ORIGINAL ARTICLE

Detection of Genetic polymorphisms of Methylene tetrahydrofolate reductase among Sudanese patients with chronic myeloid leukemia

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ABSTRACT

Background: Methylenetetrahydrofolate reductase (MTHFR) plays a crucial part in cellular biochemistry because it is involved in the metabolism of the folate critical to DNA synthesis.
Aim: To evaluate gene polymorphisms in MTHFR and the susceptibility to chronic myelocytic leukemia (CML).
Methods: Two hundred CML cases were included in this study, plus one hundred healthy volunteers as controls. All participants were genotyped for both the A1298C and C677T polymorphisms of MTHFR.
Results: Presence of the 677CC/1298AA (wild-type) group was the observational benchmark for the study. The frequency of heterozygous 677CT in CML patients was significantly higher than in the control group [23.5 vs 0.5% (*p value* = 0.000)], and it imparted a significant risk in CML development. No comparable association was found for homozygous 677TT (OR=1.514, 95% CI: 0.809-2.835, *p*=1.95). For the 1298 A>C polymorphism, a significant variation in the prevalence of the 1298AC genotype between CML patients and controls was found [55% vs 44% *(p*=0.006)]. However, the frequency of the 1298CC genotype was higher in CML patients (11%) in comparison with the controls (0%), (*p*=0.006). Also, both the MTHFR 1289AC and 1298CC genotypes were considered as genetic factors which increased the risk of CML. Furthermore, genotypic analysis revealed the following four combinations correlating to an elevated risk of CML: 677CC/1298CC, 677CT/1298AA, 677CT/1298AC and 677TT/1298AC.
Conclusions: This study found evidence of an association between CML in Sudanese patients and the C677T and A1298C polymorphisms of the MTHFR gene.
Keywords: MTHFR, A1298C and C677T polymorphisms, CML, Sudan

INTRODUCTION

Leukemia is derived from an unusual proliferation of hematopoietic tissue. The disease manifests itself in two forms - chronic and acute[1]. Chronic myeloid leukemia (CML) is also known as chronic granulocytic leukemia (CGL) and it affects genomic stability, causing an imbalance between the proliferation and apoptosis of cells. It is this which drives the leukemic changes in CML[2].

Epidemiological studies point to the association between low folate intake and elevated cancer risk, but CML pathogenesis is also assumed as related to folate metabolism[3].

The clinical manifestation and biological aspects of CML have been well reported[2], but little is known about mechanisms underlying the predisposition of individuals to the disease. Such mechanisms include hereditary, familial, geographic, ethnic, or economic factors[4].

The methylenetetrahydrofolate reductase gene (MTHFR) is identified by several polymorphisms[5]. Most prominent are single-nucleotide polymorphisms (SNPs), including C677T and A1298C[6]. The MTHFR gene regulates metabolism of folic acid critical to nucleotide biosynthesis and C677T and A1298C SNPs inhibits its enzyme activity [3].

Tong and co-workers (2018) stated that polymorphisms of C677T and A1298C have been frequently studied in other cancers[7] and that less than a third of individuals with the MTHFR 677TT genotype (homozygous state) display enzyme activity in comparison with those cases with the wild-form allele[8]. Contrastingly, enzyme activity was found in sixty percent of individuals with the heterozygous MTHFR 677CT allele[9].

Correlation in the detection of C677T and A1298C polymorphisms of the MTHFR gene and the associated risk of other leukemias has also been documented[9,10], with indications of a reduced risk of lymphoblastic leukemia among individuals with the 677TT variant[11]. However, findings about this correlation in other leukemias are unsubstantiated[9].

Another study has reported that MTHFR polymorphisms, namely C677T and A1298C in CML patients had no inherited genetic susceptibility, but that risk was higher amongst 1298C variant carriers of Asian ethnicity compared with Caucasian populations. The suggestion here therefore, is that ethnicity is a factor in MTHFR A1298C polymorphism and the risk of contracting CML[12].

Other studies also demonstrate the role of MTHFR polymorphism with the risk of CML[3,8] but the goal of the current study was to explore this association with its most common polymorphisms among diverse ethnicities of the Sudanese population.

MATERIALS AND METHODS

This study investigated two hundred CML patients (132 male, 68 female) with the mean age of 45.06(±12.34 years). The control group comprised one hundred healthy individuals (51 male, 49 female) with a mean age of 39.2

(±13.3 years). All procedures involving human subjects were approved by the research committee of Al-Neelain University.

3ml of EDTA anticoagulated blood was taken from each subject for hematological and molecular analysis. Cytogenetic, hematological and BCR-ABL transcript analysis were used to diagnose CML patients, with BCR-ABL transcript analysis undertaken at the Radiation and Isotope Center of Khartoum (RICK), between August 2014 and August 2017. One hundred and ninety nine CML patients were found to be Philadelphia *(*Ph) chromosome positive (99.5%), in the chronic phase (CP), with a single patient (Ph), in the Accelerated phase (AP) (0.5%).

MTHFR genotyping: Genotyping of MTHFR 677C>T and A1298C SNPs was performed as described previously [13, 14]. The PCR products were then treated with suitable restriction enzymes, HinfI for C677T and Mbo11 for A1298C as shown in Figures 1-A and 2-A respectively.

Statistical analysis: The Hardy-Weinberg equilibrium was used to detect allele frequency prior to analysis. Non-parametric data was undertaken using ANOVA and Kruskal Wallis. The Chi square test was also used within and between groups and logistic regression was utilized to calculate Odds ratios (ORs). Analyses were performed using SPSS, Inc, Chicago, IL, version 25.

RESULTS

There was no age related difference for genotypes of MTHFR, C677T and A1298C under the Kruskall-Wallis Test, p = 0.602 and 0.26 respectively, as shown in Table 1. Our results also revealed no statistical difference in gender for genotypes of the two polymorphisms of MTHFR in patients with CML, p =0.79 and 0.11, respectively.

Genotypic distribution (C677T and A1298C) and alleles in CML patients against the controls was demonstrated in Table 2.

Table 1: Comparison of age and gender according to C677T and A1298C genotype among patients

Variable	MTHFR genotypes					
	677CC	**677CT**	**677TT**	**12988AA**	**1298AC**	**1298CC**
Age(years) M (±SD)	45.5 (11.9)	43.1 (14.6)	45.5 (10.3)	44.6 (11.2)	45.9 (13.1)	41.8 (11.2)
Gender Male N (%)	81 (40.5)	25 (12.5)	26 (13.0)	36 (18)	85 (42.5)	11 (5.5)
Female N (%)	32 (16.0)	22 (11.0)	14 (7.0)	28 (14.0)	29 (14.5)	11 (5.5)
Total N (%)	113 (56.5)	47 (23.5)	40 (20)	64 (32)	114 (57)	22 (11)

M= mean; SD=standard deviation;

Table 2: Association between C677T and A1298Cgenotype and risk of CML

		Patient No (%)	Control No (%)	OR	95% CI
MTHFRC677T	CC	113 (56.5)	77 (77.0)	Ref	
	CT	47 (23.5)[a]	5 (5.0)	6.405	2.437-16.84
	TT	40 (20.0)[b]	18 (18.0)	1.514	.809-2.84
	C allele	273 (68.3)[a]	159 (79.5)	**0.554**	**0.37 - 0.87**
	T allele	127 (31.8)[a]	41 (20.5)		
MTHFRA1298C	AA	68 (34.0)	55 (56.0)	Ref	
	AC	110 (55.0)[a]	44 (44.0)	2.022[a]	1.23 - 3.33
	CC	22 (11.0)[a]	1 (0.0)	17.79[a]	2.33 - 136.21
	A allele	246 (61.5)[a]	156 (78)	**0.45**	**0.3 - 0.7**
	C allele	154 (38.5)[a]	44 (22)		

Key: N= total number; OR= odd ratio; (P-value) is shown in superscript parenthesis; (a) = <0.05, (b) = >0.05

Table 3: MTHFR genotype interaction analysis in CML patient versus control

MTHFRC677T and A1298C		Case		OR	95%CI	
		Control No (%)	Patient No (%)			
Combination	CC and AA	36 (36)	36 (18)	Ref		
	CC and AC	38 (38)	62 (31) [b]	1.632	.883	3.014
	CC and CC	1 (1)	15 (7.5) [a]	15.000	1.881	119.622
	CT and AA	3 (3)	16 (8) [a]	5.333	1.429	19.901
	CT and AC	2 (2)	25 (12.5) [a]	12.500	2.754	56.729
	CT and CC	1 (1)	6 (3) [b]	6.000	.687	52.383
	TT and AA	15 (15)	16 (8) [b]	1.067	.459	2.477
	TT and AC	3 (3)	23 (11.5) [a]	7.667	2.113	27.817
	TT and CC	1 (1)	1 (0.5) [b]	1.000	.060	16.611

Key: N= total number; statistical significance (P-value) is shown in superscript parenthesis; (a) = <0.05, (b) = >0.05

Prevalence of the MTHFR 677CC genotype was higher among controls than for CML patients; 77% compared to 56.5%, respectively. The frequency of the 677CT genotype was also significantly higher (OR = 6.405, 95% CI: 2.437-16.836, p = 0.000) among CML patients (23.5%) compared to the control. Frequency of the C677T

TT genotype was 20% within CML patients as opposed to 18% of the control group with *p value* = 0.195,and this was of no statistical difference.

Of MTHFR A1298C genotypes, the frequency of control subjects with the 1298AA genotype was higher at 56% compared to 34% for CML patients. Conversely, the heterozygous MTHFR 1298AC genotype was significantly higher in CML patients, 55% in comparison with controls, 44.0% (OR = 2.022, 95% CI: 1.228-3.329, *p* = 0.006).

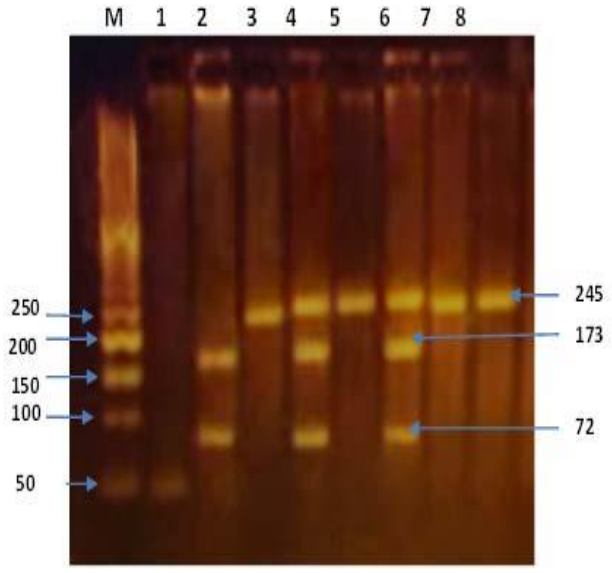
Interestingly, the mutant genotype 1298CC was dominant in 11% of CML patients and demonstrated a statistically significant relationship between MTHFR A1298C genotypes in CML patients and the control (OR = 17.794, 95% CI: 2.325-136.3205, *p* = 0.006).

The occurrence of the variant T allele for 677 C>T SNP was also higher in CML patients; 31.75% as opposed to 20.5% in the control, and this difference was also significant (*p* =0.004).

Moreover, the prevalence of the C variant allele for the 1298 A>C polymorphism was also significantly higher amongst the CML group at 38.5 % as opposed to 22 % in the control, with *p* = (0.000).

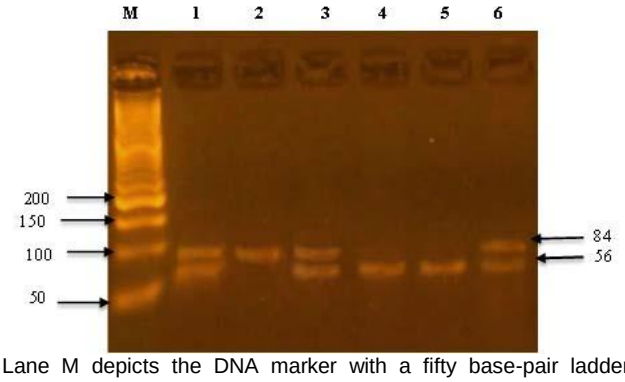
In this study, we detected significant levels of interaction between MTHFR C677T and MTHFR A1298 C (Table 3). CML patients with the compound genotypes 677CC/1298CC, 677CT/1298AA, 677 CT/1298AC and 677TT/1298AC were found to be associated with an elevated risk of CML (OR=15.000, 95% CI: 1.881–119.622, *p* = 0.011; OR=5.333, 95% CI: 1.429–19.901, *p* = 0.013; OR=12.500, 95% CI: 2.754–456.729, *p* = 0.001; OR=7.667, 95% CI:2.113–27.817, *p* =0.002 respectively).

Figure 1: Some of RFLP products of MTHFR C677T; using Hinf I.



Lane M depicts the DNA marker with a fifty base-pair. Lanes 2 is the DNA which is (TT) genotype with two fragments 173 and 72, whereas lane 3, 5 and 7 are the DNA which is (CC) genotype with one fragment 245 bp for Hinf1. Lanes 4 and 6 (CT) genotype with three fragments 245, 173 and 72 bp, Lane 5 negative control.

Fig. 2: An agrose gel electrophoresis of some of RFLP products of MTHFR C1298A; using Mob11



Lane M depicts the DNA marker with a fifty base-pair ladder. Lanes 1,3 and 6 are the DNA which is heterozygous AC with two fragments 84 and 56 bp, but 31,30 and 28 not seen. lanes 4 and 5 are the DNA which is (AA) wild type with one fragment 56 bp (also 31,30,28,18 not seen). Lane 2 shows homozygous mutant CC with 84 pb but small bands, 31, 30 and 18 not seen.

DISCUSSION

CML results from pluripotent hematopoietic stem cell transformation, displayed clinical and biological features[15], but little is known about the susceptibility to this disorder [3]. Genetic variants of both C677T plus A1298C, did however, effect a decrease in MTHFR activity[2,5].

SNPs of MTHFR C677T as well as A1298C are plausible candidates for both CML etiology, and disease progression, owing to their shared potential impact on chromosomal abnormalities connected with the clonal evolution of CML[2]. Globally, these polymorphisms differ between populations[16].

Genetic variations by subject ethnicity from evolutionary adaptation to the environment[6], affects disease susceptibility and the allele frequency of pharmacogenetically significant loci[2]. A small number of studies have investigated potential associations of MTHFR polymorphisms and CML, but produced contradictory findings between ethnic groups[3].

The current research investigates the relation between MTHFR (677 C>T plus 1298 A>C) differences and CML susceptibility and progression within the Sudanese. The study found no significant difference in the polymorphisms of MTHFR C677T and MTHFR A1298C frequency regarding gender and age[8, 17], which is in contrast to a study by Lordelo *et al*, showing a significant difference between the control and CML classes for age [2].

Our study demonstrates a statistically significant correlation between the two most common polymorphisms of the MTHFR gene, (677 C>T and 1298 A>C), and susceptibility to CML. The association was very clear in the heterozygous mutant for each MTHFR polymorphism and homozygous 1298CC, which has been associated with the altered distribution of intracellular folate metabolites[18]. Both C677T and A1298C are associated with reduced enzyme activity and rapidly replicating cell types, including hematopoietic cells, and are especially sensitive to intracellular folate availability changes[2,19]. The present study is also in agreement with Aly et al, (2014) in stating that the two common thermos-labile SNPs in the MTHFR

gene (C677T and A1298C) are associated with CML susceptibility because they result in a decrease in MTHFR activity[8].

This current study also demonstrated a relationship with the MTHFR C677T polymorphism and CML because of the higher occurrence of the CT genotype and T allele in cases. Ethnic and geographical location factors were notable because 0.5% of the control possessed a mutant heterozygous MTHFR CT genotype, and 20.5% the T allele *i.e.* the 677T allele of the MTHFR gene polymorphism.

In a study carried out by Wilcken and Co-workers on $7x10^3$ newborns in sixteen regions worldwide, the 677T allele presence was as follows: 26.6%, 46%, 25.7% and 44.2% in Italy, The Middle East and Northern China, respectively[20]. This indicates the selective effects of geographic and ethnic difference and the presence of the MTHFR677T allele[21]. These findings are contrary to the results of this study.

Consistent with an analysis on Fragile X Syndrome within Iranian populations[22], our study demonstrated a correlation between the C677T MTHFR polymorphism and an elevated frequency of the 677T allele and the 677CT genotype in CML patients (*p value* =0.010;OR=2.459 for T allele frequency; *p* =0.028; OR=2.608 for CT genotype frequency)[22]**.** These findings were also in accordance with Diakite and co-workers[23], who found an association between the T allele and the CT genotype of the MTHFR C677T polymorphism in breast carcinoma amongst Moroccan women.

Consistently, Al-Achkar *et al* in Syria [17] and Bănescu *et al* in Romania[24] are also in agreement with Aly RM and co-workers[8], in finding a relation between the TT genotype of the MTHFR C677T polymorphism and an elevated risk of CML.

Conversely, several studies have found no significant association of 1p36.3 MTHFR (C677T) locus with CML, but such studies were concerned with ethnic groups as diverse as Iranian, Brazilian and Egyptian[2,6,25].

Within Sudan there are no published studies on the role played by MTHFR genes C677T (dbSNP rs1801133) and A1298C (dbSNP rs1801131) in CML susceptibility, but there are studies concerning this polymorphism with schizophrenia, bipolar disorder (BD) and Cardiac syndrome X(CSX)[26]. There is also a reverse observed by Merghani and co-workers[27], assuming no significant association between MTHFR C677T and vaso-occlusive crisis (VOC) risk among sickle cell patients[27]. This study finds significant alterations in genotype and allele prevalence of MTHFR A1298C between the control and patients with CML in Sudan and several studies have already demonstrated this correlation, with the presence of the MTHFR 1298 A>C polymorphism (homozygous form, CC genotype [8, 12, 17]. CML susceptibility is enhanced in both forms of the MTHFR 1298 A>C polymorphism (heterozygous and homozygous), with the frequency of AC and CC genotypes among patients being 55.5% and 11.0%, respectively.

Insofar as the C allele variant is significantly more common in CML patients (38.5% as opposed to 22% in the control), such high frequencies of the A1298C allele have also been observed in Lebanese, Syrian, Egyptian and the Iranian populations[8, 17, 25]. We may assume therefore that presence of this variant allele may have a role in the risk of CML.

Another study associated the MTHFR 1298AA genotype with a notably elevated risk of CML and that 1298 AC significantly decreased this risk[2]. Also, Khoshied and Collagenous have not stated significant variation in A1298C genotype distribution or allele frequency between the control and patients with CML in Egypt[6]**.** This result accords with the findings of Vahid and his colleagues, who found no significant association between the distribution of MTHFR polymorphisms (C677T and CA1298C) in myeloid leukemia and the control among the Iranian population[25].

The synergistic (mutually reinforcing) effect of MTHFR C677T and A1298C polymorphisms was observed to decrease enzyme activity[17]. Our study showed that the compounds 677CC/1298CC, 677CT/1298AA, 677TT/1298AC677and CT/1298AC genotypes were associated with risks of CML. The combination of heterozygous of both polymorphisms, C677T and A1298C principals to features like to those found in homozygotes 677TT, and has been related with minimized enzyme activity, reduced plasma folate levels and hyperhomocysteinemia[8]. These combinations were partially in agreement with other recent studies[8,17,24], but Robien and co-workers reported that persons possessing the 677CC/1298AA genotype had an elevated relapse risk, following bone marrow (BM) transplantation[28].

CONCLUSION

This study concludes that within a Sudanese population, there is high significant levels of the CT genotype and T allele in 677 C>T and the CC and AC genotypes with the C variant allele in 1298 A>C within the MTHFR gene of CML patients.

Polymorphisms within the MTHFR gene involving the C677T and A1298C genotypes however, were found to mainly accompany a raised risk of CML.

These results should be considered exploratory, and this study welcomes further research involving a larger sample number together with the assessment of the folate situation.

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