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Association Between Combined Presence of Hepatitis C Virus and Polymorphisms in Different Genes With Toxicities of Methotrexate and 6-Mercaptopurine in Children With Acute Lymphoblastic Leukemia

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Background. The aim of the present study is to determine the correlation of hepatitis C virus (HCV) infection and polymorphisms in different genes with toxicity of either methotrexate (MTX) or 6-mercaptopurine (6-MP) administered to children with acute lymphoblastic leukemia (ALL). ***Procedure.*** One hundred children with low-risk ALL, who were treated according to the St. Jude Total therapy XV, were recruited. The recruited children were receiving MTX and 6-MP during maintenance phase. Patients were excluded from the study if they had other types of leukemia. Genotyping analyses for the thiopurine methyltransferase (TPMT), methylenetetrahydrofolate reductase (MTHFR), and glutathione S-transferase (GST) genes were performed using a combination of polymerase chain reaction (PCR) and PCR-RFLP (where RFLP is restriction fragment length polymorphism) protocols. Relevant clinical data on adverse drug reactions were collected objectively (blinded to genotypes) from the patient medical records. ***Results.*** There was a significant correlation between the combined presence of HCV and TPMT*3B G460A gene polymorphisms and grades 2–4 hepatotoxicity as aspartate aminotransferase (AST) elevation ($P < 0.04$). The same observation was seen when comparing either the presence of HCV alone or the presence of the gene polymorphism alone. A significant association between the combined presence of HCV and MTHFR C677T polymorphism and grades 2–4 hepatotoxicity as alanine aminotransferase (ALT), AST, and alkaline phosphatase (ALP) elevation was observed (P values <0.001, 0.02, and 0.001, respectively). The presence of HCV infection had a significant negative effect on hepatic transaminases. ***Conclusions.*** The present data support a role for combining analysis of genetic variation in drug-metabolizing enzymes and the presence of HCV in the assessment of specific drugs toxicities in multiagent chemotherapeutic treatment regimens. Pediatr Blood Cancer 2016;63:1539–1545.

Key words: acute lymphoblastic leukemia; chemotherapy; children; genetic polymorphism; HCV; toxicities

INTRODUCTION

Infection with hepatitis C virus (HCV) is considered a global health problem with a worldwide adult prevalence of about 3%,[1] and an Egyptian viremia prevalence of 9.8% according to the 2008 Egypt Demographic and Health survey (2008 EDHS).[2]

Acute lymphoblastic leukemia (ALL) is a malignant disease, which represents 25% of all malignancies in children.[3] About 80% of all affected patients can be cured, but resistance to the therapy and its toxic effects remain serious clinical problems.[4] As a result, the focus of research is slowly shifting from trying to increase survival rates to reduce chemotherapy-related toxicities. At the present time, the backbone of maintenance therapy for ALL consists of oral 6-mercaptopurine (6-MP) and weekly methotrexate (MTX).[3] Despite their great benefits, these drugs are associated with high degrees of hepatotoxicity and myelosuppression, which often limit their use.

In recent years, a number of polymorphisms in the genes coding for drug-metabolizing enzymes have been identified.[5] They play a significant role in modifying either the pharmacokinetics or pharmacodynamics of chemotherapy drugs. Therefore, they hold the potential to be critical for the occurrence of serious adverse effects in patients with ALL.

6-MP is metabolized by thiopurine methyltransferase (TPMT),[6,7] which is largely influenced by polymorphisms in its corresponding gene. Four common polymorphic alleles are associated with impaired activity of the enzyme. These are TPMT*2 (G238C), TPMT*3B (G460A), TPMT*3A (G460A and A719G), and TPMT*3C (A719G).[8] Variation in TPMT activity regulates thiopurine toxicity and therapeutic efficacy of thiopurine drugs.[9]

MTX inhibits the dihydrofolate reductase enzyme, which catalyzes the reduction of dihydrofolate to tetrahydrofolate

Abbreviations: 6-MP, 6-mercaptopurine; Abs, antibodies; ALL, acute lymphoblastic leukemia; ALP, alkaline phosphatase; ALT, alanine aminotransferase; ANA, antinuclear Abs; ASMA, antismooth muscle Abs; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase; GST, glutathione S-transferase; EDHS, Egypt Demographic and Health Survey; EIA, enzyme immunoassay; HCV, hepatitis C virus; LFTs, liver function tests; MTHFR, methylenetetrahydrofolate reductase; MTX, methotrexate; PCR, polymerase chain reaction; PCR-RFLP, PCR-restriction fragment length polymorphism; SPSS, Statistical Package for Social Sciences; TPMT, thiopurine methyltransferase; WBCs, white blood cells; WHO, World Health Organization

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required for the synthesis of thymidine and purine.[10] Treatment with MTX is associated with several side effects in a considerable number of patients, although some patients do not have any side effects at all.[11] Common polymorphisms have been described in folate metabolism related genes, including polymorphisms in methylenetetrahydrofolate reductase gene (MTHFR C677T). These polymorphisms have been studied in relation to the occurrence of MTX-induced side effects, but with conflicting results.[11]

Other common genetic polymorphisms occur in glutathione S-transferase (GST) gene that encodes a family of cytosolic enzymes involved in the detoxification of various exogenous and endogenous reactive species.[12] These polymorphisms are GSTT1, GSTM1, and GSTP1 A313G.[13]

Up to this point, very few studies have been concerned with the genotyping of Egyptian children in the polymorphic genes that are involved in the metabolism of chemotherapeutic agents,[14] and none addressed the combined effect of HCV infection and chemotherapeutics polymorphic genes on medications toxicities. Therefore, the aim of the present study is to determine how polymorphisms in genes coding for drug-metabolizing enzymes when combined with the presence of HCV infection would correlate with the toxicities of either MTX or 6-MP administered to children with ALL.

METHODS

Patients and Sample Collection

This was a cross-sectional/observational and parallel study consisting of two groups according to the HCV status. The subjects were children aged less than 12 who were suffering from and being managed for ALL. Diagnosis of ALL was performed using morphological, cytochemical, and immunophenotyping methods. All children were classified as low risk from white blood cell (WBC) count, morphology of leukemic cells, and other laboratory data such as immune markers. The treatment guidelines were adopted from the St. Jude Total therapy XV (St. Jude Children's Research Hospital, 2005).[15,16] All patients included in the study were in complete remission and receiving MTX 40 mg/m^2/week as intramuscular injections and 6-MP 75 mg/m^2/day as oral doses during maintenance phase. Among the inclusion criteria for patients infected with HCV was the detection of HCV RNA with or without HCV antibodies (Abs).[17] A thorough history and clinical examination as well as routine laboratory investigations including antinuclear Abs (ANA), antismooth muscle Abs (ASMA), serum ferritin, and lipid profile in addition to viral markers were done for all children at diagnosis of malignancy and those with concomitant diseases were excluded. All children tested negative for Abs against HCV (anti-HCV/EIA, where EIA is enzyme immunoassay) at diagnosis of ALL and before starting chemotherapy. Patients were excluded from the study, if they had other types of leukemia and if they were categorized as children with standard-risk or high-risk ALL. None of the patients showed abnormal renal functions or symptoms of malabsorption. Relevant medical information and clinical data on adverse effects were collected objectively (blinded to genotypes) from the patient's medical records during the week 100 and week 120 of maintenance therapy. This period was selected specifically, as all the children were only on MTX and 6-MP drugs according to the total XV protocol. Data collected included complete blood count (hemoglobin, WBCs, absolute neutrophil count, and platelets), liver function tests (LFTs; aspartate aminotransferase [AST], alanine aminotransferase [ALT], alkaline phosphatase [ALP], gamma-glutamyl transferase [GGT], total bilirubin, and albumin) and kidney function tests (creatinine). The children were followed up for drug-related toxicities every 2 weeks during the week 100 and week 120 of maintenance therapy. Grading of toxicities was registered using the World Health Organization (WHO) grading scale.[18,19] The toxicities of MTX and 6-MP included anemia, neutropenia, thrombocytopenia, and elevated ALT, AST, ALP, and bilirubin levels were evaluated. The severity of the adverse effects was graded from 0 to 4 according to the WHO scale.

The procedures of the study were approved by the local institutional ethical committee of the Faculty of Pharmacy, Cairo University. The study was conducted according to the World Medical Association Declaration of Helsinki.[20] A written informed consent was obtained from the guardians of each patient before enrollment into the study.

Genotyping Analysis of the TPMT Gene

DNA was extracted from 300 μl whole blood samples using a commercial kit (Promega, Fitchburg, WI), according to the manufacturer's recommendations. The extracted DNA was resuspended in 100 μl of the provided alkaline hydration solution at 65°C. A total of 99 DNA samples were analyzed. Total genomic DNA extracted from peripheral leucocytes was analyzed by polymerase chain reaction (PCR). To analyze the mutation in G238C (TPMT*2) allele, specific PCR was performed using primers P2W (5′-GTATGATTTTATGCAGGTTTG-3′) or P2M (5′-GTATGATTTTATGCAGGTTTC-3′) with primer P2C (5′-TAAATAGGAACCATCGGACAC-3′).[21] P2W and P2C primers produced a 254 base pair (bp) PCR fragment when G238 was present (wild type), while P2M and P2C primers produced a 254 bp PCR when C238 (mutant) was present. In the case of the G460A (TPMT*3B allele) polymorphism, primers P460F (5′-ATAACAGAGTGGGGAGGCTGC-3′) and P460R (5′-CTAGAACCCAGAAAAAGTATAG-3′) were used to amplify a 365 bp PCR fragment. Upon treatment with the restriction enzyme MwoI, it yielded fragments of 267 and 98 bp for wild-type allele and left the PCR product undigested for the mutant allele. In the case of the A719G (TPMT*3C allele), primers P719R (5′-TGTTGGGATTACAGGT-GTGAGCCAC-3′) and P719F (5′-CAGGCTTTAGCATAATTTTCAATTCCTC-3′) were used in a PCR reaction to produce a 293 bp fragment. Upon restriction enzyme digestion with AccI, the A719G mutant yielded fragments of 207 and 86 bp and left the PCR product undigested for the wild-type allele.

Genotyping Analysis of the MTHFR Gene

The MTHFR C677T polymorphism was detected using a PCR-restriction fragment length polymorphism (PCR-RFLP) as described.[22] The primers 5′-CCTTGAACAGGTGG AGGCCAG-3′ and 5′-GCGGTGAGAGTGGGGTGGAG-3′ were used to amplify a 294 bp PCR fragment. After amplification, the PCR products were digested with restriction endonuclease HinfI. Wild-type (CC) showed a single band at

TABLE I. Patients' Demographic and Clinical Characteristics

Characteristics	HCV positive (N = 39)	HCV negative (N = 61)	*P*-value
Gender, male (%)	27 (69.23%)	39 (63.93%)	0.586[a]
Age in years, mean ± SD (range)	6.9 ± 1.79 (3.2–11)	6.72 ± 1.83 (3.5–11.2)	0.63[b]
Weight in kg, mean ± SD (range)	24.28 ± 7.84 (14.3–51)	23.41 ± 0.92 (13.5–44)	0.57[b]
Height in cm, mean ± SD (range)	118.32 ± 12.59 (93–148)	118.19 ± 13.49 (94–143)	0.96[b]
BSA in m^2, mean ± SD (range)	0.88 ± 0.18 (0.6–1.45)	0.87 ± 0.18 (0.6–1.3)	0.71[b]
BMI in kg/m^2, mean ± SD (range)	16.94 ± 2.47 (11.6–23.3)	16.36 ± 1.86 (11.5–22.8)	0.18[b]
Dose of MTX in mg, mean ± SD (range)	35.32 ± 7.37 (24–58)	34.75 ± 7.29 (24–52)	0.71[b]
Dose of 6-MP in mg, mean ± SD (range)	66.23 ± 13.81 (45–108.75)	65.16 ± 13.68 (45–97.5)	0.71[b]
Estimated years of HCV acquisition, mean ± SD	2.11 ± 1.35		
History of blood transfusion (%)	39 (100%)	37 (60.65%)	<0.001[a]

SD, standard deviations; BSA, body surface area; BMI, body mass index; MTX, methotrexate; 6-MP, 6-mercaptopurine; N, number of children; HCV, hepatitis C virus [a]Chi-square test was used for comparing the groups at a level of significance of <0.05 [b]t-test was used for comparing the groups at a level of significance of <0.05.

294 bp. For heterozygous (CT) three bands of 294, 168, and 126 bp were observed. The homozygous (TT) had two bands of 168 and 126 bp.

Genotyping Analysis of the GST Gene

To detect the GSTM1 and GSTT1 gene polymorphism, a multiplex PCR method described by Abdel-Rahman et al. was used.[23] The primers 5′-GAACTCCCTGAAAAGCTAAAG C-3′ and 5′-GTTGGGCTCAAATATACGGTGG-3′ were used for amplifying a 215 bp PCR fragment. For the GSTT1 gene polymorphism, the primers 5′- TTCCTTACTGGT CCTCACATCTC-3′, and 5′-TCACGGGATCATGGCCAGC A-3′ were used for amplifying a 480 bp PCR fragment. The PCR reactions were carried out simultaneously in the same tube. The presence of either the GSTM1 or GSTT1 was detected as a PCR product band with the respective size, while the absence of the PCR product was considered as an indication of the lack of the allele (GSTM1/null and GSTT1/null). To detect GSTP1 A313G gene polymorphism, PCR-RFLP method was used as described earlier.[24] The primers 5′- ACCCCAGGGCTCTATGGGAA-3′ and 5′-TGAGGGCACAAGAAGCCCCT-3′ were used for amplifying a 176 bp PCR fragment. After amplification, the PCR products were digested with restriction enzyme Alw261. The presence of the G allele was indicated by the presence of only two fragments (91 and 85 bp), and A/G polymorphism was indicated by the presence of three fragments (176, 91, and 85 bp).

Statistical Analysis

Data were analyzed using the Statistical Package for Social Sciences (SPSS) software (Version 11.0). The occurrence of the side effects was studied in relation to the different genotypes. The Fisher exact test,[25] or Chi-square test,[26] was used for comparison of drug-related toxicities between wild-type and other genotypes, and also between children with positive HCV infection and children with negative HCV infection. Means were presented ±standard deviation. P-values of <0.05 were considered statistically significant.[27]

RESULTS

Description of the Study Population

In this study, the sample included 100 pediatric patients suffering from ALL (mean age in years 6.79 ± 1.8; range 3.2–11.2); 66 (66%) patients were males. The patient's medical records were reviewed for the LFTs at the diagnosis of malignancy and no abnormality was detected. Thirty-nine (39%) patients infected with HCV and designated by the microbiologists as genotype 4 (the most prevalent genotype in Egypt),[28] had positive history of blood and/or blood products transfusion, also some of them had history of surgical interventions with no family history of HCV infection.[29] All genotyping data were missing in a single patient infected with HCV. The characteristics of the patients at week 100 of maintenance therapy are shown in Table I.

TPMT, MTHFR, and GST Genotyping

The genotype frequencies of TPMT, MTHFR, and GST in HCV-positive and HCV-negative children with ALL are shown in Table II. Genotyping was not successful in one case for all the studied genes. The TPMT*2 (G238C) allele was not detected in any of the studied children. There was a statistical difference of genotype frequency of TPMT*3B (G460A) polymorphism between HCV-positive and HCV-negative children with ALL ($P < 0.02$). All the studied patients had mutant GSTP1 (A313G) allele. There was no statistical difference of genotype frequencies of TPMT*3C (A719G), TPMT*3A (G460A and A719G), MTHFR C677T, and GSTT1 and M1 polymorphisms between HCV-positive and HCV-negative children with ALL.

The distributions of MTHFR C677T and GSTT1 and M1 polymorphisms among the studied children are shown in Table II. With respect to the distribution of C677T MTHFR polymorphism among the studied patients, 43 (43.43%) had CT (heterozygous mutant allele), whereas 17 (17.17%) had TT (homozygous mutant allele) and 39 (39.39%) had CC (wild homozygous allele). The allele frequencies for C and T alleles of MTHFR C677T were 61.84 and 38.17%, respectively, in the children with HCV infection and were 60.65 and 39.34%, respectively, in the children without HCV infection. With respect to GSTT1 and M1 polymorphism, 45 (45.45%) patients had GSTT1 mutant allele, whereas 9 (9.09%) patients had GSTM1

TABLE II. Genotype Frequencies of TPMT*2 (G238C), TPMT*3B (G460A), TPMT*3C (A719G), TPMT*3A (G460A and A719G) in TPMT Gene, MTHFR C677T in MTHFR Gene, and GSTT1 and M1, GSTP1 (A313G) in GST Gene Among HCV-Positive and HCV-Negative Children With ALL

Genotype	HCV positive[a] (N = 38)	HCV negative (N = 61)	*P*-value
TPMT			
*TPMT*2* (*G238C*)			
Wild-type	38	61	
Mutant	0 (0%)	0 (0%)	
*TPMT*3B* (*G460A*)			
Wild-type	35	61	
Mutant	3 (7.9%)	0 (0%)	<0.02[b]
*TPMT*3C* (*A719G*)			
Wild-type	32	43	
Mutant	6 (15.8%)	18 (29.5%)	0.12
*TPMT*3A* (*G460A* and *A719G*)			
Wild-type	37	61	
Mutant	1 (2.6%)	0 (0%)	0.38
MTHFR			
C677T			
Wild-type (CC)	15	24	
Mutant (CT)	17 (44.74%)	26 (42.62%)	
Mutant (TT)	6 (15.8%)	11 (18.03%)	
Total mutant	23 (60.5%)	37 (60.6%)	0.99
GST			
GSTT1 and *M1*			
Wild-type	8	13	
Mutant GSTT1	17 (44.74%)	28 (45.9%)	
Mutant GSTM1	5 (13.16%)	4 (6.56%)	
Mutant GSTT1/M1	8 (21.05%)	16 (26.23%)	
Total mutant	30 (78.9%)	48 (78.7%)	0.97
GSTP1 (A313G)			
Wild-type	0	0	
Mutant	38 (100%)	61 (100%)	

[a]All genotyping data were missing in a single HCV positive patient
[b]Fisher exact test was used; HCV, hepatitis C virus; N, number of patients.

mutant allele, and 24 (24.24%) patients had GSTT1/M1 mutant allele.

Association Between Combined Presence of Different Genes Polymorphisms and HCV and Drug-Related Toxicities

Table III shows significant association between the combined presence of HCV and MTHFR C677T polymorphism and grades 2–4 hepatotoxicity as ALT, AST, and ALP elevation. A significant association was observed between the presence of HCV alone and grades 2–4 ALT, AST, and ALP hepatotoxicity. In addition, a significant association was detected between the presence of the T mutant allele (CT or TT) in children and grades 2–4 ALP hepatotoxicity. The presence of both HCV and MTHFR C677T polymorphism was significantly associated with elevated total bilirubin and GGT level grades 3 and 4.

Table IV represents the association of MTX-related and 6-MP-related toxicities (grades 2–4) and the combined presence of HCV and polymorphism in GSTT1 and M1 gene in the studied children. Significant association was observed between the combined presence of HCV and GSTT1 and M1 polymorphism and grades 2–4 ALT, AST, and ALP hepatotoxicity. No significant association was found between the presence of GSTT1 and M1 polymorphism alone in the studied children and any studied drug toxicities (grades 2–4 and grades 3 and 4). The presence of both HCV and GSTT1 and M1 polymorphism was significantly associated with elevated total bilirubin and GGT level grades 3 and 4.

There was a significant relation between the combined presence of HCV and polymorphism in TPMT G460A gene and grades 2–4 AST hepatotoxicity ($P < 0.04$). Two patients (66.7%) of the three with both HCV infection and TPMT G460A gene polymorphism had grades 2–4 AST hepatotoxicity, while 12 patients (12.5%) of the 96 with the absence of either HCV or genetic polymorphism in the same gene had experienced grades 2–4 AST hepatotoxicity. The same association was observed while comparing either the presence of HCV alone or the presence of the gene polymorphism alone.

No significant relation was observed between the combined presence of HCV and TPMT G460A gene polymorphism in hematologic drug toxicities occurred at any week of maintenance therapy. In addition, no significant association was found between the combined presence of HCV and TPMT A719G gene polymorphism in any studied drug toxicity. The percentage of patients (33.3%) having grades 2–4 thrombocytopenia is higher in the combined presence group, although not reaching statistical significance level ($P = 0.05$).

No significant association was found between the combined presence of HCV and TPMT*3A (G460A and A719G) gene polymorphism in any studied drug toxicity. Only one patient with HCV infection was heterozygous for the TPMT*3A allele (G460A and A719G) with allelic frequency of 1.01%.

Thirteen patients (13.13%) had mutations in the three studied genes (TPMT, MTHFR, and GST); four patients were infected with HCV whereas nine patients were HCV-uninfected. A single patient from each group showed ALT, AST, and ALP hepatotoxicity with no significant differences between them.

DISCUSSION

This was a pilot study identifying six major genetic polymorphisms in the Egyptian childhood ALL population. It provided a better understanding of the prevalence of genetic polymorphisms in genes encoding drugs metabolizing enzymes in this group. In addition, the study highlighted the role of HCV infection as a significant player in both hepatotoxicity and myelosuppression when combined with genetic polymorphisms.

The present study indicated that MTX-related and 6-MP-related hepatotoxicity was strongly associated with the combined presence of HCV and genetic polymorphisms in MTHFR C677T, GSTT1/M1, and TPMT*3B G460A genes. Drug-related hepatotoxicity presented in this study was strongly correlated

TABLE III. Association Between MTX-Related and 6-MP-Related Toxicities (Grades 2–4) and the Combined Presence of HCV and MTHFR C677T Polymorphism in Children With ALL Occurred at Week 100 of Maintenance Therapy

Toxicity	HCV and MTHFR C677T polymorphism		OR (95% CI)	*P*-value
	Combined presence (N = 23)	Absence of one/both of them (N = 76)		
	Toxicity grades 2–4* N (%)	Toxicity grades 2–4 N (%)		
ALT	12 (52.2)	14 (18.4)	4.8 (1.8–13.2)	<0.001[a]
AST	7 (30.4)	7 (9.2)	4.3 (1.3–14)	<0.02[b]
ALP	9 (39.1)	4 (5.3)	11.6 (3.1–42.9)	<0.001[b]
Total bilirubin	2 (8.7)	1 (1.3)	7.1 (0.6–82.7)	0.13[b]
GGT**	1 (12.5)	0 (0)		0.26[b]
Anemia	5 (21.7)	20 (26.3)	0.8 (0.3–2.4)	0.66[a]
Neutropenia	1 (4.3)	24 (31.6)	0.1 (0.01–0.77)	<0.001[a]
Thrombocytopenia	2 (8.7)	5 (6.6)	1.3 (0.2–7.5)	0.66[a]

*Toxicity grades were assessed using the WHO grading scale;[18,19] ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; GGT, gamma-glutamyl transferase [a]Chi-square test [b]Fisher exact test **data of eight combined presence and 23 absence of either HCV or polymorphism; N, number of patients.

TABLE IV. Association Between MTX-Related and 6-MP-Related Toxicities (Grades 2–4) and the Combined Presence of HCV and GSTT1 and GSTM1 Polymorphism in Children With ALL Occurred at Week 100 of Maintenance Therapy

Toxicity	HCV and GSTT1-GSTM1 polymorphism		OR (95% CI)	*P*-value
	Combined presence (N = 30)	Absence of one/both of them (N = 69)		
	Toxicity grades 2–4* N (%)	Toxicity grades 2–4 N (%)		
ALT	15 (50)	11 (15.9)	5.3 (2–13.8)	<0.001[a]
AST	9 (30)	5 (7.2)	5.5 (1.7–18.2)	<0.001[b]
ALP	8 (26.7)	5 (7.2)	4.6 (1.4–15.7)	<0.02[b]
Total bilirubin	2 (6.7)	1 (1.4)	4.8 (0.4–55.7)	0.19[b]
GGT**	1 (10)	0 (0)		0.32[b]
Anemia	9 (30)	16 (23.2)	1.4 (0.5–3.7)	0.47[a]
Neutropenia	3 (10)	22 (31.9)	0.2 (0.1–0.9)	<0.02[a]
Thrombocytopenia	4 (13.3)	3 (4.3)	3.4 (0.7–16.1)	0.19[b]

*Toxicity grades were assessed using the WHO grading scale;[18,19] ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; GGT, gamma-glutamyl transferase [a]Chi-square test [b]Fisher exact test ** data of 10 combined presence and 21 absence of either HCV or polymorphism; N, number of patients.

with both presence of HCV and presence of genetic polymorphisms in MTHFR C677T and TPMT*3B G460A genes. Hepatotoxicity was mainly correlated with the presence of HCV in the recruited subjects and not correlated with genetic polymorphism in GSTT1/M1 gene.

TPMT genotype influenced the safety and efficacy of ALL treatment, and genotype information may therefore be useful for optimizing 6-MP therapy.[30–32] Several studies have shown that patients with mutant TPMT alleles conferring very low enzyme activity are at high risk of developing hematopoietic and hepatic toxicity after treatment with thiopurines.[33,34]

For this reason, knowledge of the TPMT single-nucleotide polymorphism frequencies in a population is essential for estimating the proportions of risk groups under 6-MP therapy. From this point of view, in the current study, the frequencies of four variant TPMT alleles (TPMT*2, TPMT*3B, TPMT*3C, TPMT*3A), accounting for 80–95% of intermediate- or low-activity cases worldwide, were determined in pediatric patients with ALL in Egypt. TPMT*3A allele was detected in only one patient out of 99 DNA samples from the pediatric patients with ALL. The frequency of TPMT*3A allele in our subjects is thus 1.01%. The frequencies of TPMT*3B and TPMT*3C were 3.03% and 24.24%, respectively. No TPMT*2 allele was detected in the present study, which was in agreement with a Palestinian study performed on 56 childhood patients with ALL.[35] TPMT*3A allele frequency was consistent with the screened Palestinian population (0.89%),[35] and ethnically related Israeli Arab subpopulations (0.79%).[36] The frequency of TPMT*3A allele was also similar to Jordanian population (0.59%),[37] Turkish population (0.9%),[38] and Iranian population (0.87%).[39] It was lower than the frequency reported for several Caucasian, African descendants, and South Americans.[37]

In the present study, 25 (25%) patients developed anemia, 25 (25%) patients developed neutropenia, and 7 (7%) patients developed thrombocytopenia, and they all had a normal TPMT*3B (G460A) genotype. Five of the 25 patients who developed anemia, 7 of the 25 patients who developed neutropenia, and 3 of the 7 patients who developed thrombocytopenia had the heterozygous TPMT*3C (A719G) allele. The only

patient with heterozygous TPMT*3A allele did not develop any myelosuppression. It is noteworthy to mention that not all cases of myelosuppression are due to a mutation in the gene coding for the TPMT enzyme and therefore, not all cases can be prevented by screening for TPMT with either the enzymatic assay or genotype test.[40] The presence of toxicity in a number of cases and the lack of common types of mutations may result from the existence of other rare polymorphic alleles, multigenetic contribution, or other nongenetic factors.[41]

Based on the data presented in this study, hematopoietic toxicity did not differ between heterozygous (mutant) patients and homozygous wild-type for TPMT*3B G460A and TPMT*3C A719G. These results are in agreement with the results of Stanulla et al., who studied 814 German children with ALL according to Berlin-Frankfurt-Munster protocols.[42] Therefore, it seems unlikely that childhood patients with ALL and heterozygous mutant TPMT alleles treated in the Total XV protocols would benefit from dose reductions in maintenance treatment. Our results may provide a rationale for increasing 6-MP dosing according to TPMT genotype in the early course of childhood ALL. Because this rationale will affect TPMT wild-type individuals, it could have an impact on the majority of patients and, therefore, substantially influences overall treatment results.

There are few data in the literature regarding the impact of GST polymorphisms, on the toxicity of chemotherapy during maintenance treatment. Therefore, it was interesting to look at GST, and treatment toxicity in childhood ALL. In the present study, no significant relation was observed between the presence of GSTT1 and M1 polymorphism and any studied drug toxicities (grades 2–4 and grades 3 and 4). These results are in contrast to Marino et al.,[43] in which they investigated the relation between chemotherapy side effects and GST polymorphism in a small population of children with ALL. They detected an increment in the risk of bacterial infection in patients with polymorphisms in GSTM1 gene.

Another retrospective study on GSTM1 and GSTT1 polymorphisms and grades 3 and 4 gastrointestinal/hepatic/neurological toxicities and severe infections occurring in the induction and maintenance phases was performed.[44] No significant effect emerged between grade 3 and 4 toxicities reported during the induction phase and the presence of null GSTM1 and GSTT1 genotypes,[41] which is in agreement with data presented in the present study.

MTX-related hepatotoxicity was studied in relation to MTHFR C677T polymorphisms and the presence of HCV. The MTHFR C677T polymorphism was significantly correlated with hepatotoxicity during maintenance treatment of children with ALL. In addition, the presence of HCV infection had a significantly negative effect on hepatic transaminases. Thus, the combined presence of HCV and MTHFR 677 CT/TT (mutant) genotypes were associated with higher risk of hepatotoxicity (grades 2–4 and grade 3 and 4).

Studies on MTHFR C677T polymorphism, on patients receiving low-dose MTX, showed conflicting results; some in agreement with our results, found increased toxicity in patients carrying the mutant T-allele,[45] whereas others did not find such relationship, and one study reported lower toxicity in carriers of the mutant T-allele in childhood patients with ALL receiving low-dose MTX.[46] Contrasting two studies on patients with non-Hodgkin lymphoma and patients with ALL,[47,48] using a high-dose MTX containing treatment protocol, in the present study, as well as in Chiusolo et al.'s study,[45] a strong association was found between MTHFR C677T and MTX-related toxicities. Another study on 240 childhood patients with ALL receiving both low- and high-dose MTX did not show any relationship between MTHFR C677T polymorphisms and MTX-related toxicity,[49] which is in contrast to our findings. The conflicting results between studies describing patients treated with low- or high-dose MTX suggest that MTX dosage may influence the genotype-related effect on MTX-related toxicities. However, discrepancies between the different studies relating gene polymorphisms and toxicity might also be explained by environmental factors such as folate status, diet, and concurrent medications, as suggested by Krajinovic et al.[50]

Significant correlations were observed between MTX-related toxicities and MTHFR C677T genotypes, the presence of HCV and their combined presence. Since this was with only a relatively low number of included patients, and since other studies on childhood patients with ALL showed conflicting results, our conclusions need confirmation in a prospective study including higher patient numbers. Such a study has to allow correction for multiple testing and multivariate analyses, including variables such as age, sex, leucovorin rescue, or toxic MTX levels. The absence of objective evidence from histology was considered as a major limitation of the present study.

In conclusion, the present data support a role for combining analysis of genetic variation in drug-metabolizing enzymes and the presence of HCV in the assessment of specific drugs toxicities in multiagent chemotherapeutic treatment regimens. Future studies should focus on determining the mechanism by which drug toxicities are elicited by HCV infections.

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