Glutathione S Transferase T1 and M1 Gene Polymorphisms and Risk of Myeloid Leukemias

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Abstract

Leukaemias in general population result from complex interaction between multiple genetic and environmental factors over time. The interplay of xenobiotic exposure, endogenous physiology, and genetic variability of multiple loci may facilitate knowledge about leukaemia aetiology and the identification of individuals who are at increased risk of developing leukaemias. Xenobiotics are chemical substances that are foreign to the biological system, adverse effects of xenobiotics are exerted via covalent interactions between intermediate metabolites and genetic materials or proteins and their related metabolites. In order to avoid accumulation of lipophilic xenobiotics in cells and tissues, enzymatic reactions of xenobiotic metabolism that can be divided into two distinct phases are needed. Phase II enzymes known as Glutathione S Transferases (GST) catalyze the conjugation of glutathione or glucuronide with reactive electrophiles and thus detoxify procarcinogens and carcinogens. Human GSTs: GSTM1 and GSTT1 are two genes whose products can modify endogenous and exogenous toxic substrates to less reactive species through a number of mechanisms that play a critical role in the system which protects against reactive oxygen species, the breakdown of peroxidized lipid and oxidized DNA. In the present study we attempted to study null genotype frequency of GSTT1 and GSTM1 genes in myeloid leukemias by multiplex PCR. Our study included 50 newly diagnosed patients of myeloid leukemias: 25 cases with Acute Myeloid Leukemia (AML), 25 with Chronic Myeloid Leukemia (CML), and 30 healthy volunteers. A statistical significant difference was found with GSTT1 null genotypes frequency in AML patients as compared to controls 13/25 (52%) versus 4/30 (13.3%): OR=7.04 (1.9-26.1). There was no statistical significant difference in the frequencies of the GSTM1 null genotypes between AML patients and control (p-value 0.084). There was a significant association between patients achieving complete remission and GSTT1 genotype i.e. those with GSTT1 gene present were more liable to complete remission (p-value 0.008), with no statistical significant association between follow-up cases and GSTM1 genotype (p-value 0.290). There was a statistical significant difference in GSTT1 null genotypes frequency in CML patients as compared to controls 10/25 (40%) versus 4/30 (13.3%); Odds ratio [OR=4.3 (1.15-16.2)]. On the other hand there was no significant difference in frequency of the GSTM1 null genotypes between CML patients and control (p-value 0.80). In conclusion our results revealed a strong association between GSTT1 null genotypes and risk of AML and CML, with a valuable influence on the fate of AML patients where patients with GSTT1 present alleles are more liable to achieve complete remission than those with deletions of the gene. There was no association encountered between GSTM1 null genotype and risk of AML or CML.

Key Words: GST – AML – CML.

Introduction

MYELOID leukaemias are heterogenous diseases which are subdivided into acute and chronic myeloid leukaemias. Acute myeloid leukaemia is neoplastic proliferation in hematopoietic precursor cells, resulting in overgrowth of myeloblast and other immature myeloid cells. The malignant cells replace the bone marrow, circulate in the blood and may accumulate in other tissues, while chronic myeloid leukaemia is a malignancy of the hematopoietic stem cells characterized by the presence of Philadelphia chromosome and/or BCR-ABL fusion gene [1]. Environmental exposures to cytotoxic and genotoxic agents, particularly those derived from benzene and ionizing radiation, may be associated with increased risk of CML [2]. DNA damage in the hematopoietic cell is the essential prerequisite for the development of leukaemia and the body has developed a series of mechanisms in preventing such damages [3]. One such mechanism is the modification of reactive oxygen species generated either by environmental carcinogens or endogenously as a result of oxidative mechanism. Humans vary in their ability to detoxify intermediates, which in theory may explain differences in leukaemia risk as a result of exogenous exposure [4].

Xenobiotics are chemical substances that are foreign to the biological system. They include naturally occurring compounds, drugs, environmental agents and carcinogens. Adverse effects of
xenobiotics are exerted via covalent interactions between intermediate metabolites and genetic materials or proteins and their related metabolites. In order to avoid accumulation of lipophilic xenobiotics in cells and tissues, enzymatic reactions of xenobiotic metabolism that can be divided into two distinct phases are needed [8].

The key enzyme systems catalyzing phase I oxidative metabolism are enzymes of the cytochrome P450 (CYP) superfamily. Phase II enzymes catalyze the conjugation of glutathione or glucuronide with reactive electrophiles thus detoxifying procarcinogens and carcinogens. Glutathione S-transferases (GSTs) belong to the group of phase II enzymes. These are widely expressed in mammalian tissues and have a broad substrate specificity. GSTs are involved in the metabolism of a wide range of xenobiotics, including environmental carcinogens, chemotherapeutic agents and reactive species. Two widespread genetic polymorphisms that involve deletions in GSTT1 and GSTM1 have been reported to lead to loss of enzyme activity thus increasing the susceptibility to acute and chronic myeloid leukemia [6].

Numerous genetic polymorphisms have been reported for GST genes, indicating a lack of functional protein or causing either increased or reduced metabolic activity. These polymorphisms may alter the ability of enzymes to metabolize the chemical carcinogens and mutagens. It has been suggested that these differences in the ability to metabolize carcinogens and mutagens may influence the susceptibility of individuals to cancer. Thus, there may be an association between polymorphisms in genes encoding for xenobiotic-metabolizing enzymes and susceptibility to cancer. In humans GST group is known to be coded for by 16 genes in six known GST subfamilies, known as alpha (GSTA), mu (GSTM), omega (GSTO), pi (GSTP), theta (GSTT) and zeta (GSTZ) [6,7].

Human GSTs: GSTM1 and GSTT1 are two such genes whose products can modify endogenous and exogenous toxic substrates to less reactive species through a number of mechanisms that play a critical role in the system which protects against reactive oxygen species, the breakdown of peroxidized lipid and oxidized DNA. GSTs levels can be induced by exposure to foreign substances in vivo suggesting that they form part of an adaptive system to chemical stress. As a result these modified toxic materials are excreted from the body [8].

GSTM1 and GSTT1 are genetically deleted in a high percentage of the human population, with major ethnic differences [9]. Environmental exposure and genetic susceptibility play a role in the etiology of leukaemia. Benzene, ionizing radiation and cytotoxic therapy are some of the proposed causes of myeloid leukaemia through DNA damage in the hematopoietic precursor cells [10,11,12]. Individuals carrying less efficient alleles of detoxifying genes, vary in their ability to metabolize carcinogens and hence to detoxify chemicals, leading to different risk in getting cancer [13].

Chromosomal aberrations identified in AML are mostly loss of function and mutation in transcription factors that are required for normal hematopoietic development. However, these mutations are not sufficient to cause leukaemias and appear to be one hit in the multistep process of the disease. Recent studies indicate that activating mutations conferring proliferative and survival signals to the progenitor cells cooperate with the loss of function mutations in the transcription factors causing leukaemia which is characterized by uncontrolled proliferation and impaired differentiation. Most acute leukaemias appear to be the consequence of collaboration between mutations or gene rearrangements that confer a proliferative and/or survival advantage to haematopoietic cells and mutations that result in impaired differentiation and subsequent loss of apoptosis of cells [14,15].

In the present study we attempted to study null genotype frequency of GSTT1 and GSTM1 genes in myeloid leukaemias in order to evaluate their role in the susceptibility of myeloid leukaemias and their influence on treatment outcome.

Subjects and Methods

Our analysis included 50 newly diagnosed myeloid leukaemias patients: 25 AML {12 males (48%), and 13 females (52%)} with their age ranging between 15 and 61 years with a mean of 41.5±13.8; and 2 5 CML { 10 males (40%) and 15 females (60%)} with their age ranging between 17 and 73 years with a mean of 53.5±14.6, and 30 age and sex matched healthy controls. The patients were chosen from outpatient clinic of the new Kasr el Aini teaching hospital, Cairo University. Non of control cases had neither personal nor family history of malignancy.

All patients were subjected to:
1- Full medical history taking.
2- Thorough clinical examination.
3- Laboratory investigation including the following:
   • Complete blood picture.
• Bone marrow aspirate, with subsequent cytochemistry staining for proper FAB typing including myeloperoxidase, specific esterase and non specific esterase.

• Immunophenotyping for acute leukaemia by flow cytometry.

• Neutrophils alkaline phosphatase for chronic myeloid leukaemia.

• Philadelphia chromosome & cytogenetic analysis (BCR-ABL fusion gene) for chronic myeloid leukaemia diagnosis.

• Genotyping of glutathione-S-transferase T1 (GSTT1) and glutathione-S-transferase M1 (GSTM1) genetic polymorphism by multiplex PCR [16].

DNA isolation:
Venous blood samples (10 ml) were collected in EDTA containing vacutainers. Genomic DNA used for polymorphic analysis was extracted from white blood cells using DNA purification kit provided by *Promega corporation, catalogue number A1 120. Isolated DNA was stored at -20°C till use.

GSTT1 and GSTM1 polymorphism: [16,17]
GSTT1 and GSTM1 genes were genotyped using the multiplex PCR approach. The primers used for GSTT1 and GSTM1 amplification were:
F5’-TTC CTT ACT GGT CCT CAC ATC TC-3’, R5’-TCA CCG CCT GCT CAT CAT GGC CAG C-3’; R5’-GTT GGG CTC AAA TAT ACG GTG G-3’, respectively. The b-globin gene primer pairs were F5’-CAA CCT CAT CCA CGT TCA TCA-3’, R5’- GAA GAG CCA AGG ACA GGT GC-3’. PCR Master Mix was provided by Promega corporation, size: 100 reactions, catalogue number # 7502. In the thermocycling procedure, initial denaturation at 94°C for 4 minutes was followed by 35 cycles of 1 minute at 94°C, 45 seconds at 55°C, 1 minute at 72°C and final extension was 10 minutes at 72°C. Genotyping of the genes (null genotypes) is revealed by the absence of the 480 bp for GSTT1 and 219 bp for GSTM1 PCR products respectively, using the b-globin amplification (268 bp) as an internal positive control. PCR product for the genotyping of polymorphisms was visualized by 2% agarose gel electrophoresis with ethidium bromide. The absence of b-globin amplification indicated a failure of PCR reaction.

Statistics:
Data were described using the median, minimum, maximum and range or number of cases and percentage when appropriate. Comparison of quantitative variables between the study groups was done using Mann-Whitney test. Comparison of categorical variables was done using the Pearson chi-square test (X²) and the Fischer’s exact test. The relative risk of the association between risk factor and disease was measured by odds ratios (OR). OR among patients compared with controls was calculated by simple cross-tabulation, with 95% confidence intervals (CI). A probability value (p-value) <0.05 was considered statistically significant. All statistical calculation were done using computer SPSS statistical program version 10.0 (Statistical Package for the Social Science, SPSS Inc; Chicago, IL, USA).

Results
The 25 cases diagnosed as AML were 12 males and 13 females. Their age ranged between 15 and 61 years. As regards FAB typing, 8% (2 cases) of our patients were M0, 16% (4 cases) were M1, 20% (5 cases) were M2, 20% (5 cases) were M3, 12% (3 cases) were M4, 16% (4 cases) were M5 and 8% (2 cases) were M6. The 25 cases diagnosed as CML were 10 males and 15 females. Their age ranged between 17 and 73 years. Seventy two % (18 cases) of our patients were in the chronic phase and 28% (7 cases) were in the accelerated or crisis phases.

AML patients:
Statistical analysis of the laboratory data of AML patients was as follows:
A- The total leukocytic count ranged between 1.100/cm^3 and 198.000/cm^3 with mean count 44.296/cm^3.
B- Blast percentage ranged from 20% to 100% with mean percentage 69.08%.

Treatment criteria of AML patients:
Patients with AML were treated according to the seven and three regimen with combination chemotherapy consisting of: Cytosine arabinoside and Danrubcine. Patients with M3 were treated with All-transretinoic acid (ATRA).

Patients were followed up for 3 months and were evaluated for:
1- Toxic death: That was defined as death occurring after the start of treatment and before bone marrow evaluation on day +28.
2- Complete remission: That was defined as cellular marrow with less than 5% blast cells, neutrophils count ≥ 1.5 x 10^9/L, platelet count ≥ 10^11/L and no evidence of leukaemia in other sites.
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3- **Primary resistance:** >5% blasts or evidence of leukemia in other sites.

4- **Early relapse:** Relapse within 6 months of remission [18]. Twenty percent (5 cases) responded by complete remission after induction chemotherapy; 32% (8 cases) showed failure to induction chemotherapy in the form of, primary resistance and relapsed cases; 28% (7 cases) of patients were dead and 20% (5 cases) were missed.

**CML patients:**

Statistical analysis of the laboratory data of CML patients were as follows:

A- The total leukocytic count ranged between 17,000/cm\(^3\) and 328,000/cm\(^3\) with mean count 122.320/cm\(^3\).

B- Blast percentage ranged from 0% to 100% with mean percentage 10. 12%.

**Treatment criteria of CML patients:**

Patients with CML were all treated with hydroxyurea, zyloric, busulfan. A minority of patients were treated with Interferon-Alpha in addition to the above regimen. As regards response to induction chemotherapy: there was 24% (6 cases) responded by complete haematologic remission on induction chemotherapy; 48% (12 cases) showed failure to induction chemotherapy in the form of incomplete remission or resistant cases and 28% (7 cases) developed complications: accelerated phase or blastic crisis of CML.

Patients were followed up for 3 months and were evaluated for:

1- **Complete haematological remission:** Which was defined as the normalization of peripheral blood cell counts and differential counts and the disappearance of all signs and symptoms of CML.

2- **Chronic phase:** That was defined as blast cells are less than 10% in peripheral blood or in bone marrow, basophils count <20%, platelet count >100x10\(^9\)/L, normal splenic size and no additional genetic abnormalities.

3- **Accelerated phase:** That was defined as blast cells 10-19% in peripheral blood or in bone marrow, basophils count >20%, platelet count < 100 x 10\(^9\)/L, increasing splenic size unresponsive to therapy and additional genetic abnormalities that were not present at time of diagnosis; and.

4- **Blastic crisis:** That was defined by the WHO as blast cells are more than 20% in P.B or in B.M, extramedullary proliferation, or large foci or clusters of blasts in the B.M biopsy [19].

**Gene expression in GSTT1 & GSTM1 in normal individuals:**

There were 30 control individuals, age and sex matched and none of them had personal or family history of malignancy, 13 males and 17 females, their age ranged from 25-60 years. Genotypic analysis of GSTT1 was: 13.3%(4/30) cases were null genotype and 86.7% (26/30) cases were present; on the other hand, genotypic analysis of GSTM1 was: 36.7% (11/30) cases were null genotype and 63.3% (19/30) cases were present. 6.7% (2/30 cases) only showed double null genotype.

**Gene expression in GSTT1 & GSTM1 in AML patients:**

1- **Genotypic analysis of GSTT1:**
   - 52% (13/25 cases) were null genotype.
   - 48% (12/25 cases) were present.

2- **Genotypic analysis of GSTM1 was:**
   - 60% (15/25 cases) were null genotype.
   - 40% (10 cases) were present.
   - 3.44% (11/25 cases) showed double null genotype.

As regard age, sex, total leukocytic count, blast count and FAB subtyping, there was no statistical association between GSTT1, GSTM1 genotypes and the studied group of AML patients.

There was a significant association between patients achieving complete remission and GSTT1 genotype i.e. those with GSTT 1 present were more liable to complete remission (p-value 0.008), with no statistical significant association between follow-up cases and GSTM1 genotype (p-value 0.290) (Table 1).

A statistical significant difference was found with GSTT 1 null genotypes frequency in AML patients as compared to controls 13/25 (52%) versus 4/30 (13.3%): OR= 7.04 (1.9-26.1) it projects a 7.04 folds increased risk for AML in individual with GSTT1 null genotypes comparing to those possessing both allele of the gene. There was no statistical significant difference in the frequencies of the GSTM1 null genotypes between patients and control in AML patients (Table 2).

**Gene expression in GSTT1 & GSTM1 in CML patients:**

1- **Genotypic analysis of GSTT1:**
   - 40% (10/25 cases) were null genotype.
   - 60% (15/25 cases) were present.
2. Genotypic analysis of GSTM1:
   - 40% (10/25 cases) were null genotype.
   - 60% (15/25 cases) were present.
   - 3-16% (4/25 cases) showed double null genotype.

As regard age, sex, blast count, chronic/accelerated phase subtyping, and follow-up of patients achieving complete haematological remission there was no statistical association between GSTT1, GSTM1 genotypes and the studied group of CML patients.

There was a statistical significant difference in GSTT1 null genotypes frequency in CML patients as compared to controls 10/25 (40%) versus 4/30 (13.3%): Odds ratio (OR = 4.3 (1.15-16.2)) it projects a 4.3 folds increased risk of CML in individuals with GSTT1 null genotypes comparing to those possessing both alleles of the gene. On the other hand there was no significant difference in frequency of the GSTM1 null genotypes between patients and controls in CML patients (Table 3).

A representative example of Densitometer scan for GSTT1 and GSTM1 expression in AML, CML patients and control groups are illustrated in Fig. (1).

Table (1): Statistical analysis of follow-up in relation to GSTT1 & GSTM1 genotypes in AML patients.

<table>
<thead>
<tr>
<th></th>
<th>T-ve (n=20)</th>
<th>T+ve (n=20)</th>
<th>M-ve (n=20)</th>
<th>M+ve (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CR</td>
<td>0/11</td>
<td>5/9</td>
<td>2/13</td>
<td>3/7</td>
</tr>
<tr>
<td>Toxic death</td>
<td>11/11</td>
<td>4/9</td>
<td>11/13</td>
<td>4/7</td>
</tr>
<tr>
<td>p-value</td>
<td>0.008</td>
<td>0.290</td>
<td></td>
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</tr>
</tbody>
</table>

CR: complete remission.
Toxic death: on induction chemotherapy.
Data are presented as frequency (percentage %).

Table (2): Risk estimate in AML cases.

<table>
<thead>
<tr>
<th></th>
<th>AML (n=25)</th>
<th>Control (n=30)</th>
<th>p-value</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-ve</td>
<td>13/25 (52%)</td>
<td>4/30 (13.3%)</td>
<td>0.002</td>
<td>7.04</td>
</tr>
<tr>
<td>+ve</td>
<td>12/25 (48%)</td>
<td>26/30 (86.7%)</td>
<td></td>
<td>(1.9-26.1)</td>
</tr>
<tr>
<td>M:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-ve</td>
<td>15/25 (60%)</td>
<td>11/30 (36.7%)</td>
<td>0.084</td>
<td>2.591</td>
</tr>
<tr>
<td>+ve</td>
<td>10/25 (40%)</td>
<td>19/30 (63.3%)</td>
<td></td>
<td>(0.87-7.71)</td>
</tr>
</tbody>
</table>

OR: Odd’s ratio.

Discussion

Leukaemias probably result from complex interaction between multiple genetic and environmental factors over time. An understanding of the interplay of xenobiotic exposure, endogenous physiology, and genetic variability of multiple loci will facilitate knowledge about leukaemia aetiology and the identification of individuals who are at increased risk of developing leukaemias [16].

Human leukaemias are characterized by acquisition of mutations and chromosomal translocations. Chromosomal aberrations identified in AML are mostly loss of function due to mutation in transcription factors that are required for normal hematopoietic development. However, these mutations are not sufficient to cause leukaemias and appear to be one hit in the multistep process of the disease. Recent studies indicate that activating mutations that confer proliferative and survival signals to the progenitor cells cooperate with the loss of function mutations in the transcription factors leading to uncontrolled proliferation and
impairment. Most acute leukaemias appear to be the consequence of collaboration between mutations or gene rearrangements that confer a proliferative and/or survival advantage to haematopoietic cells and mutations that result in impaired differentiation and subsequent loss of apoptosis of cells [14,15]. In addition biological and epidemiological data implicate exogenous toxins, including cytotoxic drugs, benzene, radiation and cigarette smoking as contributing factors in aetiology of acute leukemia [20,21,22].

Acute myeloid leukaemia (AML) in adults has a 20% 5-years disease-free survival, despite treatment with aggressive cytotoxic chemotherapy. Recent studies on molecular characterization of specific defects in acute leukaemias have provided new avenues for targeted therapy. In AML patients, the occurrence of a sizeable number of deaths related to drug toxicity is an important limitation for the success of treatment [14,18,23].

Chronic myeloid leukaemia is a malignancy of the haematopoietic stem cell, Characterized by the presence of Philadelphia chromosome and/or BCR-ABL fusion gene. DNA damage in the haematopoietic precursor cell is the essential prerequisite for the development of leukaemia and the body has developed a series of mechanisms aimed at preventing such damage [16]. It is known that environmental exposure to cytotoxic and genotoxic agents particularly derived from benzene may be associated with increased risk of CML [24].

Glutathione-s-transferases (GSTs) are a family of multifunctional detoxifying enzymes that catalyze the conjugation of glutathione with large number of electrophilic xenobiotics, such as chemical carcinogens, environmental pollutants, and antitumor agents [2-25]. Glutathione-S-transferases are closely related with cancer as they are increased in blood and tissues of cancer patients. GSTs have been recognized as a tumor marker [26,27].

There are three mammalian GST families, namely cytosolic, mitochondrial, and microsomal GST. Cytosolic GST isoenzymes are broadly cytoprotective, where as microsomal proteins have pro inflammatory activities [25].

In year 2000, it was proved that the human cytosolic GST super family contains six distinct classes namely: known as alpha (GSTA), mu (GSTM), omega (GSTO), pi (GSTP), theta (GSTT) and zeta (GSTZ) based on their structural and functional properties with Mu being the most widely expressed [28,29]. Cytosolic human GST exhibit genetic polymorphisms and this variation can increase susceptibility to carcinogenesis and inflammatory diseases [25]. Knowledge of variation in frequency of GSTT1 and GSTM1 null genotypes in different populations may help to explain differential responses to toxic chemicals [30].

In this study, we aimed to define GSTT1 & GSTM1 genotypes in de-novo cases of (acute & chronic) myeloid leukaemias through a multiplex PCR technique and correlate this expression with the laboratory data and clinical outcome following induction chemotherapy.

Statistical significant difference was found with GSTT 1 null genotypes frequency in AML patients as compared to controls; where the former group had a higher level of null genotypes, thus possessing an increased risk of AML. However there was no difference in the frequencies of the GSTM1 null genotypes between AML patients and control group.

In AML cases, expression of GSTT 1 null genotypes was 52%, this frequency was higher than that reported in several studies, one in the Egyptian population By Abdel Rahman et al. [31] which was 14.4%, that reported by Vaso et al. [32] which was 28% and the one published by Cotton et al. [33] which was between (10%–2 1 %).

Our results are in accordance with Chen et al. [34]and Naeo et al. [35] who reported an increased risk of MDS and acute leukaemia associated with the GSTT1 null genotype. In contrast, Basu et al. [36], Preudhomme et al. [37] and Rollinson et al. [38] failed to identify an association between GST T1 null genotypes and AML.

On the other hand, no evidence of association could be detected in our study between GSTM1 null genotypes and the incidence of AML. The frequency of GSTM 1 null genotypes in our patients was 60% i.e. higher than that reported by Mossalam et al. [18] which was 56%, and the one reported by Vaso et al. [32] which was 42%. This frequency was also beyond previously reported in normal Egyptian population by Abdel Rahman et al. [31] which was 44%, and also above the published range of 42%–57% reported by Hasse et al. [39].

This difference between studies could indicate differences between populations in the influence of these genetic polymorphisms on genetic susceptibility to leukaemia or in exposures involved in leukaemogenesis [40].

As regard age, sex, total leukocytic count, blast count and FAB subtyping, there was no statistical
association between GSTT1, GSTM1 genotypes and the studied group of AML patients. On the other hand the study carried by Mossallim et al. \[18\] reported a negative correlation between GSTT 1 & GSTM1 null genotypes with age, sex, TLC and percentage of blasts in peripheral blood.

Another study by Vaso et al., reported an increased frequency of GSTM1 null genotypes in patients with AML over 60 years. Prolonged exposure of haematopoietic progenitor cells to toxic agents in combination with a reduced capability of detoxification might contribute to the pathogenesis in the elderly \[32\].

Davis et al. \[40\] reported a significantly increased frequency in GSTT 1 null genotype in FAB M3 and M4 subtypes. These data indicate that these subtypes may be particularly influenced by exposure to environmental agents detoxified by GSTT1.

In the current study, regarding the rate of early death during induction of chemotherapy due to drug toxicity there was a significant difference between GSTT 1 null compared to GSTT 1 present in AML patients, yet there wasn’t a significant difference between GSTM1 null and GSTM1 present in AML patients. Davis et al. \[40\] and Naoe et al. \[38\] reported an increased rate of early death in GSTT1 null compared to GSTT1 present group. On the other hand, Mossallam et al. \[18\] and the south west oncology group (SWOG) did not report such association in a group of AML patients \[41\]. This could be explained by the fact that GSTs enzymes play an important role in the detoxification of chemotherapeutics. GSTT1 & GSTM1 enzyme polymorphism in the form of deletion of either or both genes affect pharmacodynamic of chemotherapeutics used in treatment of leukemia. The importance of genetic polymorphism in drug metabolism may differ based on the nature and intensity of the treatment regimen. When drugs are being dosed at levels that are near those that produce toxicity, the inheritance of deficiency of an enzyme involved in the detoxification of such drug can lead to a worse outcome because of greater toxicity \[42\].

In the current work, the rate of complete remission in AML patients did not differ significantly in groups with and without deletion in GSTM1, yet a statistical significant difference was found between GSTT 1 present and null genotype, where GSTT 1 present are more liable to achieve complete remission \(p\)-value: 0.008). On the other hand, Davies et al. \[40\], Naoe et al. \[38\], Mossallam et al. \[18\] reported no difference in complete remission between GSTT1 null and GSTT1 present genotypes in adult AML patients. In contrast, Vaso et al. \[32\] indicated that AML patients with deletion of M 1 or T 1 or both had a lower probability to achieve complete remission on induction therapy as compared with intact GST genes.

Statistical significant difference was found with GSTT 1 null genotypes frequency in CML patients as compared to controls; where the former group had a higher level of null genotypes, thus possessing an increased risk of CML. However there was no difference in the frequencies of the GSTM1 null genotypes between patients with CML and control group.

Expression of GSTT1 null genotypes was (40%), which is lower than that described in Taspinar et al. \[2\] that was (40.2%), but higher than Bajpai et al. \[16\] in adult CML which was (20%). On the other hand, no evidence of association could be detected in our study between GSTM1 null genotypes and the incidence of CML. The frequency of GSTM 1 null genotypes in our patients was (40%), which is higher than (30%) reported by Bajpai et al. \[16\], but lower than (44.9%) reported in CML cases by Taspinar et al. \[2\].

Our results are in accordance with Mondal et al. \[43\] and Bajpai et al. \[16\] who reported an increased risk of chronic leukaemia associated with the GSTT 1 null genotype. In contrast Loffler et al. \[44\] failed to find an association between GSTT 1 null genotypes and incidence of CML. The difference between our results and those of Loffler et al. \[44\] might be attributed to polymorphism of the gene and ethnic differences, Loffler also suggested that genetic susceptibility and environmental effects might be additive in CML.

As regard age, sex, blast count, chronic/accelerated phase subtyping, and follow-up there was no statistical association between GSTT 1, GSTM 1 genotypes and the studied group of CML patients.

In our study, the rate of complete remission in CML patients did not differ significantly in groups with and without deletions in GSTT1 and GSTM1, this was in accordance with Mondal et al. \[43\], Bajpai et al. \[16\] and Taspinar et al. \[2\].

In conclusion our results revealed a strong association between GSTT1 null genotypes and risk of AML and CML, with a valuable influence on the fate of AML patients where patients with GSTT1 present alleles are more liable to achieve complete remission than those with deletions of
the gene. There was no association between GSTM1 null genotype and risk of AML and CML.

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