were

![Fig. 1. Representation of multiplex PCR products analyzed on 2% agarose gel, consists of GSTM1 (M + 219 bp) and GSTT1 (T + 459 bp).](image-url)
investigated by an $\chi^2$ test. The odd’s ratios upper and lower limits of the 95% confidence intervals (95% CI) for GSTM1, GSTT1 and GSTP1 variants were determined. P values < 0.05 ($p < 0.05$) were considered statistically significant.

3. Results

3.1. Demographic details of the study subjects

In this study, we recruited 100 AML adult patients and 100 healthy controls. AML patients included 61 males and 39 females, whereas there were 54 males and 46 females in the control. The minimum and maximum ages of the recruited subjects were 19 and 82 years in AML cases and 18 and 63 years in the controls. The mean age of the participants was 38.9 ± 15.1 and 39.9 ± 12.06 years in AML cases and controls, significantly associated between both the groups ($p = 0.02$) (see Table 2.).

3.2. HWE tests and genotypic analysis

The distributions of GSTP1 polymorphism did not demonstrate any deviation from HWE in cases and controls. The frequency of the null genotype of GSTT1 and GSTM1 was 13% and 34%, respectively in AML cases, whereas it was 21% and 44%, respectively in the control subjects. However, the frequency of the positive genotype of GSTT1 and GSTM1 was 87% and 66%, respectively in AML cases and 79% and 56%, respectively in the control subjects. None of the genotypes showed positive association either with GSTT1 [OR 0.56 (95% CI 0.26–1.19); $p = 0.31$] and GSTM1 with AML cases versus controls [OR 0.65 (95% CI 0.37–1.16); $p = 0.14$]. The frequency of the GSTP1 genotypes—AA, AG, and GG—in AML cases was 53%, 37%, and 10%, respectively, and it was 46%, 49%, and 5%, respectively in the controls. The frequency of the A and G alleles in AML cases was 71.5% and 28.5, respectively, whereas it was 70.5% and 29.5% in the control subjects [OR 0.95 (95% CI 0.61–1.46); $p = 0.82$]. The dominant genotype also failed to show any significant association when compared between AML cases and control subjects [OR 0.75 (95% CI 0.43–1.31); $p = 0.82$]. The genotypic and allelic distributions of GSTT1, GSTM1, and GSTP1 polymorphisms in AML cases and controls are documented in Table 3.

3.3. Haplotype analysis

The disease associated with the haplotype (GSTT1 + GSTM1 + GSTP1) was found to be similar association. None of the genotypes showed positive association individually when compared between AML cases and controls. The detailed genotypic frequencies are shown in Table 4. The T1 (+)/M1 (+)/P1 (AA) genotype was used as a reference genotype to compare with other genotypes [OR 0.74 (0.33–1.65); $p = 0.46$] when compared with T1 (+)/M1 (+)/P1 (AG or GG) genotypes.

4. Discussion

This is an initial genetic study carried out in a Saudi population to examine the genotypic and allelic distributions of GST gene polymorphisms in AML cases and control subjects. This study aims to test the genetic association of GSTT1, GSTM1, and GSTP1 gene polymorphisms with AML in the Saudi Arabia. The study results confirm non-significant association between the GSTT1, GSTM1 and GSTP1 gene polymorphisms and AML in the studied population. There was no significant difference in the genotypic/allelic distributions of these polymorphisms between cases and controls. The mean age of AML cases (38.9 ± 15.1 years) was lower when com-
pared with that of the controls (39.9 ± 12.06 years), and we found a significant difference in the age distribution (p = 0.02). Similarly, the gender distribution was found to differ between AML cases and controls. The haplotype also failed to show any significant association with the disease.

The cytosolic enzymes are encoded by the GST genes, and its genetic and functional variants belong to the superfamily of metabolizing enzymes in phase-II. Reduced glutathione with electrophilic compounds is highly soluble in water permitting their elimination, and this detoxification activity prevents cells from DNA damage, genomic instability, and cancer development. GSTs have the ability to modulate the non-enzymatic proteins and signaling pathways that control cell proliferation, differentiation, and apoptosis (Weich et al., 2016). Various types of GSTs translate internal and external carcinogenic compounds and ROS to nontoxic substances. The GST polymorphisms such as GSTP1, GSTT1, and GSTM1 have been classified into three families such as Mu family, Theta family, and Pi family. GSTM1 and GSTT1 are considered loss-of-function mutations as they involve the loss of structural homozygosity. With this deletion, the enzymes with detoxifying functions are modified predominantly in smoking participants or in those exposed to carcinogenic pollutants. The two chromosomal regions, 1p13.3 and 22q11.2, map to the genes GSTM1 and GSTT1, which are expressed in different areas of the human body (Barjui and Reiisi, 2017). In the GSTP1 gene, the common A-to-G transition at 1578 nucleotide position within exon 5 reverts the isoleucine residue (A allele) with valine (G allele) at codon 105 and affects the conjugative ability of reducing glutathione. The presence of the G allele decreases the enzymatic efficiency of GST and in turn decreases the antioxidant capacity and increases the oxidative stress and subsequent cellular damage in the cells. This polymorphism results in reduction of the enzyme activity and is associated with the presence of a high level of hydrophobic DNA adducts (Chuelle et al., 2017; Nomani et al., 2016). Considering the established relation between GST gene variants and AML and the results from prior meta-analysis-based studies, this study was carried out the association study in an adult AML patient from Saudi Arabia. A meta-analysis involves collection of prior association studies on the subjects from different ethnicities and unifying them to reach a consensus conclusion. It is a statistical study for clubbing the results of replication studies from different ethnic populations (Martin and Austin, 2000). A meta-analysis, which included 29 association studies on AML and GST gene polymorphisms and categorized the subjects into East-Asian and Caucasian populations, reported that the GSTM1 and GSTT1 variants were associated with the risk of AML in East-Asian population and in Caucasians. However, GSTP1 polymorphism was not associated in either East-Asians or Caucasian population (He et al., 2014). The first meta-analysis on AML and the GST polymorphism included 15 different global case-control studies and reported a significant association of AML risk with GSTM1 and GSTT1 variants but not with the GSTP1 polymorphism (Das et al., 2009). Both the meta-analyses confirmed the negative association of AML risk with the GSTP1 polymorphism globally. The current study was designed as a case-control study, and results indicated non-significant association of AML with GSTM1, GSTT1, and GSTP1 polymorphisms. In general, case-control studies are retrospective and observational studies carried out either in hospitals or in institutions. Based on the inclusion and exclusion criteria, the cases or the diseased subjects (patients affected with the disease under study) are recruited along with the controls, which are either healthy participants or subjects negative for the disease under study. The case-control genetic studies represent the disease risk in term of the odds ratio in a 95% confidence interval and the statistical differences between cases and controls in terms of p-values. These tests compare the genotypic and allelic distributions of targeted markers such as single nucleotide polymorphisms (SNPs) in cases and controls, and investigate whether the genetic marker is associated with the disease-risk (Clarke et al., 2011). Recently, the GST variants were studied in AML patients from different populations of Spain, and no association was found between the GSTT1, GSTM1, and GSTP1 variants but not with the GSTP1 polymorphism (Das et al., 2009). Both the meta-analyses confirmed the negative association of AML risk with the GSTP1 polymorphism globally. The current study was designed as a case-control study, and results indicated non-significant association of AML with GSTM1, GSTT1, and GSTP1 polymorphisms. 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### Table 3
Genotypic distribution between AML cases and controls with GST gene polymorphisms.

| Genotypes       | Cases (n = 100) | Controls (n = 100) | OR (95%CI) | p-Value |
|-----------------|----------------|-------------------|------------|---------|
| GSTT1 (+)       | 87 (87%)       | 79 (79%)          | Reference  | 1.00    |
| GSTT1 (-)       | 13 (13%)       | 21 (21%)          | 0.56 (0.26–1.19) | 0.31    |
| GSTM1 (+)       | 66 (66%)       | 56 (56%)          | Reference  | 1.00    |
| GSTM1 (-)       | 34 (34%)       | 44 (44%)          | 0.65 (0.37–1.16) | 0.14    |
| GSTP1 (AA)      | 53 (53%)       | 46 (46%)          | Reference  | 1.00    |
| GSTP1 (AG)      | 37 (37%)       | 49 (49%)          | 0.65 (0.36–1.17) | 0.15    |
| GSTP1 (GG)      | 10 (10%)       | 05 (5%)           | 1.73 (0.55–5.44) | 0.34    |
| GSTP1 (AG + GG vs AA) | 47 (47%) | 54 (54%) | 0.75 (0.43–1.31) | 0.32    |
| GSTP1 (A)       | 143 (71.5%)    | 141 (70.5%)       | Reference  | 1.00    |
| GSTP1 (G)       | 57 (28.5%)     | 59 (29.3%)        | 0.95 (0.61–1.46) | 0.82    |

### Table 4
Triple genotype frequencies between AML cases and control in GST (GSTT1/GSTM1/GSTP1) genes.

| Genotypes       | Cases (n = 100) | Controls (n = 100) | OR (95%CI) | p-Value |
|-----------------|----------------|-------------------|------------|---------|
| T1 (+) /M1 (+) /P1 (AA) | 31 (31%)   | 22 (22%)          | Reference  | 1.00    |
| T1 (-) /M1 (+) /P1 (AA) | 02 (2%)    | 05 (5%)           | 0.28 (0.05–1.99) | 0.13    |
| T1 (-) /M1 (-) /P1 (AA) | 15 (15%)   | 15 (15%)          | 0.70 (0.28–1.74) | 0.45    |
| T1 (+) /M1 (+) /P1 (AA) | 04 (4%)    | 04 (4%)           | 0.70 (0.16–3.14) | 0.65    |
| T1 (+) /M1 (+) /P1 (AG or GG) | 23 (23%)  | 22 (22%)          | 0.74 (0.33–1.65) | 0.46    |
| T1 (+) /M1 (-) /P1 (AG or GG) | 17 (17%)  | 20 (20%)          | 0.60 (0.25–1.40) | 0.24    |
| T1 (-) /M1 (+) /P1 (AG or GG) | 01 (1%)    | 08 (8%)           | 0.08 (0.01–0.76) | 0.008   |
| T1 (-) /M1 (+) /P1 (AG or GG) | 07 (7%)    | 04 (4%)           | 1.24 (0.32–4.76) | 0.75    |
AML cases and 100 control subjects were included which is native of Saudi Arabia. Genotyping was performed with multiplex PCR for GSTT1 and GSTM1, which worked as an internal control, and for GSTP1, genotyping was carried out by PCR-RFLP. However, the present study has certain limitations such as BMI, smoking, and family history were avoided in this study, and skipping the validation for genotyping results through Sanger sequencing. Although the purpose of recruiting the patients from the hospital is to ensure the complete geographical coverage of the Kingdom of Saudi Arabia, the present study results may not reflect the trend of the entire Saudi population.

To the best of our knowledge, this is the first genetic study that investigated the association of GSTT1, GSTM1, and GSTP1 gene polymorphisms with AML risk in Saudi Arabia. These results confirm the negative association; therefore, the GST gene polymorphisms may not be associated with susceptibility to AML. Further studies would be required in different ethnic populations to facilitate a meta-analysis–based investigation in the future. The present study strongly recommends employing next-generation sequencing–based examination in a larger cohort of AML cases with elaborated clinical information of the patients.

Conflict of interest

None.

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Table 5

| S.No | GSTM1 | GSTT1 | GSTP1 | Refs. |
|------|-------|-------|-------|-------|
| 1    | Yes   | Yes   | Yes   | 32    |
| 2    | No    | No    | No    | 13    |
| 3    | Yes   | No    | No    | 39    |
| 4    | No    | Yes   | No    | 15    |
| 5    | No    | No    | N/A   | 32    |
| 6    | Yes   | No    | No    | 24    |
| 7    | Yes   | Yes   | No    | 30    |
| 8    | No    | No    | Yes   | 38    |
| 9    | N/A   | Yes   | N/A   | 35    |
| 10   | No    | No    | N/A   | 20    |
| 11   | Yes   | N/A   | N/A   | 36    |
| 12   | No    | No    | No    | 54    |
| 13   | No    | Yes   | No    | 17    |

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