Journal of the Egyptian National Cancer Institute 29 (2017) 127–133

Contents lists available at ScienceDirect

Journal of the Egyptian National Cancer Institute

journal homepage: www.sciencedirect.com

Full length article

Impact of CYP1A1, GSTP1 and XRCC1 genes polymorphisms on toxicity and response to chemotherapy in childhood acute lymphoblastic leukemia

Asmaa Abo-Bakr [a], Ghada Mossallam [a,*], Nevin El Azhary [a], Hanafy Hafez [b], Ragia Badawy [a]

[a] Department of Clinical Pathology, National Cancer Institute, Cairo University, Egypt
[b] Department of Pediatric Oncology, National Cancer Institute, Cairo University, Egypt

ARTICLE INFO

Article history:
Received 31 March 2017
Received in revised form 21 July 2017
Accepted 23 July 2017
Available online 24 August 2017

Keywords:
ALL
CYP1A1
GSTP1
XRCC1
Toxicity

ABSTRACT

Background: Acute lymphoblastic leukemia (ALL) is the most common childhood malignancy. The interindividual genetic variations in drug metabolizing enzymes and DNA repair genes influence the efficacy and toxicity of numerous chemotherapeutic drugs affecting the treatment outcome.
Aim of the work: The aim of the study was to investigate the impact of drug metabolizing CYP1, GSTP1 and DNA repair (XRCC1) genes polymorphisms on the toxicity and response to chemotherapy in childhood ALL.
Patients and methodology: Ninety seven ALL pediatric patients were genotyped for CYP1A1, GSTP1 ILe105 Val and XRCC1 Arg194Tryp single nucleotide polymorphisms (SNPs) using PCR-RFLP.
Results: No statistically significant differences were observed between the wild and variant (homozygous and heterozygous) genotypes of the polymorphisms studied in CYP1A1, GSTP1 or XRCC1 genes regarding age, total leukocyte count, immunophenotyping, cytogenetic or risk group. The SNPs in CYP1A1, GSTP1 and XRCC1 genes did not show significant association with complete remission (CR) rate, overall survival (OS) or event free survival (EFS). However, XRCC1 Arg194Trp SNP was associated with higher drug toxicity; carriers of variant genotypes (CT and TT) had a significantly higher frequency of myelosuppression compared to those with the wild CC genotype (21/43[48.8%]) compared to (14/54[25.9%]) ($p = 0.020$). The analysis of the combined effect of studied SNPs did not show any significant association with patient outcome.
Conclusion: Our study reported a significant association between the DNA repair gene polymorphism and myelosuppression in childhood ALL patients. Adjustment of the dose of chemotherapeutic agents according to XRCC1 Arg194Trp polymorphism may improve outcome in cases with risk of toxicity.

Introduction

Acute lymphoblastic leukemia (ALL) is the most common childhood malignancy, constituting about 25–30% of all childhood malignancies [1,2]. During the last decades, there has been a marked increase in survival rates for childhood leukemia. Recently, nearly 80% of the children with ALL are expected to have a long-term event free survival (EFS) and an overall survival (OS) close to 90%. This dramatic increase in survival is largely attributed to better risk stratification using prognostic markers that guide the intensity of the treatment protocol [3].

The inter-individual variation in activities of enzymes involved in xenobiotic metabolism pathways showed that they play role in the susceptibility to childhood ALL and in the processing of chemotherapeutics used in therapy influencing the treatment outcome [4,5]. Phase I xenobiotic metabolizing enzymes, such as cytochromes and of phase II detoxifying enzymes such as glutathione-s-transferases (GSTs) have variant alleles associated with substrate-dependent change in their enzymatic activity [5,6]. DNA repair systems are important to correct carcinogens or anticancer drugs induced DNA damage. Changes in the efficiency of repair may affect both cancer risk and responsiveness of cancer cells to chemotherapeutics. Base excision repair (BER), mismatch repair, and nucleotide excision repair mechanisms, including poly-

Peer review under responsibility of The National Cancer Institute, Cairo University.
* Corresponding author at: National Cancer Institute, Cairo University, Fom El-Khalig Square, Kasr El-Aini St., 11796 Cairo, Egypt.
E-mail address: ghadamossallam@hotmail.com (G. Mossallam).

http://dx.doi.org/10.1016/j.jnci.2017.07.002
1110-0362/

morphisms in the coding sequences of XRCC1 were shown to contribute to drug resistance [7].

Various studies reported the association of polymorphisms in genes involved in drug metabolism and DNA repair with increased risk of ALL [8–10]. However, few studies are available concerning their impact on clinical outcome. Krajinovic et al. [11], observed poor outcome with CYP1A1 polymorphism, while GSTP1 105 Val allele was associated with reduced risk of relapse in one study and worse prognosis in the other [5,12]. The aim of our study was to investigate the impact of CYP1A1, GSTP1 and DNA repair (XRCC1) genes polymorphism on toxicity and response to chemotherapy in childhood ALL.

Patients and methods

Patients

This prospective study included 97 consecutive pediatric ALL patients who presented to the Department of Pediatric Oncology, National Cancer Institute, Cairo University, from January 2012 to November 2013. Patients were categorized according to ST Jude Children's Research Hospital (SJCRH) risk stratification system into low, standard and high risk groups [13]. Written consent was obtained from the parents and the protocol was approved by the Institutional Review Board. All patients were monitored regularly in the pediatric oncology outpatient clinics and treated with the ST Jude Children's Research Hospital (SJCRH) total XV protocol, which consisted of induction using (prednisone, vincristine (VCR), Doxorubicin, L-asparaginase, Cyclophosphamide, Cytarabine and 6-Mercaptopurine), consolidation, intensification (for high risk only) and maintenance phases.

Complete remission was defined by <5% leukemic blasts in the marrow with restoration of normal hematopoiesis. Immunologic remission defined as leukemic involvement of <0.01% of nucleated bone marrow cells was evaluated at day 14 after induction therapy. Assessment of toxicities of the different chemotherapeutic agents used for these patients included myelosupression defined by repeated leucopenia and neutropenia that required cessation or modification of chemotherapy doses. Vincristine induced neurotoxicity (foot drop, illeus, vocal cord paralysis and ptosis) and cerebrovascular adverse events (superior sagittal sinus or transverse sinus thrombosis, assessed by MRI/MRV of the brain) were done. The individual incidents of various toxicity of the chemotherapeutic agents within this protocol were graded according to National Cancer Institute guidelines [14].

Methods

Genomic DNA was extracted from EDTA peripheral blood samples using the salting out technique [15]. Identification of gene polymorphism was performed using PCR-RFLP.

CYP1A1 (T6235C) genotyping

PCR was performed using Dream Taq Green PCR Master Mix (ThermoScientific, Fermentas). The reaction for CYP was performed in 25 µl reaction containing 100 ng of genomic DNA, 1x master mix, 25 pmol of each primer F: 5′-GGCTGAGCAATCTGACCCTA-3′ and R: 5′-TAGGAGTCTTGTCTCATGCCT-3′ [16]. The cyclic condition consisted of initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 63 °C for 1 min and extension at 72 °C for 1 min. Final elongation at 72 °C for 10 min. Amplification resulted in 899 bp fragment, each PCR (10 µl) product was subjected to *Msp*1 (SibEnzyme) digestion and analyzed by gel electrophoresis (2%). The presence of the polymorphic *Msp*1 restriction site yields 693 and 206 bp in case of variant C allele (CYP1A1*2A).

GSTP1 Ile105Val genotyping

Primer sequences were: F5′- CCAGTGACTGTGTGTTGATC-3′ and R: 5′-CAACCCTGGTGCAGATGCTC-3′ [17]. The cyclic condition consisted of initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 62 °C for 30 s and extension at 72 °C for 30 s Final elongation at 72 °C for 7 min. Amplification resulted in a 189-bp fragment, each PCR product was subjected to BstMAI (SibEnzyme) digestion and analyzed by gel electrophoresis (3%). The presence of the polymorphic BstMAI restriction site yields 148-bp and 41-bp fragments, indicating the presence of the G allele (GSTP1 Val/Val).

XRCC1 (Arg194 Tryp) genotyping

The XRCC1 (Arg194 Tryp) genotype was analyzed according to Lee et al. [18], primer sequences were: 5′GTTCCGTGTGAAGGAGG AGGA-3′ and R5′-CGAGTCTAGGTCTCAACCCTACTCACT-3′. The cyclic condition consisted of initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 62 °C for 30 s and extension at 72 °C for 30 s Final elongation at 72 °C for 7 min. Amplification resulted in 138 bp fragment. Ten microliters of the PCR products were digested separately with 10 units of Pvu II (for codon 194) and analyzed by gel electrophoresis (2%). The presence of the polymorphic Pvu II (New England Biolabs) restriction site yields: 75 bp, 63 bp in case of variant T allele.

Statistical methods

Data was analyzed using IBM SPSS advanced statistics version 22 (SPSS Inc., Chicago, IL). Numerical data were expressed as mean and standard deviation or median and range as appropriate. Chi-square (Fisher's exact) test was used to examine the relation between qualitative variables. Survival analysis was done using Kaplan-Meier method and comparison between two survival curves was done using log-rank test. All tests were two-tailed. A p-value < 0.05 was considered significant.

Results

The present study included 97 patients with median age of 5 years (range from 1 to 18 years) and male to female ratio was1.4:1. The patients' characteristics and clinical prognostic fac-

Table 1
The patients' Characteristics.

Parameter		Number	Percentage (%)
Sex	Male	57	58.8
	Female	40	41.2
Age (year)	<1 year	1	1
	1–10 years	76	78.4
	>10 years	20	20.6
Organomegaly	Hepatomegaly	67	69.0
	Splenomegaly	58	59.7
	Lymphadenopathy	17	17.5
TLC ($\times 10^3$/ul)*	19.6 (2.0–622.8)		
Cytogenetics	Good	13	14.1
	Intermediate	74	80.4
	Poor	5	5.4
IPT	B-cell	76	78.4
	T-cell	21	21.6
Risk group	Low risk	38	41.3
	Standard risk	49	53.2
	High risk	5	0.05

* Median (range).

tors were evaluated as shown in Table 1. Immunophenotypic classification of ALL patients showed that 78.4% of patients were B-ALL and 21.6% were T-ALL (Table 1). Among the B-ALL cases, pre B-ALL was the commonest subtype (56.7%) followed by the common-ALL (20.6%) and Pro B-ALL (1.0%). Cytogenetic analysis was carried out on 92 patients in this study and we found that 69/92 (75.0%) of patients had normal karyotype, while 23(25%) had cytogenetic abnormalities in the form of hyperdiploid in 3 (3.3%), t(12,21) in 10patients (11.0%), t(1,19) in 5 (5.4%) and t(9,22) in 5 patients (5.4%). Cytogenetic abnormalities associated with good prognosis includes t(12,21) and hyperdiploidy, intermediate group includes patients with normal karyotype and t(1,19) while cytogenetic abnormalities associated with poor prognosis includes t(9,22). According to SJCH risk criteria (13), 49 patients (53%) were stratified as low risk, 38 (41%) were standard risk and 5 cases were high risk (Table 1).

Single gene polymorphism (SNP) analysis

Frequency of SNPs

CYP1A1(T6235C) SNP analysis showed that the wild TT genotype was present in 64/97 patients (66.0%), while the variant genotypes T/C and CC were present in 31/97 patients (31.9%) and 2/97 patients (2.1%), respectively. GSTP1 ILe105Val SNP analysis detected the Ile105 (AA) genotype in 49/97 patients (50.5%), while the variant genotypes, (Ile105/Val105) (A/G) and Val105/Val105 (GG) were present in 45/97 patients (46.4%) and 3/97 patients (3.1%), respectively. For XRCC1 Arg194 Tryp, the wild CC genotype was present in 54/97 patients (55.7%), while the variant genotypes C/T and TT were present in 41/97 patients (42.2%) and 2/97 patients (2.1%), respectively.

Association of SNPs with other characteristics and prognostic factors

No statistical significant difference was observed between the wild and variant (homozygous and heterozygous) genotypes of the studied polymorphism in CYP1A1, GSTP1 or XRCC1 genes regarding age, total leucocytic count, IPT, cytogenetics and risk group (Table 2).

Association of SNPs with clinical outcome

SNPs and response to induction therapy at day 14. Nine patients lost follow-up or died before evaluation at day 14. Eighty-two/88 patients (93.1%) achieved morphological remission. Difference in CR rate between patients carrying wild type and those carrying variant genotypes was not significant for each studied SNP as shown in Table 3.

SNPs and minimal residual disease (MRD) at day 14. Minimal residual disease analysis at day 14 was available for 84 patients. MRD $\leq$ 0.01% was achieved in 73/84 patients (86.9%). No significant difference was observed in rate of immunological remission in patients carrying wild genotype compared to those carrying variant genotypes for the three studied SNPs as shown in Table 3.

SNPs and toxicity. Toxicities to chemotherapy were grouped into myelosuppression, VCR induced neurotoxicity and cerebrovascular thrombosis. Of the 97 patients evaluated for toxicity of induction chemotherapy, myelosuppression was found in 35/97 patients (36.0%), vincristine induced foot drop was found in 4/97 patients (4.1%), while cerebrovascular thrombosis in the form of superior sagittal sinus and or transverse sinus thrombosis, was found in 9/97 patients (9.0%).

Carriers of XRCC1 variant genotypes (CT and TT) had a significantly higher frequency of myelosuppression [21/43 (48.8%)] compared to the wild CC genotype [14/54 (25.9%)] (p = 0.020) (Table 4). An equal distribution between low and standard risks was observed in patients with myelosuppression (11 and 10 patients, respectively) and in patients without myelosuppression (7 patients in each category). While there were no significant associations between different CYP1A1 and GSTP1 genotypes and any of these toxicities as shown in Table 4. Other types of toxicities were not analyzed due to lack of sufficient data.

SNPs and survival. The follow-up period was ranging between 3 and 45.6 months with a median of 18.9 months. The causes of death varied between sepsis, chest infection and renal failure in one patient.

Disease outcome was assessed by estimating overall survival (OS) and event free survival (EFS) probabilities for patients with and without the variant genotypes.

For CYP1A1, the cumulative OS at 2 years was 78% in patients with TT genotype compared to 77.4% in patients with variant genotypes TC + CC, the difference was not statistically significant (p = 0.9) (Fig. 1). The cumulative EFS at 2 years was 76% in patients with TT genotypes compared to 77.4% in patients with variant genotypes TC + CC, the difference was not statistically significant (p = 0.98) (Fig. 2).

Table 2
Association of CYP1A1, GSTP1 and XRCC1 SNPs with prognostic factors in childhood ALL patients.

Parameter	Genotype N (%) CYP1A1		P value	Genotype N (%) GSTP1		P value	Genotype N (%) XRCC1		P value
	TT	TC + CC		AA	AG + GG		CC	CT + TT	
Age									
<1+> 10	14/21 (66.7)	7/21 (33.3)		9 (42.9)	12 (57.1)		9 (42.9)	12 (57.1)	
1–10 year	50/76 (65.8)	26/76 (34.2)	0.940	40 (52.6)	36 (47.4)	0.428	45 (59.2)	31 (40.8)	0.182
TLC									
<50	44/65 (67.7)	21/65 (32.3)	0.612	32 (49.2)	33 (50.8)	0.718	3 (55.4)	29 (44.6)	0.936
≥50	20/32 (62.5)	12/32 (37.5)		17 (53.1)	15 (46.9)		18 (56.3)	14 (43.8)	
IPT									
B-cell	49/76 (64.5)	27/76 (35.5)	0.552	39 (51.3)	37 (48.7)		43 (56.6)	33 (43.4)	0.732
T-cell	15/21 (71.4)	6/21 (28.6)		10 (47.6)	11 (52.4)	0.764	11 (52.4)	10 (47.6)	
Cytogenetics									
Good	8/13 (61.5)	5/13 (38.5)		6/(46.2)	7 (53.8)		8 (61.5)	5 (38.5)	0.574
Intermediate and Poor	52/79 (65.8)	27/79 (34.1)	0.763	42 (53.1)	37 (46.8)	0.632	42 (53.1)	37 (46.8)	
Risk group									
Low risk	24/38 (63.2)	14/38 (36.8)		20 (52.6)	18 (47.6)		22 (57.9)	16 (42.1)	
Standard and High risk	36/54 (66.6)	18/54 (33.3)	0.727	28 (51.8)	26 (48.1)	0.941	28 (51.8)	26 (48.1)	0.566

Table 3
Association of CYP1A1, GSTP1 and XRCC1 SNPs with clinical outcome in childhood ALL patients.

	CR at day14	p value	MRD day + 14 (positive > 0.01)	p value
CYP1A1				
TT	56/64 (87.5%)	0.391	7/64 (10.9%)	0.819
TC + CC	26/33 (78.7%)		4/33 (12.1%)	
GSTP1				
AA	43/49 (87.7%)	1.000	8/49 (16.3%)	0.147
AG + GG	39/48 (81.2%)		3/48 (6.2%)	
XRCC1				
XRCC1CC	45/54 (83.3%)	1.000	6/54 (11.1%)	0.877
CT + TT	37/43 (86%)		5/43 (11.6%)	

Table 4
Association of CYP1A1, GSTP1 and XRCC1 SNPs with toxicity in childhood ALL patients.

	Myelo-suppression	p value	CNS toxicity	p value	Thrombosis	p value
CYP1						
TT	24/64 (37.5%)	0.686	3/64 (4.6%)	*	5/64 (7.8%)	0.488
TC + CC	11/33 (33.3%)		1/33 (3.0%)		4/33 (12.1%)	
GSTP1						
AA	19/49 (38.7%)	0.577	2/49 (4.0%)	*	5/49 (10.2%)	1.000
AG + GG	16/48 (33.3%)		2/48 (4.1%)		4/48 (8.3%)	
XRCC1						
CC	14/54 (25.9%)	0.020	2/54 (3.7%)	*	5/54 (9.2%)	0.994
CT + TT	21/43 (48.8%)		2/43 (4.6%)		4/43 (2.3%)	

* No p value because of small number of cases within subgroups.

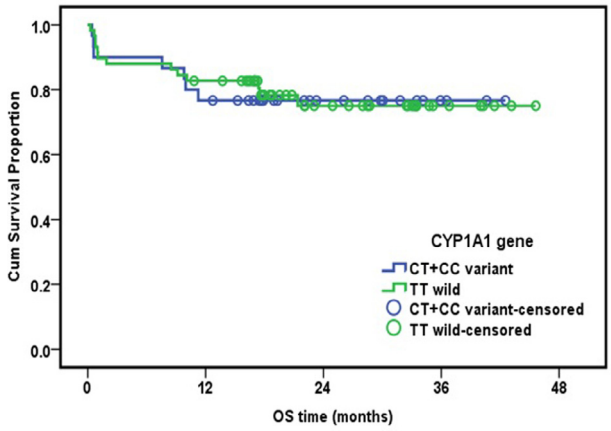

Fig. 1. Overall survival of ALL patients according to CYP1A1 polymorphism.

The cumulative OS at 2 years was 79.2% in patients with GSTP1 AA genotype compared to 72.3% in patients with variant genotypes AG + GG, the difference was not statistically significant (p = 0.597) (Fig. 3). The cumulative event free survival (EFS) at 2 years was 76.7% in patients with AA genotypes compared to 72.7% in patients with variant genotypes AG + GG, the difference was not statistically significant (p = 0.747) (Fig. 4).

The cumulative OS at 2 years was 74% in patients with XRCC1 CC genotype compared to 82.9% in patients with variant genotypes CT + TT, the difference was not statistically significant (p = 0.263) (Fig. 5). The cumulative EFS at 2 years was 72% in patients with CC genotype compared to 82.9% in patients with variant genoytpes CT + TT, the difference was not statistically significant (p = 0.199) (Fig. 6).

Combined gene polymorphisms analysis

Combined analysis of drug metabolizing enzymes genes (CYP1A1 and GSTP1genes), drug metabolizing and DNA repair

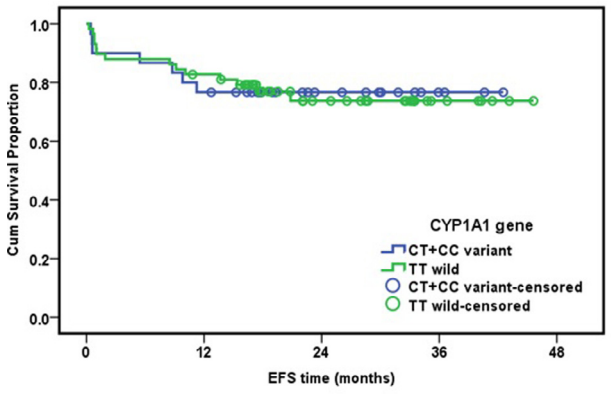

Fig. 2. Event-free survival of ALL patients according to CYP1A1 polymorphism.

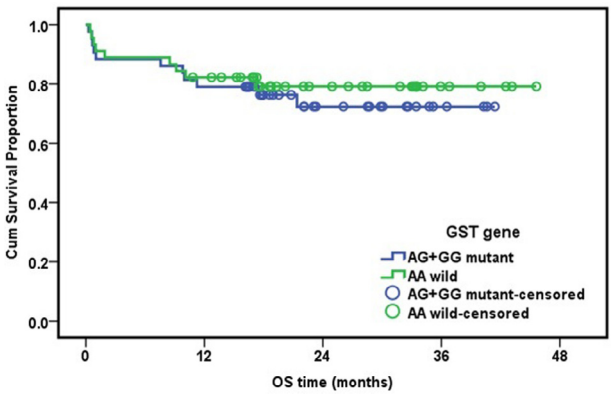

Fig. 3. Overall survival of ALL patients according to GSTP1 Ile105Val polymorphism.

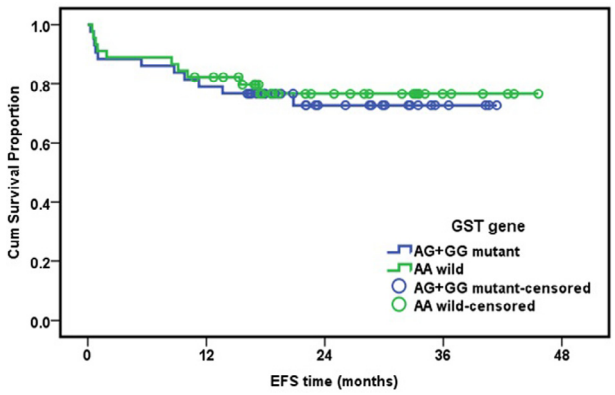

Fig. 4. Event-free survival of ALL patients according to GSTP1 Ile105Val polymorphism.

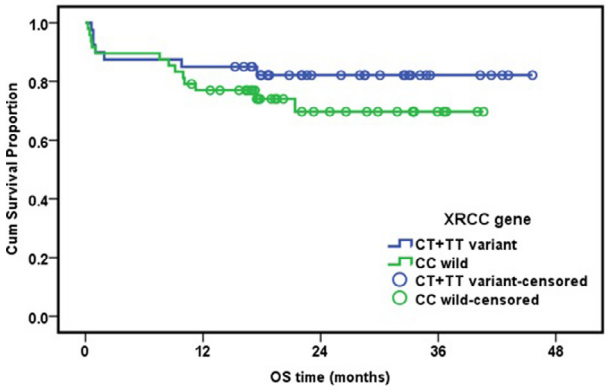

Fig. 5. Overall survival of ALL patients according to XRCC1 (Arg194 Tryp) polymorphism.

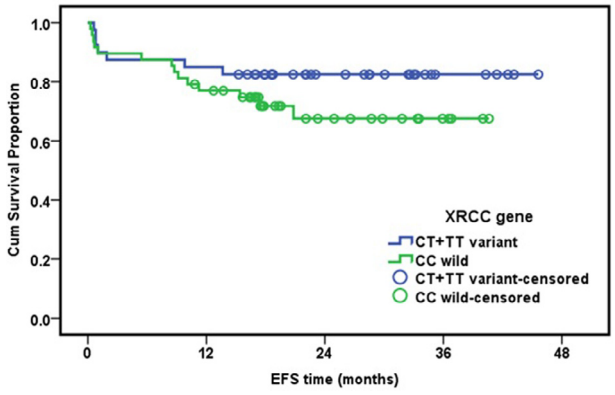

Fig. 6. Event-free survival of ALL patients according to XRCC1 (Arg194 Tryp) polymorphism.

genes (CYP1A1 and XRCC1) or (GSTP1 and XRCC1); showed no statistical significant difference between both or either variant compared to their wild genotypes regarding other prognostic factors, clinical outcome or survival (data not shown). A near significant association was observed between combined GSTP1 and XRCC1 SNPs and MRD at day 14, where patients carrying the variant genotypes of either or both genes had a higher frequency of MRD on day 14 [7/84 (8.3%)] compared to those carrying wild genotypes [4/84 (4.7%)] with p = 0.086. However, this association did not affect EFS with p = 0.53.

Discussion

The interindividual genetic variations in drug metabolizing enzymes and DNA repair genes influence the efficacy and toxicity of numerous drugs used in the treatment of malignancy influencing the treatment outcome. Of the drug metabolizing enzymes, CYP1A1 participates in the activation and inactivation of chemotherapeutics. Our studied polymorphism is known to be associated with elevated enzymatic activity [19]. The frequency of wild TT genotype was 64 / 97 patients (66%) in our series, while the variant genotypes T/C and CC were present in 31/97 (31.9%) and 2/97 patients (2.1%), respectively. Krajinovic et al. [20] in a study involving French–Canadian pediatric ALL patients, found TT genotype in (80.6%) of their patients, while T/C and CC genotypes were present in (18.8%) and (0.6%), respectively. While Suneetha et al. [5] in a study on Indian ALL children found the wild TT genotype in only 42.6%, while T/C and CC genotypes were present in (47.7%) and (9.9%), respectively. The observed variation in frequency may be due ethnic variation.

Our results did not show significant association between the studied CYP1A1 polymorphism and other prognostic factors. It did not affect the response to therapy or the patients' survival. Similar to our results, Suneetha et al. [5] reported that this polymorphism did not affect the outcome in their study on Indian ALL children. In contrast to our results, Krajinovic et al. [11] have reported that children with CYP1A1 variant genotype had worse therapeutic outcome and shorter survival probabilities. It was claimed that enhanced enzyme activity could promote activation and thus toxicity of CYP1A1 substrates if an individual is exposed to such procarcinogens. CYP1A1 variant genotype was reported to be associated with increased risk of leukemia [8].Because this polymorphism may affect drug activity; it would be valuable to analyze its impact on drug toxicity. In our study, no significant association was observed between CYP1A1 polymorphism and toxicity to chemotherapeutics.

The second studied drug metabolizing enzyme, GSTP1 is involved in the metabolism of a wide range of chemicals including environmental carcinogens and anticancer drugs. GSTP1ILe105Val SNP is associated with reduced enzymatic activity for certain substrates. The frequency of the wild AA genotype was 49 / 97 patients (50.5%), while the variant genotypes, AG andGG were present in 45/97 patients (46.4%) and 3/97 patients (3.1%), respectively. In the study conducted by Stanulla et al. [12] the wild genotype was present in (50.8%), while the variant genotypes, AG and GG were present in 38.3% and10.9%, respectively.

In our study GSTP1ILe105Val SNP was not found to be associated with other prognostic factors or the outcome of patients. Similar to our results, Krajinovic et al. [11] did not observe any association between GST genotypes and ALL. Stanulla et al. [12], studied the association between polymorphisms within glutathione S-transferase genes (GSTM1, GSTT1 and GSTP1ILe105Val) and risk of relapse in childhood B-cell precursor ALL and reported a decreased risk of CNS relapse with Val105/Val105 genotype compared to the combined of Ile105/Val105 and Ile105/Ile105 genotypes. In contrast, in another study on Indian ALL children, GSTP1 (Val allele) either in heterozygous or homozygous condition was associated with significant poor outcome [5]. High glutathione levels in blasts were found to have significant resistance to vincristine and ifosfamide [5]. While there are limited number of studies discussing the relation between GSTP1 polymorphism and ALL outcomes, there are several studies concerning the role of the GSTP1Ile105Val polymorphism in susceptibility to risk of developing childhood AL. Suneetha et al. [21] found no significant risk associated with the GSTP1 gene for the development of ALL. Similar results were obtained by Ye Z and Song H [22].

The third studied gene XRCC1 is one of the most important genes involved in DNA repair, specifically in the base excision repair (BER) pathway and in single-strand break repair activity. It facilitates the repair of the damaged bases produced by endogenous or exogenous factors. In our studied patients, XRCC1 Arg194Trp wild CC genotype was present in 54/97 patients (55.7%), while the variant genotypes CT and TT were present in 41/97 patients (42.2%) and 2/97 patients (2.1%), respectively. Batar et al. [10], have found the CC genotype in (74.2%), while the variant genotypes CT and TT were present in (22.2%) and (3.6%) of the patients, respectively.

We could not find a significant association between this polymorphism and other prognostic factors or clinical outcome in our ALL patients. However, a significant association was observed between the XRCC1 variant allele and the chemotherapeutic toxicity in our patients in form of myelosuppression (p-value = 0.02). Although little attention has been paid to the relationship between XRCC1 polymorphism and ALL outcomes, several studies were concerned with XRCC1 polymorphism in solid tumors and other hematological diseases such as acute myeloid leukemia (AML). Chemotherapeutic drugs including alkylating agents and antimetabolites are able to induce DNA strand breaks. Polymorphisms of DNA repair genes including XRCC1 and XPD were related with toxicity of platinum-based chemotherapy in lung cancer patients treated with the alkylating platinum-based chemotherapy, where higher risk of hematologic toxicity was reported in patients with XRCC1 399 variant allele [23]. In contrast, Kuptsova et al. [24] reported associations between reduced toxicities and XPD and XRCC3 polymorphisms in AML patients and significantly shorter survival in those harboring XRCC1 variant allele compared to the wild type. DNA repair polymorphisms have been proved to be associated with variation in rates of DNA repair and genotoxic damage [25]. Functional DNA repair capability was previously observed to be significantly defective in those carrying XRCC1 399Gln, XRCC3 241Met and XPD 312Asn, 751Gln variant alleles [26]. In our patients, possible defect in DNA repair associated with variant allele may explain its association with marrow toxicity in the form of myelossuppression.

Allelic polymorphism of a single gene may not be enough to clarify its effect in xenobiotic metabolism. Several studies showed that combined polymorphism in CYPs and GSTs genes was associated with increased risk for several types of cancers and influenced treatment outcome [27]. An effect that may be related to their combined effect on the formation of DNA adducts in human white blood cells [11].In our study, combined analysis of GSTP1 and CYP1A1 polymorphisms did not show prognostic or clinical difference between ALL patients carrying both or either variant genotypes and those carrying wild genotypes. Similarly, study of combined GSTP1 and XRCC1 polymorphisms or CYP1A1 and XRCC1 polymorphisms did not show significant association in terms of prognosis or clinical outcome. A border line significant association was observed in response to treatment between patients carrying combined GSTP1 and XRCC1 variant genotypes compared to those carrying wild genotypes, where variant alleles were associated with trend to MRD positivity at day 14 (p = 0.086), this trend, however, did not impact the EFS. Although analysis of either gene was not associated with significant clinical outcome, decreasing the cells sensitivity towards chemotherapeutic drugs associated with increasing levels of GSH in the cells resulting from reduced activity of GSTP1 together with diminished DNA repair efficiency associated with XRCC1 variant allele may result in resistance to cytotoxic drugs. In line with our findings, one study reported reduced EFS in association with the lower activity of GSTP1, while the other noted resistance to cytotoxic drugs, with reduced DNA mismatch repair efficiency [5,11].

To conclude, our study reported a significant association between the DNA repair gene polymorphism and toxicity in childhood ALL patients. Adjustment of the dose of chemotherapeutic agents according to XRCC1 Arg194Trp polymorphism may improve outcome in cases with risk of toxicity. The study of other polymorphisms that may have possible synergistic effect with XRCC1Arg194Trp is warranted.

Conflict of interest

The authors report no conflict of interest.

References

[1] Coebergh JW, Reedijk AM, de Vries E, Martos C, Jakab Z, Steliarova-Foucher E, et al. Leukemia incidence and survival in children and adolescents in Europe during 1978–1997. Report from the Automated Childhood Cancer Information System project. Eur J Cancer 2006;42(13):2019–36.
[2] Esparza SD, Sakamoto KM. Topics in pediatric leukemia-acute lymphoblastic leukemia. MedGenMed 2005 Mar 7;7(1):23.
[3] Schmiegelow K, Forestier E, Hellebostad M, Heyman M. Kristinsson J, Soderhall S. Long term results of NOPHO ALL-92 and ALL-2000 studies of childhood acute lymphoblastic leukemia. Leukemia 2010;24(2):345–54.
[4] Sinnett D, Krajinovic J, Labuda D. Genetic susceptibility to childhood acute lymphoblastic leukemia. Leuk Lymphoma 2000;38:447–62.
[5] Suneetha KJ, Nancy KN, Rajalekshmy KR, Rama R, Sagar TG, Rajkumar T. Role of glutathione-s-transferase and CYP1A1*2 A polymorphisms in the therapy outcome of south Indian acute lymphoblastic leukemia patients. Indian J Med Paediatr Oncol 2011 Jan–Mar;32(1):25–9.
[6] Board PG, Menon D. Glutathione transferases, regulators of cellular metabolism and physiology. Biochim Biophys Acta 2013;1830(5):3267–88.
[7] Chou WC, Wang HC, Wong FH, Ding SL, Wu PE, Shieh SY, et al. Chk2-dependent phosphorylation of XRCC1 in the DNA damage response promotes base excision repair. EMBO J 2008;27:3140–50.
[8] Swinney RM, Beuten J, Collier AB, Chen TT, Winick NJ, Pollock BH, et al. Polymorphisms in CYP1A1 and ethnic-specific susceptibility to acute lymphoblastic leukemia in children. Cancer Epidemiol Biomarkers Prev 2011 Jul;20(7):1537–42.
[9] Canalle R, Burim RV, Tone LG, Takahashi CS. Genetic polymorphisms and susceptibility to childhood acute lymphoblastic leukemia. Environ Mol Mutagen 2004;43:100–9.
[10] Batar B, Guven M, Bariş S, Celkan T, Yildiz I. DNA repair gene XPD and XRCC1 polymorphisms and the risk of childhood acute lymphoblastic leukemia. Leuk Res 2009;33:759–63.
[11] Krajinovic M, Labuda D, Mathonnet G, Labuda M, Moghrabi A, Champagne J, et al. Polymorphisms in genes encoding drugs and xenobiotic metabolizing enzymes, DNA repair enzymes, and response to treatment of childhood acute lymphoblastic leukemia. Clin Cancer Res 2002;8:802–10.
[12] Stanulla M, Schrappe M, Brechlin AM, Zimmermann M, Welte K. Polymorphisms within glutathione S-transferase genes (GSTM1, GSTT1, GSTP1) and risk of relapse in childhood B-cell precursor acute lymphoblastic leukemia: a case-control study. Blood 2000;95:1222–8.
[13] Pui CH, Sandlund JT, Pei D, Campana D, Rivera GK, Ribeiro RC, et al. Improved outcome for children with acute lymphoblastic leukemia: results of Total Therapy Study XIIIB at St Jude Children's Research Hospital. Blood 2004;104:2690–6.
[14] http://ctep.cancer.gov/reporting/ctc.html. Accessed July 25/2017.
[15] Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1988;16:1215.
[16] Cascorbi I, Brockmöller J, Roots I. A C4887A polymorphism inexon 7 of human CYP1A1: population frequency, mutation linkages, and impact on lung cancer susceptibility. Cancer Res 1996;56:4965.
[17] Kiyohara C, Shirakawa T, Hopkin JM. Genetic polymorphism of enzymes involved in xenobiotic metabolism and the risk of lung cancer. Environ Health Prev Med 2002 May;7(2):47–59.
[18] Lee JM, Lee YC, Yang SY, Yang PW, Luh SP, Lee CJ, et al. Genetic polymorphisms of XRCC1 and risk of the esophageal cancer. Int J Cancer 2001;95:240–6.
[19] Georgiadis P, Topinka J, Vlachodimitropoulous D, Stoikidou M, Gioka M, Stephanou G, et al. Interactions between CYP1A1 polymorphisms and exposure to environmental tobacco smoke in the modulation of lymphocytes bulky DNA adducts and chromosomal aberrations. Carcinogenesis 2005;26:93–101.
[20] Krajinovic M, Labuda D, Richer C, Karimi S, Sinnett D. Susceptibility to childhood acute lymphoblastic leukemia: influence of CYP1A1, CYP2D6, GSTM1, and GSTT1genetic polymorphisms. Blood 1999;93:1496–501.
[21] Suneetha KJ, Nancy KN, Rajalekshmy KR, Sagar TG, Rajkumar T. Role of GSTM1 (present/null) and GSTP1 (Ile105Val) polymorphisms in susceptibility to acute lymphoblastic leukemia among the South Indian population. Asian Pac J Cancer Prev 2008;9:733–6.
[22] Ye Z, Song H. Glutathione S-transferase polymorphisms (GSTM1, GSTT1 and GSTP1) and the risk of acute leukemia: a systematic review and meta-analysis. Eur J Cancer 2005;41:980–9.

[23] Peng Y, Li Z, Zhang S, Xiong Y, Cun Y, Qian C, et al. Association of DNA base excision repair genes (OGG1, APE1 and XRCC1) polymorphisms with outcome to platinum-based chemotherapy in advanced nonsmall-cell lung cancer patients. Int J Cancer 2014 Dec 1;135(11):2687–96.
[24] Kuptsova N, Kopecky KJ, Godwin J, Anderson J, Hoque A, Willman CL, et al. Polymorphisms in DNA repair genes and therapeuticoutcomes of AML patients from SWOGclinicaltrials. Blood 2007 May 1;109(9):3936–44.
[25] Vodicka P, Kumar R, Stetina R, Sanyal S, Soucek P, Haufroid V, et al. Genetic polymorphisms in DNA repair genes and possible links with DNA repair rates, chromosomal aberrations and single-strand breaks in DNA. Carcinogenesis 2004;25:757–63.
[26] Giachino DF, Ghio P, Regazzoni S, Mandrile G, Novello S, Selvaggi G, et al. Prospective assessment of XPD lys751gln and XRCC1 Arg399gln single nucleotide polymorphisms in lung cancer. Clin Cancer Res 2007;13:2876–81.
[27] D'Alo F, Voso MT, Guidi F, Scardocci A, Sica S, Pagano L, et al. Polymorphisms of CYP1A1 and glutathione s-transferase and susceptibility to adult acute myeloid leukemia. Haematologica 2004 Jun;89(6):664–70.