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Detection of Methylene Tetrahydrofolate Reductase (MTHFR C677T) Mutation among Acute Lymphoblastic Leukemia in Sudanese Patients

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Abstract

Background: A genetic polymorphism that causes abnormal folate metabolism may lead to genomic instability and increase susceptibility to malignancies such as Acute Lymphoblastic leukemia (ALL). The purpose of this research is to identify methylene tetrahydrofolate reductase (MTHFR C677T) (NCBI ID: 4524) mutation in ALL patients.

Methods: The study was a descriptive case-control hospital-based study with one hundred Sudanese participants divided equally into fifty (50) Sudanese ALL diagnosed patients as cases and fifty (50) Sudanese individuals as controls. The MTHFR C677T mutant allele was detected using conventional PCR, with the primer sequence of MTHFR C677T F-TGAAGGAAGGTGTCTGCGGGA R-AGGACGGTGCGGTGAGAGTG. The study was conducted from January to March 2023, and samples were collected from the Radiation and Isotops Center at Khartoum Hospital.

Results: The investigation revealed that 12 of the 50 patients in the case group (24%) had the MTHFR C677T mutant allele, and the study also revealed that there is significant correlation with the control group. There is no significant relationship between socio-demographic variables and MTHFR mutation detection in ALL patients. Also, the sociodemographic variables predictors of MTHFR mutation among ALL patients adjusted for smoking habit revealed no significant relationship**.**

Conclusion: According to the findings of this study, the mutant allele of the Methylene Tetra Hydro Folate Reductase C677T was detected and demonstrated varying degrees of significance. It was concluded that the MTHFR C677T gene mutation was associated with acute lymphoblastic leukemia in Sudanese patients.

Keywords: ALL, MTHFR C677T, MTHFR protein, Mutation.

Introduction

ALL is a group of lymphoid disorders characterized by swiftly growing clones of hematopoietic cells with high DNA synthesis demands. Lymphoid cancers arise from hematopoietic cell point mutations, chromosome rearrangements, and epigenetic changes. Imperfections in the genes of folate-dependent enzymes, as well as micronutrient deficiencies, could affect cancer susceptibility (1-3).

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The MTHFR gene is found at the terminus of the short arm of chromosome 1, 1p36.3 (4), and the protein that it encodes, MTHFR, is an essential enzyme in folate metabolism. It changes 5,10- methylTHF, the primary circulatory type of folate which offers a methyl group for homocysteine methylation, to 5,10-methyleneTHF, which serves as a donor for methylating uridylate to thymidylate (dTMP) in DNA synthesis (5). C677T and A1298C are common MTHFR gene polymorphisms. The variants 677T and 1298C both affect the activity of the enzyme, with 677T affecting the catalytic MTHFR domain and 1298C affecting the regulation MTHFR domain. In 677TT and 1298CC homozygotes, as well as to a smaller degree in heterozygotes, increased thermolability and reduced activity of enzymes were noticed (6). MTHFR C677T polymorphic allele genotypes included TT, CT, and CC. MTHFR 1298C polymorphic alleles were AA, AC, and CC (7).

A variety of investigations have found that these prevalent polymorphisms of the MTHFR gene protect against Acute Lymphoblastic Leukemia in children and adults. According to one study, the impact of MTHFR polymorphisms on the likelihood of Acute Lymphoblastic Leukemia may be affected by folate status (8) Reduced MTHFR activity may reduce homocysteine methylation to methionine and thus the amount of S-adenosyl-methionine (SAM), leading to DNA hypomethylation. The decreased MTHFR substrate level, 5,10-methylene-THF, necessary for thymidylate synthesis, on the opposite, might result in uracil misincorporation into DNA, decreased DNA repair, and raised incidence of chromosomal ruptures and destruction (9-12). Malignancies that originate from rapidly proliferating tissues, with the greatest need for DNA synthesis, must be more vulnerable to folate deficiency and the resulting DNA damage. In fact, the DNA variants that cause decreased MTHFR activity have been linked to a lower risk of leukemia, lymphoma, and colorectal carcinoma (13-16). The purpose of this study is to look for MTHFR polymorphism in Sudanese with ALL patients.

Materials and Methods

Study design and area

The study was a descriptive case control study conducted at a hospital. From January to March 2023, the research was carried out in Khartoum state. The samples were taken at the Radiation and Isotopes Center of Khartoum Hospital. Exon molecular biology laboratory in Khartoum state conducted laboratory experiments. Sudanese patients who have been diagnosed with ALL and are being treated at the Radiation and isotopes center Khartoum hospital. This study collected 100 samples, which were divided into two groups: 50 Sudanese ALL patients served as the case group, and 50 seemingly healthy people served as the control group. Data were obtained utilizing a straight structured questionnaire at the time of sample collection.

Participants selection

Patients professionally diagnosed ALL, according to radiation and isotopes center protocol for leukemia diagnosis identification (17) were included. Patients diagnosed with other Myeloproliferative disorders were excluded from the study.

DNA extraction

Following strict sterile procedures, 3 ml of venous blood from each participant was aseptically collected in Ethylene Diamine Tetra Acetic Acid (EDTA) anticoagulant for DNA extraction. DNA extraction was conducted using the EZ1 Tissue Kit (Qiagen, Hilden, Germany). Following this, 300 µl of whole blood was mixed with 900 µl of red blood cell lysis solution in a 1.5 ml tube, vortexed vigorously, and incubated for 5 minutes at room temperature. Following centrifugation at 10,000 x g for 1 minute, the supernatant was removed. To lyse the cells, 300 ul of cell lysis solution was added to the re-suspended cells and pipetted. After reaching room temperature, 100 ul of MgCl2 buffer was added, vortexed for 20 seconds at high speed, and centrifuged at 13,000-16,000 x g for 3-5 minutes. The supernatant containing DNA (300 ul) was transferred to a new 1.5 ml tube.

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To wash the DNA pellet, 300 ul of 100% isopropanol was added, and the sample was gently inverted. After centrifugation at 13,000-16,000 x g for 1 minute, the tube was air-dried for 10-15 minutes on absorbent paper. Subsequently, the dehydrated DNA was rehydrated by adding 150 ul of DNA rehydration buffer after 30 minutes of incubation at 65 °C.

The polymerase chain reaction (PCR)

The detection of the MTHFR C677T mutation was conducted using the primers F-TGAAGGAGAAGGTGTCTGCGGGA and R-AGGACGGTGCGGTGAGAGTG.
Following established protocols from previous publications, PCR and gel electrophoresis were carried out for mutation analysis (DNA Mastercycler; Eppendorf, Sigma-Aldrich Handels Gmbh, Wien, Austria) (18, 19).

Data analysis

Before being analyzed with Statistical Package for the Social Sciences (SPSS) version 23 statistical software [SPSS Inc, USA], data had been entered and organized into a Microsoft Office Excel 2010 data sheet. Chi squire t-test analysis was carried out using the analysis of variance. $P < 0.05$ was regarded as statistically significant.

Results

The case population, aged 3 to 20 years, was divided into two groups: Group (1) comprised individuals aged 3-11 years (76%), while Group (2) included those aged 12-20 years (24%). In the case group, 72% were male, and 28% were female. The mean age of the case population was 8.5 years, with a standard deviation of 4.01. Participants were also categorized based on smoking status, with 4% being smokers and 96% nonsmokers. No significant association ($p > 0.05$) was observed between patient and control samples (Table 1).

The study identified the MTHFR C677T gene mutation in 24% of the case group (12 out of 50 participants), indicating a significant prevalence compared to the control group ($p=0.001$) (Table 2). No significant correlation ($p > 0.05$) was found between sociodemographic characteristics and MTHFR mutation identification in ALL patients (Table 3). Additionally, sociodemographic variables, when controlling for smoking habits, did not show a significant association with MTHFR mutation in ALL patients ($p > 0.05$) (Table 4).

Table 1. The socio-demographic characteristics of the patientss in the study.

Sociodemographic variables	Responses	Case (50)		Control (50)		p value
		Count	%	Count	%	
Age Group	3– 11 Year	38	76.0	28	56.0	0.028*
	12 – 20 Year	12	24.0	22	44.0	
Gender	Male	36	72.0	39	78.0	0.322
	Female	14	28.0	11	22.0	
Smoking habit	Smokers	2	4.0	3	6.0	0.691
	non smoker	48	96.0	47	94.0	

Mean ± SD for age of the control group was (12.5±4.6) years while mean ± SD for age of cases was (8.5±4.1) years. *p value ≤ 0.05, gender and age are matched between case and control.

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Table 2. The prevalence of MTHFR mutation in ALL cases and its association with control group.

MTHFR mutation	Responses	Case (50)		Control (50)		p value
		Count	%	Count	%	
Detection	Yes	12	24.0	0	0	0.001*
	No	38	76.0	50	100	

The prevalence of MTHFR mutation among cancer patents is 24.0%. *p value ≤ 0.05.

Table 3 The association between Socio-demographic variables and detection of MTHFR mutation among 50 ALL patients.

Sociodemographic variables	Responses	Cancer patients Number (%)		X^2	p value
		Yes	No		
Age Group (year)	3– 11	9 (18.0)	29 (58.0)	0.009	0.602
	12 – 20	3 (6.0)	9(18.0)		
Gender	Male	11(22.0)	25 (50)	3.03	0.080
	Female	1 (2.0)	13 (26.0)		

Table 4. Socio demographic variables Predictors of MTHFR mutation among 50 ALL patients adjusted by smoking habit.

Predictors	Responses	Cancer patients Number (%)		Adjusted model			
		Yes	No	β	OR	95% CI	p-value
Age Group	3– 11 Year	9 (18.0)	29 (58.0)	R	-	-	-
	12 – 20 Year	3 (6.0)	9(18.0)	1.7	1.5	0.25-8.5	0.119
Gender	Male	11 (22.0)	25 (50)	R	-	-	-
	Female	1 (2.0)	13 (26.0)	0.38	5.6	0.64-49.3	0.674
Smoking habit	nonsmoker	11 (22.0)	37(74.0)	R	-	-	-
	Smokers	1 (2.0)	1(2.0)	1.2	3.2	0.13-8.4	0.473

R: Reference (control) group.

Discussion

Acute Lymphoblastic Leukemia (ALL) is a disorder characterized by monoclonal proliferation and the development of immature lymphoid cells in the bone marrow, blood, and other tissues. It is the most frequent type of pediatric cancer and the second most common type of cancer in Sudan. Polymorphisms in folate pathway genes are risk factors for developing acute leukemia. Changes in folate metabolism may impact cancer risk (20). This is congruent with the present study, which showed a highly significant relationship between the folate pathway gene mutation (MTHFRC677T) and ALL (p=0.001).

The age range of the case population in this study was 3-20 years, divided into two groups: Group (1) with ages 3-11 representing 76%, and Group (2) with ages 12-20 representing 24%. In the case group, 72% were male, and 28% were female. The age group mean in the case population was 8.5, with a standard deviation of 4.07. Patients were also classified based on their smoking status, with smokers accounting for 4%, and nonsmokers

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accounting for 96%. There was no significant correlation between patient and control samples.

Previous research conducted in Sudan by Ali et al., 2020 (21), revealed that leukemia is the second most common cancer in Sudan, and ALL is the first malignancy of childhood cancer in Sudan. ALL subjects had a mean age of 13.6 years. Their gender distribution was 63% male and 36% female. The incidence of ALL was slightly higher (41%) among adolescents (7-18 years of age) than among children (1-6 years) (32%) and the elderly (>18 years of age) (22%). The majority of ALL patients (75.5%) had B immunophenotyping.

The present study identified the MTHFR C677T gene mutation in 24% (12 out of 50) of patients in the case group, while the control group showed no mutations. Significantly, differences were observed between the patient and control groups, indicating an association between MTHFR polymorphism and susceptibility to ALL. This aligns with the findings of Li et al., 2020 (22), who studied the association of MTHFR polymorphism and susceptibility to ALL in Asia and supported this conclusion. Comparison across various genetic models, including allele, dominant, recessive, homozygous, heterozygous, and in Caucasian children, revealed no significant differences, further supporting the linkage of MTHFR polymorphism to ALL.

In a 2018 study conducted in Turkey by Umay et al., which involved 91 ALL patients and 101 healthy controls, results indicated that patients carrying the 1298C allele or the 1298CC genotype had a higher risk of ALL (23). Additionally, in the recessive model, patients with the 677TT genotype had a lower risk of ALL compared to patients with the 677CC and 677CT genotypes (24). Tantawy et al., 2010, studied 40 ALL patients in Egypt, revealing that the MTHFR gene was associated with an increased relapse rate in childhood ALL (25). Another study in India by Reddy et al., 2006, focused on children with ALL and found a link between the MTHFR gene polymorphism and ALL (26).

Our findings diverged from those of Zanrosso et al., 2006, who, in a study on patients in Brazil, found no association between MTHFR polymorphism and the risk of ALL in their total case-control sample (27).

Despite adjusting for smoking habits, the sociodemographic variables predicting MTHFR mutation among ALL patients showed no significant relationship in our study. This result contrasts with a study conducted in Italy by De Stefano et al. (2002), which indicated that the detectable MTHFR mutation in 10% of case studies increases the risk of venous thromboembolism in ALL patients (28).

Based on our study's findings, the MTHFR C677T mutation was present in 12 patients, leading to the conclusion that the MTHFR mutation is associated with acute lymphoblastic leukemia in Sudanese patients.

Ethical approval

The National University of Sudan's (Faculty of Graduate Studies and Scientific Research) ethical committee approved this study (NU/FGSSR/163/01/2023). Before collecting samples, all participants in this study provided written informed consent. The data and genetic materials were kept private and were only used for research purposes.

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Conflict of interest

Author declared that there is no conflict of interest in this research.

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