ORIGINAL ARTICLE

Evaluation of Relationship of the Methylene Tetrahydrofolate Reductase Enzyme Polymorphisms with Serum Methotrexate Concentration and Toxicity in Acute Lymphoblastic Leukemia Patients Treated with High Dose Methotrexate Infusion

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Abstract:

Background: Methotrexate (MTX) blocks Methylene Tetrahydrofolate Reductase (MTHFR) Enzyme thereby, interrupt folate metabolism, it is used in the treatment of cancer and autoimmune disorders. *Aim and Objectives:* The present study aimed to evaluate the relationship of the MTHFR polymorphisms with serum MTX concentration and its toxicity in Acute Lymphoblastic Leukemia (ALL) patients treated with high dose MTX infusion. *Material and Methods:* Level of Serum MTX was measured, along with the detection of MTHFR polymorphisms viz. C677T and A1298C by Polymerase Chain Reaction (PCR) followed by DNA sequencing. The percentages of toxicity developed in patients were calculated among the wild type and carriers for both polymorphisms and were compared between the groups. *Results:* The majority of patients 36 (72 %) were wild type for the C677T polymorphism and 32 (64 %) of patients were carriers for the A1298C polymorphism [48% heterozygous (AC), and 16 % homozygous (CC)]. Among 50 ALL patients studied, significant difference was noted in the genotype and allele frequencies for C677T polymorphism, while only allele frequencies differed significantly for A1298C polymorphism. The serum MTX level at 48 hours after the start of High Dose MTX (HDMTX) infusion of the C677T variant (CT) was slightly high in all four cycles however, in the first cycle, there was a significant increase in the level of MTX. There was no significant difference in the serum MTX level found in all four cycles between patients wild type and carriers for A1298C polymorphism. For A1298C polymorphism, the mean SGPT level in carriers was significantly high as compared to wild type. *Conclusion:* The present study concludes that patients with C667T variant had elevated serum MTX concentration at 48 hours after the start of HDMTX infusion.

Keywords: Methylene Tetrahydrofolate Reductase Enzyme, Polymorphism, Methotrexate, Acute Lymphoblastic Leukemia

Introduction:

The enzyme Methyl Tetrahydrofolate Reductase (MTHFR) plays an important role in one-carbon metabolism, which converts 5,10–methylene-tetrahydrofolate to 5-methyltetrahydrofolate, an active form of folate. Methotrexate (MTX) is a folate antagonist that has been commonly used in different types of cancer treatments and autoimmune disorders. MTHFR is blocked by antifolate MTX, which interrupts folate metabolism by inhibiting the synthesis of deoxythymidine monophosphate (dTMP) needed

for DNA replication [1]. The activation of folate is created in the folic acid pathway which is required for methylation of DNA, homocysteine and DNA synthesis.

Two important single nucleotide polymorphisms are identified in the MTHFR gene, C667T, and A1298C, the presence of these polymorphisms is known to lower the MTHFR enzyme activity [2-3]. Individuals carrying CT and TT genotypes of C677T, exhibit 60% and 30% of the normal MTHFR activity respectively, [4-5] whereas individuals in the case of A1298C, with CC genotype, showed 60% of the normal activity [6]. Therefore, decreased activity of MTHFR due to mutant alleles may increase the availability of 5,10 methylenetetrahydrofolate and decrease 5-methyltetrahydrofolate. Also, decreased MTHFR enzyme activity leads to an increase in MTX induced toxicity and imbalance in plasma folate concentration.

The patients with mutant alleles may not be able to tolerate MTX and because of this, patients may manifest various toxicities. As per the recent meta-analysis the correlation of MTX infusion with MTHFR polymorphisms and its toxicities has been investigated in many of the published studies. Most of the studies have reported no association of MTHFR polymorphisms and toxicities in pediatric ALL patients. The studies have shown that the association could not prove the relationship between MTHFR and toxicities due to conflicting results to each other. The reason for the variability in results could be ethnicity and different treatment protocols [7]. However, the correlation between serum methotrexate concentration, MTHFR polymorphisms, and related toxicities has not been studied yet in India. We hypothesized that the polymorphisms in the MTHFR gene may influence serum methotrexate concentration after administration of High Dose Methotrexate (HDMTX) via infusion, thereby causing MTX associated toxicities. In view of this, the present study was aimed to elucidate the association of the MTHFR gene polymorphisms and toxicities in response to serum MTX drug level in children with Acute Lymphoblastic Leukemia (ALL).

Material and Methods:

The cross-sectional study was carried out in Bharati Hospital, Pune and KEM Hospital and Research Center, Pune, for a period of one year from 1st September 2018 to 30th September 2019. Children of either gender below 18 years with ALL at the time of enrollment who received high dose MTX intravenous infusion as a 2 g/m^2 and 5 g/m^2 dose over 24 hours were included in the study. ALL patients who were undergoing bone marrow transplantation were excluded from the study. The study was approved by the Institutional Ethics Committee (BVDUMC/IEC/74 and KEMHRC, LFG/EC/2423). Prior to the study participation, every patient's parents or guardian received extensive standardized information material about the aim and the content of the study. The informed consent was obtained from the patient's parents or guardian and assent was obtained from children above 13 years. As per the Berlin-Frankfurt-Munster (BFM) based protocol, HDMTX infusion has to be delivered to Central Nervous System (CNS) positive patients. In present study, 50 ALL CNS positive children undergoing HDMTX treatment were recruited by convenient sampling between the period of 1st September 2018 to 30th September 2019.

Treatment Protocol and Drug Administration:
The selected patients were treated as per the BFM treatment-based protocol that includes four courses of HDMTX. For each course, 10% of the total MTX dose (maximum to 0.5 g) was given intravenously in the first half an hour, and the rest was administered evenly during the subsequent hours. Patients were hydrated for 48 hours with intravenous 0.45% Dextrose Normal Saline, 20 mmol KCl/liter and 40 mEq $NaHCO_3$/liter until the urine pH was 7.0 before HDMTX infusion. Hydration is continued during and after HDMTX infusion.

Blood Sampling and MTX Assay:
Two mL blood samples were collected in a plain vacutainer following 48 hours of MTX infusion and allowed to clot. Coagulated blood was centrifuged for 15 minutes at 3000 rpm and serum was separated for analysis. Serum MTX drug levels were estimated by using ARCHITECT MTX assay kit by (Abbott Laboratories, USA). The measuring range of the assay kit is from 0.040 µmol/L to1.500 µmol/L. Specimens with MTX concentration more than 1.500 µmol/L were diluted with the manual dilution procedure provided by the manufacturer. Following high dose MTX treatment with calcium folinic acid rescue, serum MTX concentrations above safety values, characterized as >1.0 µmol/L at 48 hours demonstrated greater susceptibility to adverse reactions [8]. Complete blood count including neutrophil count, total leukocyte, platelet counts were quantitated by hematology analyzer DxH 800 (Beckman Coulter, US) and Serum Glutamate Pyruvate Transaminase (SGPT) was estimated using Randox SGPT kit (Randox Laboratories Ltd, UK) before and after administration of HDMTX infusion as per the oncology protocol.

Extraction of Genomic DNA:
Three mL blood samples were collected in the EDTA vacutainers for the isolation of genomic DNA. Genomic DNA was extracted from the white blood cells of blood samples from each participant by using the DNeasy Blood and Tissue DNA kit (Qiagen, Germany). Samples were first lysed using lysing buffer Proteinase K. Buffering conditions were adjusted to provide optimal DNA binding conditions and the lysate was loaded onto the DNeasy Mini spin column. During centrifugation, DNA was selectively bound to the DNeasy membrane as contaminants pass through. Remaining contaminants and enzyme inhibitors were removed in two efficient wash steps and DNA was then eluted in the buffer. DNA purity was checked on nano-drop measuring ratio of ODs at 260 nm and 280 nm. The concentration of DNA was estimated by Qubit Fluorometer (Life technologies, USA) using the Qubit BR dsDNA quantification kit.

Genetic Analysis:
The polymorphisms C677T and A1298C of MTHFR were detected by Polymerase Chain Reaction (PCR) followed by DNA sequencing. The primer pairs used to amplify MTHFR C677T (rs1801133) polymorphism were Forward 5'-CCCAGCCACTCACTGTTTAGTTCAGGC-3' and reverse 5'-GTGAGAGTGGGGTGGA GGGAGCTTATG-3', producing 406 bp product. Primer pair used to amplify MTHFR A1298C (rs1801131) polymorphism were Forward 5'-GGCCTGCAGACCTTCCTTGCAAATACAT-3' and reverse 5'- ACTTACCCTTCTCCCTTT GCCATGTCCA -3', giving 509 bp product. The PCR reaction was performed in a final volume of 25 µl, consisting 100 ng of genomic DNA, the

PCR mix (containing 2.5 µl of 10× PCR buffer (200 mMTris-HCl – pH 8.4, 500 mMKCl), 0.75 µl MgCl2 (50 mM), 0.5 µl dNTPs (10 mM), 0.2 µl of Taq DNA polymerase (5 U/µl) (Life technologies, USA), 10 pM each of primers) and final volume was adjusted to 25 µl with sterile nuclease-free water. PCR reaction conditions for amplification of MTHFR polymorphisms used were, an initial denaturation for 5 min at 95°C, followed by 35 cycles of denaturation for 30 s at 94°C, annealing for 30 s at 60°C, and elongation for 30 s at 72°C, with a final extension for 7 min at 72 °C using a Veriti 96-well Thermal cycler (Applied Biosystems, USA). For quality checks, one negative control reaction without a DNA sample was run with every batch of reactions. PCR products generated were separated with a 100 bp DNA ladder on a 2% agarose gel (Promega Corporation, Madison, USA) followed by DNA sequencing for detection of C677T and A1298C polymorphisms for MTHFR.

Amplicon Purification and Sequencing:

PCR products generated from PCR were run with 100 bp DNA ladder on a 2% agarose gel spiked with ethidium bromide (Promega Corporation, Madison, USA). The separated bands were visualized and documented using Quantity One software on Gel Doc XR System (Bio-Rad). PCR products were purified using ExoSAP IT reagent (ThermoFisher). Purified PCR amplicons were sequenced using Big Dye® Terminator v3.1 Cycle Sequencing reaction kit (Applied Biosystems, USA), as per manufacturer's instructions followed by capillary electrophoresis performed on an ABI 3130 Genetic Analyzer (Applied Biosystems, USA). The sequence data is retrieved in FASTA format, aligned with reference sequences of each SNP locus to find out whether there is a mutation or not at the specified rsID SNP locations. The Chromas Pro v3.1 software was used for sequence assemble. Sequence alignment of reference and samples was done using Clustalw online sequence alignment tool (http://www.genome.jp/tools/clustalw/).

Statistical Analysis:

The age was presented as Mean ± Standard Deviation (SD). The other continuous variables were calculated as Mean ± Standard Error (SE). The genotype and allele frequencies of C677T and A1298C polymorphisms of MTHFR were calculated as proportions. The distributions of genotype and allele frequencies were compared using the chi-square test. Then the percentages of toxicity developed in patients were calculated among the wild type, and carriers for both polymorphisms and were compared by the chi-square test. The patients were further divided based on wild type and carriers for both the polymorphisms and the methotrexate level at cycle 1 to 4, and means of total leukocyte count, platelet count, neutrophil count, and SGPT level were compared between these groups using the student's 't' test. The association of both the polymorphisms with the methotrexate levels at cycles 1 to 4 was tested by logistic regression analysis. The percentages of possible haplotypes were calculated for C677T and A1298C, the haplotype with a frequency of less than 0.1% was omitted. The phenotypes were compared between the haplotype groups considering haplotype CA as the baseline. The p values of <0.05 were considered significant. The statistical analysis was done using SPSS and SNP stats software.

Results:

The present study comprised of total 50 pediatric CNS positive ALL patients. In those 50 children a total of 200 HDMTX infusion were delivered, each child with 4 cycles of infusion. Out of 50 patients, 36 (72%) were boys and 14 (28%) were girls, with mean body surface area of 0.76 m^2, and among ALL patients 46 (92%) belonged to ALL B-cell phenotype, while 4 (8%) belonged to T-cell phenotype. The mean age group of study subjects was 7.7 ± 4.5 years. The total leukocyte count, platelet, neutrophil, SGPT are summarized in Table 1.

Out of the total 50 patients studied 36 (72%) were wild type for C677T polymorphism, and 14 (28%) were carrier for T allele (CT). When compared the genotype and the allele frequencies of C677T among ALL patients differed significantly ($p<0.0001$). In case of A1298C polymorphism18 (36%) patients were wild type (AA), 24 (48 %) were heterozygous (AC) and 8 (16%) were homozygous (CC) and there was no significant difference noted in the genotype frequencies of A1298C polymorphism, but the allele frequencies differed significantly ($p=0.0466$). The results of the genotype and allele frequencies are depicted in Table 2.

Table 1: Demographic and Laboratory Parameters among 50 ALL Patients

Variables	Number of patients n (%)	
Gender	**Boys**	36 (72)
	Girls	14 (28)
Mean body surface area (BSA) (m^2)		0.76
Immunotype	**B-cell**	46 (92)
	T-cell	4(8)
HDMTX dose	**2 (g/m^2)**	46 (92)
	5 (g/m^2)	4(8)
Mean age at the time of diagnosis (years) (mean ± SD)		7.7 ±4.5
Total leukocyte count (/cumm) (Mean ± SE)		4864.14 ± 239.38
Platelet count(/cumm) (Mean ± SE)		272896.0 ± 17828.9
Neutrophil count (%) (Mean ± SE)		51.16 ± 1.79
SGPT (IU/L) (Mean ± SE)		48.68 ± 14.33

HDMTX: High dose methotrexate, SE: Standard error, SGPT: Serum glutamic pyruvate transaminase

Table 2: Allele and Genotype Frequencies of C677T and A1298C SNPs of MTHFR Gene in Study Subjects

	Study group	*P*	
C677T	Genotype frequencies n (%)		
	CC	36 (72)	< 0.0001
	CT	14 (28)	
	Allele frequencies		
	C	0.86	< 0.0001
	T	0.14	
A1298C	Genotype frequencies n (%))		
	AA	18 (36)	0.1077
	AC	24 (48)	
	CC	8 (16)	
	Allele frequencies		
	A	0.60	0.0466
	C	0.40	

The serum MTX levels of four cycles at 48 hours after the start of HDMTX infusion means of platelet count, total leukocyte count, and neutrophil count were compared among the patient groups based on the presence (wild type) or absence (carriers) of C677T polymorphism. (Table 3). The serum MTX level at 48 hours after the start of HDMTX infusion was found to be slightly high in all four cycles however, in the first cycle only, there was a significant increase in the level of methotrexate (p=0.016). The levels of SGPT, platelet count, total leukocyte count and neutrophil count in individuals' carriers (CT) did not differ significantly than the wild type.

There was no significant difference observed in the serum methotrexate level in all four cycles at 48 hours after the start of HDMTX infusion, between wild type and carriers for MTHFR A1298C patients, while SGPT level in carriers (AC and CC) was significantly elevated (p=<0.0001) as compared to wild type (AA), with no significant difference in platelet, total leukocyte and neutrophil counts (Table 4).

Table 3: Association of MTHFR C677T Variant with Serum Methotrexate Level and Hematological Indices in ALL Patients

Characteristics	Serum methotrexate level at 48 hrs after start of infusion(micromol/L)		*P*
	CC N=36 (Mean ± SE)	CT N=14 (Mean ± SE)	
MTX level at cycle 1	0.34 ± 0.03	0.51 ± 0.09	0.016
MTX level at cycle 2	0.45 ± 0.06	0.51 ± 0.09	0.56
MTX level at cycle 3	0.39 ± 0.03	0.49 ± 0.08	0.18
MTX level at cycle 4	0.60 ± 0.09	0.92 ± 0.16	0.074
Total leukocyte count (/cumm)	5108.60 ± 274.26	4235.54 ± 456.56	0.1148
Platelet count (/cumm)	277351.80 ± 19117.19	261439.69± 41651.06	0.69
Neutrophil count (%)	52.79 ± 1.94	46.96 ± 3.88	0.14
SGPT (IU/L)	43.01 ± 15.06	63.27 ± 34.2	0.53

MTX: Methotrexate, SGPT: Serum glutamic pyruvate transaminase, SE- standard error, MTX- Methotrexate

Table 4: Association of MTHFR A1298C Variant with Serum Methotrexate Level and Hematological Indices in ALL Patients

Characteristics	Serum methotrexate level at 48 hrs after start of infusion (micromol/L)			*P*
	AA N= 18 (Mean ± SE)	AC N= 24 (Mean ± SE)	CC N= 8 (Mean ± SE)	
MTX level at cycle 1	0.65 ± 0.12	0.7 ± 0.11	0.76 ± 0.28	0.9
MTX level at cycle 2	0.45 ± 0.07	0.45 ± 0.09	0.53 ± 0.1	0.87
MTX level at cycle 3	0.44 ± 0.06	0.41 ± 0.06	0.37 ± 0.06	0.78
MTX level at cycle 4	0.41 ± 0.05	0.37 ± 0.06	0.37 ± 0.06	0.86
Total leukocyte count	4618.06± 359.12	5224.23 ± 370.69	4337.57 ± 577.24	0.33
Platelet count	268943.87 ± 21767.45	296329.8 ± 27888.7	211489.6 ± 53590.08	0.26
Neutrophil count	51.82 ± 3.01	50.87 ± 2.62	50.53 ± 4.67	0.96
SGPT	25.02 ± 2.29	56.77± 26.59	101.46 ± 66.41	<0.0001

SGPT: Serum glutamic pyruvate transaminase, SE- standard Error, MTX- Methotrexate

When the proportions of the patients were calculated as per the cut-off values derived [8] CT genotype of C667T was found to be higher serum MTX level in 10.7 % cycles of MTX infusion and dose is reduced in 3 patients (6%) after the first methotrexate cycle (Table 5).

The logistic regression analysis of polymorphisms with MTX levels at all 4 cycles revealed, positive association of presence of T allele of C677T with MTX level at cycle 4, while no significant difference found between the polymorphisms of C677T & A1298C, and MTX levels at all 4 cycles.

The haplotype analysis of C677T and A1298C revealed the percentages of 0.46, 0.4 and 0.14 for CA, CC and TA haplotypes. The difference in the phenotypes of methotrexate levels from cycle 1 & cycle 4, leukocyte count and platelet count has significantly differed among the haplotypes. The linkage disequilibrium of C677T and A1298C shows the D' statistic of 0.9989, r -value of 0.3291 with a corresponding p value of 0.001, depicting significant chances of inheriting both polymorphisms together in next progeny from the parent carrying them.

Table 5: Distribution of Serum MTX Level at 48 Hours as Per Cut Off Value of 1 µmol/L After HDMTX Infusion as per the Genotypes for MTHFR Polymorphisms

	Serum methotrexate level	MTX level at				Total cycles n (%)	Dose reduction (n)
		cycle 1	cycle 2	cycle 3	cycle 4		
		Number of cycles (%)					
C677T							
CC (N=36)	<1 µmol/L	31	34	35	36	136 (94.4)	0
	>1 µmol/L	5	2	1	0	8 (5.6)	
CT (14)	<1 µmol/L	11	13	13	13	50 (89.3)	3
	>1 µmol/L	3	1	1	1	6 (10.7)	
A1298C							
AA (N=18)	<1 µmol/L	17	18	18	18	71 (98.6)	0
	>1 µmol/L	1	0	0	0	1(1.4)	
AC (N=24)	<1 µmol/L	20	24	24	24	92 (95.8)	4
	>1 µmol/L	4	0	0	0	4(4.2)	
CC (N=8)	<1 µmol/L	6	7	8	8	29(90.6)	2
	>1 µmol/L	2	1	0	0	3(9.4)	

Table 6: Logistic Regression Analysis of C667T and A1298T Polymorphisms of MTHFR with Methotrexate Levels at Different Cycles in Leukemia Patients

Variable	Coefficient	SE	*P*
MTHFR C677T			
MTX level at cycle 1	0.86886	0.60693	0.1523
MTX level at cycle2	-1.90775	1.89983	0.3153
MTX level atcycle3	-1.67603	2.48860	0.5006
MTX level at cycle 4	6.03316	2.77614	0.0298
MTHFR A1298C			
MTX level at cycle 1	0.37555	0.60538	0.5350
MTX level at cycle2	0.87765	1.20872	0.4678
MTX level atcycle3	-0.45581	1.99187	0.8190
MTX level at cycle 4	-1.54858	2.07967	0.4565

SE- standard Error, MTX- Methotrexate

Table 7: Expected Phenotypic Means according to Possible Haplotypes for SNPs C677T and A1298C of the MTHFR gene in Leukemia Patients Considering CA as Baseline

	CC (0.4)	TA (0.14)	*P*
MTX level at cycle 1	0.14 (-0.09 - 0.37)	0.39 (0.03 - 0.75)	0.039
MTX level at cycle2	0.05 (-0.1 - 0.2)	0.09 (-0.14 - 0.33)	0.45
MTX level atcycle3	-0.01 (-0.12 - 0.09)	0.1 (-0.06 - 0.25)	0.24
MTX level at cycle 4	0.01 (-0.08 - 0.11)	0.18 (0.04 - 0.33)	0.019
Leukocyte count	-202.79 (-442.56 - 36.99)	-977.66 (-1034.53 - -920.8)	<0.0001
Platelet count	-23588.74 (-Inf - Inf)	-28080.91 (-Inf - Inf)	<0.0001
Neutrophil count	20.45 (-10.79 - 51.69)	-4.6 (-52.8 - 43.61)	0.85
SGPT level	-11.19 (-44.87 - 22.5)	30.98 (-17.06 - 79.01)	0.21

SGPT: Serum glutamic pyruvic transaminase, SE standard error, MTX- Methotrexate

Discussion:

ALL is common cancer among children reported for 72% of all cancers [8]. The highest reported cases of ALL in the developed countries were between the age group of 2 to 5 years and were boys. [9] In the present study, majority of patients were boys (72%) and the mean age was 7.7 ±4.5 years. As per the BFM 2002 protocol, the dose of HDMTX in B cell ALL was 2 g/m^2 and maximum patients (92%) of our study were B cell ALL.

HDMTX is an important treatment modality for CNS prophylaxis in ALL patients. The drug MTX acts as an anti-folate agent blocking the conversion of the 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate in the folic acid cycle. Two polymorphisms in the MTHFR gene, C677T and A1298G, are known to lower the MTHFR activity along with increased levels of homocysteine [10-11]. The distribution of these polymorphisms of MTHFR differ by ethnicity, the MTHFR C677T mutant allele is present in 34% of Caucasians, 14% of African Americans and 40% in the Italian population [12-14]. In the present study, majority of patients (72%) were wild type (CC) for C677T polymorphism of MTHFR, while 28% were heterozygotes (CT), while no patient was reported to be homozygous for the mutant (T) allele with significant change in the frequency, the results were compared with various studies, which reported the absence of homozygous genotype (TT) for MTHFR C677T [15-17]. The frequencies of CC and CT genotype of C677T were, 4.9% and 15.1% of C677T respectively reported in the study from India by Adiga *et al*. [15] in which frequencies of alleles were comparatively less as compared to present study.

The second polymorphism studied in the present study was A1298C, out of all patients studied 36% of patients were wild type (AA), 48% were heterozygotes (AC) and 16% were homozygous (CC) for mutant C allele. Another study from India by Nikbakht *et al.* [18] reported slightly deviated results than the present study, with genotype frequencies of 52%, 41.6 % and 11.6 % for AC, AA, and CC respectively. The variation in the frequencies of A1298 polymorphism was also found in several studies reported from the other countries [19-21]. This diversity in each study might be the result of geographic and ethnic distributions.

The kinetics response and efficacy of HDMTX are influenced by polymorphisms in the MTX pathway genes such as SLCO1B1, ABCC2, SLC19A1, MTHFR, TYMS [23]. Many studies have reported that the two frequent functional polymorphisms of MTHFR, C677T, and A1298C, are connected with the reduced enzyme activity of MTHFR which leads to MTX toxicity, the individuals with CT and TT genotype for C677T showed a reduction of 60% and 30% in MTHFR activity respectively. Whereas CC genotype for polymorphism A1298C, reported to have 60% of total MTHFR normal activity [23-25]. As a result, the patients with the variant alleles, may lead to impaired clearance of MTX and may be susceptible to the its toxicity [26].

The highest toxicity was developed in patients carriers for C677T polymorphism as compared to patients wild type (CC), and dose reduction was required in 6% of patients. The MTX level in the 4th cycle was found to be significantly higher in

patients carriers (CT) than wild type (AA) for MTHFR A1298C polymorphism, while in the remaining three cycles the levels were higher but not significant. Contradictory results were observed by Haase *et al.* [26] who reported higher serum MTX levels in patients' wild type for C677T compared to carriers for polymorphism. Toxicity in patients who were carriers for C677T polymorphism could delay the elimination of MTX since most of these patients had an increased level of serum MTX ($\geqslant$ 1.0 µmol/L). According to a meta-analysis, many of the studies have found no association between C667T polymorphism and toxicities [7]. The genotype distribution of patients on the basis of development of toxicities i.e. wild type and carriers, revealed no significant difference in the number of patients and toxicity developed.

In the present study, higher incidence of thrombocytopenia, leucopenia episodes and statistically significant elevation in liver enzymes were noted in patients' homozygotes for C allele for A1298C. Maximum dose reduction i.e. 25% was required in patient's mutant for A1298C polymorphism. These findings of present study are consistent with the study conducted by Kantar *et al.* [27] reporting a higher incidence of thrombocytopenia, anemia and higher serum MTX levels in patient's carrier (AC+CC) for MTHFR A1298C polymorphism.

Conclusion:

The present study concludes CT genotype of C667T is associated with higher serum MTX level at 48 hours in ALL patients treated with HDMTX infusion. There was no significant difference in the serum methotrexate level of all four cycles at 48 hours after the start of HDMTX infusion between wild and variant MTHFR A1298C patients. However, the liver toxicities were reported in patients with CC genotype of A1298C polymorphism of MTHFR. The metabolic pathway for MTX is very complex, and the genetic variants may influence the toxicity and outcomes. Identifying a single genetic difference is not sufficient for a proper prognostic factor of the kinetics and toxicity of MTX. A large prospective study examining the effects of several SNPs is required to understand the role of genetic variation.

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