ORIGINAL ARTICLE

The role of genetic polymorphisms of the MTHFR (C677T and A1298C) gene in the incidence of Acute Myeloid Leukaemia

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ABSTRACT

Background: The burden of cancer is growing globally as one of the leading fatal diseases. Methylenetetrahydrofolate reductase (*MTHF* is a central enzyme involved in the metabolism of folate methylation of DNA and synthesis.
Aim: To investigate the role of genetic polymorphisms of the MTHFR (C677T and A1298C) gene in the incidence of acute myeloid leukemia (AML) in different populations from Sudan**.**
Methods: K_3EDTA blood samples of 3 ml were collected from patients and controls and recorded at the flow cytometry Unit of the Radiation and Isotope Centre Khartoum (RICK), from 06/2016 to 06/2018. Polymorphisms of the MTHFR gene, C677T and A1298C were genotyped by polymerase chain reaction /restriction fragment length polymorphism (PCR-RFLP) in 200 Sudanese AML patients, and 65 apparently healthy controls.
Results: The study showed cases with the A1298C polymorphism had no associated risk of AML (1298 AC: p = 0.262; 1298CC: *p* = 0.063). However, individuals with the C677T polymorphism had a significantly reduced risk of AML (677CT: p = 0.00; 677TT: p = 0.559).
Conclusions: In summary, this case-control study demonstrates that A1298C rather than C677T MTHFR polymorphisms may have a protective effect in AML carcinogenesis among the Sudanese study group.
Keywords: AML, MTHFR, C677T and A1298C genes, Sudan

INTRODUCTION

Despite improvements in health education and facilities for health care, the burden of cancer is increasing internationally - especially across Africa and The Middle East. To date, data is largely unavailable for Leukemia incidence and its predisposing factors[1,2].

Leukemia is a neoplastic hematopoietic disease characterized by the monoclonal atypical expansion of one progenitor cell. It results in permanent genetic alteration, which disturbs the process of cell development and hematopoiesis[3].

AML is a heterogeneous group of hematological malignancies, and clinical treatment and duration requires a different style of management action and protocol. AML is highly variable in incidence with a broad spectrum of non-specific signs and symptoms. Malignancy affects all age groups, but mainly adults[4].

Carcinogenesis is a complex multistep process, in which both genetic predisposition and environmental factors are strongly associated with cancer development[5]. However, the incidence rate in the majority of leukemic cases is not strongly affected by such risk factors[6]. MTHFR however is an essential folate metabolizing enzyme that may influence and disrupt DNA methylation, synthesis and repair[7].

Several analytical studies have evidenced the association between genetic polymorphisms of gene encoding enzymes (involved in xenobiotic biotransformation), and cancer. However, such results have been inconclusive[3].

A known risk factor for leukemia is a change in the quantity and quality of folic acid. Folate and its metabolites are necessary for the processes of DNA methylation, synthesis and repair[8]. Epidemiological studies suggest that folate deficiency and consequent cell signaling pathway disruption, play a significant role in multiple DNA aberration and leukemia development[9].

Mutations and other genetic alterations associated with CML include deletion, inversion and translocation. The folate metabolites of carcinogens can influence gene expression and DNA instability. DNA translocations, inversions or deletions in hematopoietic progenitor cells will lead to leukemia[3].

MTHFR plays a significant role in cellular metabolism by regulating the levels of folate and homocysteine[3,7]. A drop in MTHFR activity contributes to many genetic changes and events, including changes in chromosomal recombination, abnormal chromosome segregation and global hypo-methylation of DNA[10].

The main function of 5, 10-methylenetetrahydrofolate is the conversion of dUMP to dTMP in the donation of a methyl group, which enables DNA proliferation and enhances DNA repair. Decreases in the MTHFR enzymatic activity leads to falling levels of 5,10-methylenetetrahydrofolate causing an elevation in homocystine and impairment of DNA methylation. This reduction is associated with two common gene polymorphisms; MTHFR - C677T and A1298C[11].

Two common MTHFR single nucleotide polymorphic sites have been identified, namely rs1801133 (C677T) and rs1801131 (A1298C). The C677T variant results in a transition of cytosine to thymine at position 677 within exon 4 of the MTHFR gene. This variant results in a substitution of alanine-to-valine at codon 222 of the enzyme, while the

A1298C mutation causes a glutamate-to-alanine substitution at codon 429[12].

Along with A1298C variant presence, studies have demonstrated reduced enzyme activity in MTHFR polymorphisms involving C677T and A1298C[3,13,14]. Presence of MTHFR 677CT and 677TT genotypes inhibit enzyme activity of the wild type genotype 677CC by 60% and 30%, respectively. Reductions in MTHFR enzyme activity lead to high concentrations of homocysteine in the plasma. This affects the folate metabolic balance with a subsequent increase in the availability of 5,10-MTHF, for thymine synthesis[10].

The Sudanese ethnicity is distinguished by several tribal groups of Arab and African origin, who live within a diverse environment. AML patients and healthy controls participant in this study were drawn from all such groups, according to guidelines approved by the Scientific Research Committee of the Faculty of Medical Laboratory Sciences at Al Neelein University.

In this study, we critically elaborate the association between genetic variation and xenobiotic exposure as well as endogenous physiology. In the wake of an increasing incidence of leukemia in Sudan, we will propose educational initiatives to raise public awareness in the disease. We also aim to elaborate more on leukemia risk factors and their associations with genetic polymorphism, to further disease prognosis. Cumulatively, these initiatives will add to scientific understanding in the development of a national protocol for biologically based leukemia therapies.

METHODS

This is a case control study conducted in Khartoum State Sudan, involving local participants. Participants included 200 patients with AML and without previous treatment, and 65 apparently healthy controls. Three milliliter (3ml) of venous blood was collected in EDTA containers from all participants with each sample then being subject to molecular analysis. All patients had been professionally diagnosed as AML, using full blood count, blood morphology and flow cytometry.

Molecular analysis: The Blood Genomic DNA 'Miniprep' Kit (QIAGEN) was used for DNA extraction. This kit is a simple and convenient method for isolating high-quality genomic DNA, with a proven track record in the analysis of fresh or frozen whole blood.

Genotyping of MTHFR was then performed by PCR (Polymerase Chain Reaction) and RFLP (Restriction Fragment Length Polymorphism). PCR products were then treated with suitable restriction enzymes, HinfI for C677T and Mbo11 for A1298C[15, 16] and the results analyzed.

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

In this study, 200 patients with AML and 65 healthy individual's age matched as a control group. All the patient and control participants were successfully genotyped by PCR-RFLP as shown in Table 1.

This table demonstrates the frequency of gene types/variants among patients and controls and compares their genetic profile using odds ratio, confidence intervals and allele frequency for of MTHFR polymorphisms C677T and A1298C. For AML patients the most frequent C677T genotype was the wild type CC (86.5%), followed by heterozygous CT (12.5%). The homozygous TT genotype was recorded in a low frequency for both AML patients (1.0%) and the control (1.5%), as shown in Table 1.

Interestingly, the 677 CT genotype was statistically associated with a decreased risk for AML (OR = 0.267, 95% CI: 0.142–0.536, *p value = 0.00*), whereas in 677TT no significant differences were observed in this genotype (OR = 0.486, 95% CI = 0.043–5.482, *p value*= 0.559), as shown in Table 3. Table 2 demonstrates the frequency of specific alleles across patients and controls and the allele of MTHFR 677 C was high in both groups.

With regard to MTHFR A1298C, Table 1 records the highest frequency with AC (95.0%), followed by the AA (5.0%). The CC genotype was found with low frequency in both AML patients and the control participants.

Table 3 shows No significant differences were observed for 1298AC or 1298CC compared with 1298AA (1298 AC: OR = 0.305, 95% CI = 0.038–2.429, P = 0.262; 1298CC: OR = 0.050, 95% CI = 0.002–1.179, *p value = 0.063*).

For the MTHFR A1298C polymorphism the A and C alleles were almost equally frequent in both groups, AML (A: 52.5%, C: 47.5%) and Control (A: 48.5%, C: 51.5 %). A p-value of 0.423 indicated that there was no significant difference between alleles in patients and controls.

Additionally, no significant differences were observed for combination analysis between MTHFR A1298C and MTHFR C677T in patient and control, as shown in Table 4.

Table 1: Comparison between Patients and control in genetic data

		Cases	
		Patient N (%)	**Control N (%)**
MTHFR 1298	AA	10 (5.0)	1 (1.5)
	AC	189 (94.5)	62 (95.4)
	CC	1(0.5)	2 (3.1)
MTHFR 677	Heterozygous CT	25 (12.5)	22 (33.8)
	Homozygous TT	2 (1.0)	1 (1.5)
	Wild type CC	173 (86.5)	42 (64.6)

Table 2: Comparison between Patients and control in Alleles frequency

		Cases		P-value	Odd Ratio	95% CI	
		Patient N (%)	**Control N (%)**				
MTHFR 1298	A	210 (52.5)	63 (48.5)	0.423	1.2	0.79	1.75
	C	190 (47.5)	67 (51.5)				
MTHFR 677	C	371 (92.75)	108 (83.08)	0.001	2.606	1.44	4.72
	T	29 (7.25)	22 (16.92)				

Table 3: Odd ratio + Confidence interval for genotypes of MTHFR genes, 1298 and 677

		Case		Exp(B)	95%CI		P-value
		Control N (%)	Patient N (%)		Lower	Upper	
MTHFR 1298	1.00 AA	1 (1.54)	10 (5.0)	Ref			
	2.00 AC	62 (95.4)	189 (94.5)	0.305	0.038	2.429	0.262
	3.00 CC	2 (3.1)	1 (0.5)	0.050	0.002	1.179	0.063
MTHFR 677	1.00 CC	42 (64.6)	173 (86.5)	Ref			
	2.00 CT	22 (33.9)	25 (12.5)	0.276	0.142	0.536	0.000
	3.00 TT	1 (1.5)	2 (1.0)	0.486	0.043	5.482	0.559

Table 4 Combination analysis between MTHFR 1298 and MTHFR 677 in study population

MTHFR 1298 and MTHFR 677	Control	Patient	Exp(B)	95%CI		P-value
1.00 AA and CC	1 (1.5)	9 (4.5)	Ref			
4.00 AC and CC	40 (61.5)	163 (81.5)	0.45	0.06	3.68	0.458
5.00 AC and CT	21 (32.3)	25 (12.5)	0.13	0.02	1.138	0.065
6.00 AC and TT	1 (1.54)	2 (1)	0.22	0.01	5.28	0.352
7.00 CC and CC	2 (3.08)	1 (0.5)	0.06	0.00	1.32	0.074

DISCUSSION

There has been little research regarding MTHFR polymorphisms and the susceptibility to developing cancer and Leukemia in particular. Arising specifically from MTHFR C677T polymorphism within AML cases, 86.5% had the wild type CC genotype and 12.5% the heterozygous CT genotype.

Conversely and as a result of the MTHFR C677T polymorphism in Sudanese subjects, homozygous TT genotype presence was low for both AML patients (1.0%) and the control (1.5%). Similar low frequencies of the homozygote variant genotype (677TT) were also obtained by *Deligezer et Al* in their studies on patients with AML, Acute Lymphocytic Leukemia (ALL), and Chronic Myelogenous Leukemia (CML)[17].

Other studies however have found a higher presence of the TT genotype, suggesting that genotypes arising from MTHFR polymorphisms will vary as a result of geographical location and ethnicity. Homozygous TT presence was found in 20% of CML cases in a study undertaken in N China[18]. And studies published by *Vahid et Al*. and *Rizwan et Al,* found frequencies of the MTHFR 677TT genotype in AML patients to be 8.4% in Iranians and 9.82% in North Indians, respectively [13]. Further studies have evaluated 677TT homozygosity within the Arab world and outside, with a frequency of 11.0% amongst Lebanese cases, 10.4% in German patients and 10.0% in Greek patients[19].

Our findings however, are in agreement with contemporary knowledge on the protective effect of *MTHFR* 677TT to acute leukemia[20, 21].

A recent review of published studies supports the association between MTHFR C677T (but not MTHFR A1298C) polymorphisms and the risk of developing leukemia [22]. Prior to this review however, it was believed that MTHFR C677T polymorphisms were not significantly associated with a risk of AML development[23, 24].

Concerning MTHFR 677 CT (OD 0.37) presence, our findings are similar to a case controlled study undertaken on AML child patients in Brazil, in associating this genotype with a decreased risk of contracting the disease; [OR, 0.37; CI 95%, 0.14 – 0.92][14]. Again, such findings are dissimilar to studies conducted in other parts of the World e.g. UK, who found no such association[25].

Interestingly, our study demonstrates a statistically strong relationship between 677CT genotype presence and a decreased risk of AML development (OR = 0.267, 95% CI: 0.142–0.536, p value = 0.00), whereas no significant differences were observed in 677TT genotype, (OR = 0.486, 95% CI = 0.043–5.482, p value= 0.559). This can be explained by a possible 'heterozygous advantage' in certain MTHFR variants. Although heterozygous MTHFR variants have higher enzyme activity than homozygous variants, this may be one of the prophylactic effects of 677CT.

Our results also highlighted a high allele frequency for MTHFR 677C in both patients (83.08%) and controls (92.75%), whereas low allele frequency was noticed for the T allele, 16.92% in patients and 7.25% in the control. This agrees with similar case studies in India and in Iran, which demonstrated elevated allele frequencies for MTHFR 677C genotypes in both AML patients and the controls[13, 23].

Frequencies of A- and C- alleles were similar in MTHFR A1298C polymorphism for both AML patients (A: 52.5%, C: 47.5%) and the control (A: 48.5%, C: 51.5 %), showing no significant relation between these alleles and AML susceptibility. Furthermore, the most frequent genotype in MTHFR A1298C polymorphism was found to be AC (95.0%), followed by AA (5.0%). The lowest allele frequency was found in the CC genotype for both AML patients and the Control (0.5% and 3%, respectively), and interestingly, previous studies have reported significant heterogeneity in this polymorphism concerning C-allele distribution across geographical regions.

The CC genotype and C allele frequencies observed were: Mexico city CC (2.3%), C (14.7%), West Africa CC (1.9%), C (13.9%), Italy CC (7.5%) and C (28.1%), France CC (11.5%), C (35.7%), Algeria CC (7.4%) and C (21.81%)[26], Iran CC (22.6%) and C (41%) (8). Globally, A1298C C-allele frequency ranges from 10% to 70% in Asia, 24% to 46% in Europe, 13% to 32.2% in Africa, and 0% to 15% in America[27].

Research published in 2015 has indicated that individuals with the MTHFR 1298AC variant were associated with a decreased risk of AML[28]. However, in other types of leukemia, studies have suggested that 1298AC variants significantly increased the risks of ALL[20, 29] and decreased the risks CML, when compared with 1298AA presence [30]. In addition, MTHFR 1298 CC and AC polymorphisms were protective against ALL in children from Europe, as well as CML in adults. It was also found

that MTHFR 677TT and CT polymorphisms played protective roles, while the CC wild type was a risk genotype for ALL leukemia in child populations worldwide[31]. Conversely, some studies find no association between MTHFR677CT and ALL[23]. In a study undertaken in Kashmir it was found that C677T SNP strongly increases the risk of CML whilst A1298C SNP has a protective effect[32].

However, our study noted no statistically significant difference in the presence of genotypes 1298 AC and 1298CC, when compared with 1298 AA (1298 AC: OR = 0.305, 95% CI = 0.038–2.429, P = 0.262; 1298CC: OR = 0.050, 95% CI = 0.002–1.179, p value = 0.063).

In our study, we are able to show that 677CT provides protective effect for the AML, and that there is no significant association with 1298 AC and 1298CC homozygotes and AML. Other studies concur, finding no association with the MTHFR A1298C polymorphism and Acute Leukemia[20, 22].

A study undertaken in 2015 demonstrated that individuals with MTHFR 1298AC were associated with a decreased risk of AML[14]. Additionally, a study undertaken on adult AML patients in China found that MTHFR1298 AC genotypes were significantly less at risk of AML compared with AA genotypes[28]. Another study also stated that European children with AML were significantly associated with a decreased risk of AML[31] .

Several meta-analyses have been performed to clarify the association between MTHFR (C677T and A1298C) and AML risk. These studies suggested that MTHFR (C677T and A1298C) polymorphisms were not significantly associated with this risk [33]. And a recent meta-analysis undertaken in 2019 demonstrated no association with CML, AML, or MM for either polymorphism[20].

We also examined Combination Analysis between MTHFR 1298 and MTHFR 677 in patients and controls and found no statistical difference in the joint effects of the two polymorphisms. Conversely, studies amongst adult AML patients in China exposed to low-doses of carcinogens (non-smokers and non-drinkers), proposed that MTHFR 1298AC genotype and 677CC/1298AC haplotype presence is associated with a significantly reduced risk of AML, compared to cases with an AA genotype and 677CC/1298AA haplotype presence[28]. Additionally, a study conducted in Poland found that the MTHFR 677T allele alone, or in combination with the MTHFR 1298C allele, significantly increases the risk of AML development in subjects under 18 years of age[29].

Core reasons for the general lack of consensus in the aforementioned study results can be explained by several factors. The strength of the relationship between MTHFR polymorphism and AML development varies, both ethnically and geographically. The level of carcinogenic exposure will also affect MTHFR (C677T and A1298C) pathways and this possibly in combination with other environmental triggers. Additionally, other unknown factors may also alter the pathway in folate metabolism. Moreover, some of the aforementioned studies have limited their results on account of small sample size.

On the basis of our results and those from previous studies concerning AML, we can speculate that the decreased MTHFR polymorphic activity associated with A1298C and C677T genotypes, may result in an increased stability of DNA and an enhanced protection against malignancy for MTHFR 677 CT genotypes.

CONCLUSION

Our analysis indicates that MTHFR 677TT**,** 1298AC and CC genotypes provided no notable defence against AML, but that MTHF677CT may have a protective effect against the disease.

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