Association of methionine synthase reductase (MTRR A66G) polymorphism with susceptibility to acute lymphoblastic leukemia

Mashaer Taha Edris,[1,2] Maye Mohammad Merghani,[2] Shaza Salih Gafar,[3] Ahmed Mohamed Asmali,[4] Elrashed B. Yasin,[5] Raed Alserihi,[6,7] Heba Alkhatabi,[6,7] Haitham MH. Qutob,[5] Rowaid Qahwaji,[6] Elshazali Widaa Ali[8]

[1]Department of Hematology, Faculty of Medical Laboratory Sciences, National Ribat University, Khartoum, Sudan; [2]Department of Hematology, Faculty of Medical Laboratory Sciences, Nahda College, Khartoum, Sudan; [3]Department of Microbiology, Faculty of Medical Laboratory Sciences, Nahda College, Khartoum, Sudan; [4]Department of Clinical Chemistry, Faculty of Medical Laboratory Sciences, Nahda College, Khartoum, Sudan; [5]Department of Medical Laboratory Technology, Faculty of Applied Medical Sciences, King Abdulaziz University, Rabigh, Saudi Arabia; [6]Department of Medical Laboratory Sciences, Faculty of Applied Medical Sciences, King Abdulaziz University, Jeddah, Saudi Arabia; [7]Hematology Research Unit (HRU), King Fahad Medical Research Center (KFMRC), King Abdulaziz University, Jeddah, Saudi Arabia; [8]Department of Hematology, Faculty of Medical Laboratory Sciences, Al Neelain University, Khartoum, Sudan

Correspondence: Elrashed B. Yasin, Department of Medical Laboratory Technology, Faculty of Applied Medical Sciences, King Abdulaziz University, Rabigh, Saudi Arabia.
E-mail: eyasin@kau.edu.sa

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ABSTRACT

Background and Objectives. The enzyme methionine synthase reductase is involved in cellular methylation reactions, DNA synthesis, and epigenetic processes. It is encoded by the MTRR gene, which garnered a lot of attention in current medical genetics research. This study was conducted to study the association between MTRR (A66G) polymorphism and the risk of developing acute lymphoblastic leukemia among Sudanese patients. Materials and Methods. This is a case-control study in which 150 patients with acute lymphoblastic leukemia (ALL) and 150 healthy participants as a control group were enrolled. DNA was extracted and analyzed for the MTRR (A66G) polymorphism using the real-time polymerase chain reaction. Results. Based on flow cytometry results, B-ALL was more common (79%) than T-ALL (21%). The comparison of hematological parameters in acute lymphoblastic leukemia subtypes showed a statistically significant high mean total white blood count (P=0.000) and mean blast percentage (P=0.050) in patients with T-ALL. The molecular analysis showed that the incidence of the MTRR homozygous genotypes AA and GG were higher in the patients (44% and 9.3%, respectively) compared to the control group (40% and 6.7%, respectively). In comparison, the heterozygous genotype AG was lower in the patients (46.7%) than in the control group (53.3%). However, the association between the polymorphism and acute lymphoblastic leukemia risk was not statistically significant (OR: 1.179, 95% CI 0.7459-1.865, P=0.445). Conclusions. This study concluded that MTRR A66G polymorphism was not associated with the risk of acute lymphoblastic leukemia among the Sudanese population.

Introduction

Acute lymphoblastic leukemia (ALL) is the most frequent type of juvenile cancer, accounting for 30% of all malignancies identified in children.[1] Being widely known that changes in the genes involved in DNA synthesis and methylation may be the first step in the development of malignant hemopoietic transformation, it is crucial to maintain an appropriate concentration of folate, which provides a methyl group for proper DNA synthesis, as well as homocysteine in the methionine cycle, which is crucial in the DNA methylation process.[2] The availability of folate in cells may be affected by genetic polymorphisms of genes encoding essential regulators and transport enzymes of the folate cycle, such as MTHFD1, MTRR, and RFC1. This could change the susceptibility to ALL and the way the treatment works.[3-5]

According to a recent study, physical activity and diet both increase the risk of developing cancer.[6] Cancer risk and folate intake have been linked, according to research by Kawakita *et al*.[6] In addition, interactions between genetic factors and environmental exposure are thought to cause different types of cancer.[7]

The etiology and development mechanisms of ALL have not yet been elucidated. However, it is known that different people have different susceptibilities to the same type of cancer even under comparable environmental exposures.[8] Genetic predisposition, including polymorphisms in genes involved in ALL etiology, can be used to account for this variation.[9,10] Several studies have been performed using genetic polymorphisms involved in folic acid metabolism in various types of cancer.[11-13] This suggests that methionine and folic acid metabolism play an essential role in epigenetic processes, DNA synthesis, and cellular methylation reactions by providing one-carbon donors for purine and pyrimidine synthesis, and associated methylation and remethylation (of homocysteine) involved in the DNA methylation.[14,15]

One of the important regulating enzymes in the folate metabolic pathway is MTRR. The MTRR gene is located on chromosome 5; a polymorphism (rs1801394) was identified in this gene, causing a G to A transition and leading to the replacement of methionine for isoleucine at codon 22, position 66.[16] MTRR polymorphisms have been stated to be closely related with leukemia risk and associated with a reduced enzymatic affinity for the widely studied methionine synthase.[17] Previous studies have suggested a relationship between the MTRR gene and its polymorphisms and the risk of many types of cancers.[15,18] such as acute leukemias (lymphoblastic and myeloid), lung cancer, and colorectal cancer;[16,19-21] however, the results remain controversial.[15]

This study was conducted to further elucidate the link between the MTRR A66G polymorphism and ALL risk among the Sudanese population.

Materials and Methods

Study subjects

This case-control study was carried out at the flow cytometer center for diagnosis of leukemia and lymphoma, central laboratory-ministry of higher education, and National University Biomedical Research Institute, Khartoum, Sudan, in the period from May 2017 to April 2021. A total of 300 participants were recruited for the study, 150 patients diagnosed with ALL using flow cytometry and 150 age and sex-matched apparently healthy participants as a control group.

Hematological analysis

An automated blood counter (Sysmex KX 21) and peripheral blood picture were used to evaluate the hematological biomarkers. ALL was diagnosed according to the white blood cell (WBC) count at varying degrees and blood cell morphology and further confirmed using antigen-specific antibodies to analyze the immune cell subsets by flow cytometer (Coulter EPICS X -Mcl TM flow cytometer Miami, Florida, USA).

Analysis of MTRR A66G polymorphism

Genomic DNA was isolated from peripheral leucocytes using the Spin-Column Extraction method. DNA concentration and purity were assessed using a Nanodrop (NanoDrop™ Lite-UV Spectrophotometer-ThermoFisher Scientific). The MTRR A66G polymorphism was analyzed using the TaqMan Real-Time PCR (qTOWER3G, Anlitka Jena, Germany). The probes and primers sequences used were as follows: MTRR primers forward: AGG CAA AGG CCA TCG; MTRR primers reserved: ATC CAT GTA CCA CAG CT; and MTRR Probes: G- CAG AAG AAA TGT GTG A; A- CAG AAG AAA TAT GTG A. Touchdown the reaction mix was cycled at 95°C for 10 minutes, 95°C for 15 seconds and 60°C for one minute (40 cycles), then the analysis was done by using absolute analysis method.

Data collection and analysis

Patients' data were collected through a structured questionnaire and the statistical analysis was performed by the Statistical Package for Social Sciences, version 25. The chi-square test was used to compare the distribution of genotypes among study groups, and regression analysis was performed to investigate the association between the polymorphism and ALL risk.

Ethical considerations

The study was approved by the Ethical Committee, Ministry of Health, Khartoum State, and informed consent was obtained from all adult participants or the parents or guardians of children participants before sample collection.

Results

Demographic data

In this study, 300 people were included, 150 of whom were ALL patients identified using flow cytometry, and 150 volunteers who were matched for age and sex and appeared to be in good health as a control group. 100 (66.7%) of each of the study groups were males, and 50 (33.3%) were females. For both groups, the age range was 2-78 years (Mean±SD: 20.7±18.3). 86 (57.3%) of the participants were children (up to 16 years), and 64 (42.6%) were adults.

Immunophenotyping

Immunophenotyping was carried out by flow cytometer, it confirmed the diagnosis of ALL and revealed that B-ALL was more prevalent 119 (79%) than T-ALL 31 (21%). B-ALL was more predominant in both children and adults than T-ALL and also in males than females, however, there was no statistically significant association between any subtype and either age group or gender (Table 1).

Hematological data

Comparison of the hematological parameters among ALL subtypes showed statistically significantly higher mean total WBCs count and blast percentage in patients with T-ALL than those with B-ALL. No statistically significant difference was found in the mean red blood cells (RBCs) count, hemoglobin, and platelets (PLTs) count (Table 2).

Genotyping of MTRR (A66G) polymorphism

The genotype distribution of MTRR A66G was consistent with Hardy-Weinberg equilibrium (P>0.05). The MTRR heterozygous AG genotype was more prevalent in both study groups, after that the homozygous AA and GG genotypes. The comparison of genotypes distribution among the study groups showed that both the homozygous GG and AA genotypes were more frequent in the patients than the control group, while the heterozygous genotype AG genotype was more frequent in the control group, However, the distribution of genotypes among the study groups was not statistically significant (Table 3). The regression analysis showed no statistically significant association between MTRR

Table 1. Association of acute lymphoblastic leukemia subtypes with age group and gender.

Variable		B-CLL	T-CLL	P
Age group	Children Adults	66 (44.0%) 53 (35.3%)	20 (13.3%) 11(7.3%)	0.364
Gender	Male Female	79 (52.7%) 40 (26.7%)	21 (14.0%) 10 (6.7%)	0.887

Table 2. Comparison of the hematological biomarkers according to acute lymphoblastic leukemia subtypes.

Parameter	B-CLL Mean±SD	T-CLL Mean±SD	P
Total WBCs count ($X10^9$/l)	43.3±52.1	162.2±139.4	0.000
RBCs count ($X10^{12}$/l)	2.7±0.8	2.7±0.9	0.843
Hemoglobin (g/dl)	8.7±6.2	8.6±2.7	0.942
PLT count ($X10^9$/l)	45.4±4.3	51.3±39.9	0.525
Blast %	60.9±18.3	68.3±19.6	0.050

WBC, white blood cell; RBC, red blood cell; PLT, platelets.

Table 3. Distribution of MTRR polymorphic genotypes among the study groups.

Genotype	Patients	Control	P
AA	66 (44.0%)	60 (40.0%)	
AG	70 (46.7%)	80 (53.3%)	0.445
GG	14 (9.3%)	10 (6.7%)	

polymorphism and risk of ALL (OR: 1.179, 95% CI: 0.745-1.865, P=0.445).

The comparison of the MTRR genotypes among children and adult groups showed that both the homozygous AA and heterozygous AG genotypes were more frequent in the children than in the adult group. In contrast, the homozygous genotypes GG were more frequent in the adult group. However, the distribution of genotypes among the study groups was also not significantly different (OR: 1.118, 95% CI: 0.583-2.143, P=0.071) (Table 4).

Comparison of the hematological parameters in ALL patients with wild type allele (AA) and mutant type allele (AG+GG) showed a statistically significant difference in mean RBCs count but not in mean Hb, total WBCs count, PLTs count, and blast (%) (Table 5).

Discussion

ALL is the most prevalent form of childhood cancer, ALL affects people of all ages, with a peak incidence occurring between the ages of 2 and 5. As a result, age is a key indicator of when a disease will manifest.[22] There are various suggestions that environmental and genetic variables contribute to the beginning of leukemogenesis, despite the fact that its etiology is still not fully understood.[23] Single nucleotide polymorphisms were highlighted. Due to their crucial involvement in the detoxification and occasionally inactivation of carcinogens as well as their defense against oxidative stress, a number of gene polymorphisms have attracted a lot of attention for their potential to influence the risk of ALL development.[24]

The significance of the folate-related gene MTRR (A66G) polymorphism in the metabolism of numerous environmental chemicals is the subject of this study, which focuses on it as a risk factor for the development of ALL.

The results showed that most of ALL patients were males, with a male: female ratio of about 2:1. This result agrees with the results reported by Jawaid *et al*. in Pakistan, who stated that males were more predominant than females.[25] Also, Sultan *et al*., and Shahab and Raziq in Pakistan showed that most ALL populations in their studies were males with a ratio of 2:1.[26,27] The finding is also in agreement with the results reported by the Saudi Cancer Registry and the American Cancer Society.[28,29]

According to the findings of this study, the majority of patients (57.3%) were under the age of 16; this finding is consistent with a report published by the American Cancer Society and a study in Sudan by Ebrahim *et al*. who reported that two third of their cases were between 6-16 years.[30] The analysis of complete blood count in ALL patients revealed the presence of leukocytosis, anemia, thrombocytopenia, and high blast percentage; these findings were consistent with the results of many studies in Sudan, Mexico, Iran, Egypt, and Pakistan.[31-35]

In this study, B-ALL was more frequent than T-ALL; this outcome agrees with many studies conducted in Jordan, Brazil, Italy, Egypt, and Mexico; all of them reported higher frequency for B-ALL than T-ALL.[26,36-40] On the other hand, our finding disagrees with two studies conducted in Iran and Pakistan; both found that the frequency of T-ALL is greater than B-ALL.[41] This variation may confirm

Table 4. Distribution of MTRR (A66G) genotypes among children and adults.

Genotype	Children	Adult	P
AA	39 (45.3%)	27 (42.2%)	
AG	43 (50.0%)	27 (42.2%)	0.071
GG	4 (4.7%)	10 (15.6%)	

Table 5. Comparison of hematological parameters in acute lymphoblastic leukemia patients with wild and mutant type MTRR alleles.

Parameter	Wild (N=66) Mean±SD	Mutant (N=84) Mean±SD	P
Total WBCs count ($X10^9$/l)	63.5±9.0	71.3±11.4	0.606
RBCs count ($X10^{12}$/l)	2.6±0.9	2.8±0.8	0.058
Hemoglobin (g/dl)	8.9±8.2	8.6±2.1	0.727
PLT count ($X10^9$/l)	40.0±3.9	51.8±5.9	0.188
Blast %	60.4±19.6	64.1±18.0	0.241

WBC, white blood cell; RBC, red blood cell; PLT, platelets.

the heterogeneous behavior of the disease and appear to be proportionate with an interplay of environmental and biological factors.

In the current study, the comparison of hematological parameters according to the ALL subtype showed a significantly higher mean total white blood count and mean blast percentage among patients with T-ALL than those with B-ALL, while the means of other parameters (RBCs count, PLTs count, and Hb concentration) were not statistically significant; this finding agrees with that reported in China by Dai *et al*., who reported that patient with T-ALL showed significantly higher total white blood cells count and blast count at diagnosis than patients with B-ALL.[42] Also, our finding agrees with two studies in Brazil by De Sousa *et al*., and in Iraq, by Jaafar and Kadhom both found that total white blood cell count is significantly increased in T-ALL than B-ALL.[43,44]

In this study, the genotyping of MTRR (A66G) polymorphism showed that the homozygous genotypes AA and GG were more frequent in the patients compared to the control group. The heterozygous genotype AG was lower in the patients compared to the control group. However, the association between MTRR (A66G) polymorphism and ALL risk was not statistically significant in all age groups. This result agrees with many studies that also found no significant differences in susceptibility to ALL regarding the MTRR (A66G) polymorphism.[45] A German study by Gast *et al.* found no statistically significant differences between the cases and the controls in allele and genotype frequencies regarding the MTRR polymorphisms.[46] Also, two studies in Korea and Western Europe found no significant association between polymorphism and susceptibility to ALL.[23,47] Furthermore, many studies in Russia, the United Kingdom, and Italy found that MTRR polymorphism can reduce the risk of ALL.[48-50] The finding disagrees with a Chinese study which found a significantly increased risk for ALL by 2.15 times with the MTRR 66 GG variant.[19] A marginally increased risk was also observed in Korea by Hee Nam *et al.* for ALL and the MTRR66 GG genotype. The conflict findings regarding the association of MTRR polymorphism with the risk of ALL development could indicate that the effect of this polymorphism on susceptibility to ALL is different among various populations and this can be due to interaction with some environmental factors.

In the present study, the comparison of hematological parameters in ALL patients with MTRR (A66G) wild and mutant types showed no statistically significant difference in mean RBCs count, Hb concentration, TWBCs count, PLTs count, and blast percentage; no published data concerning the comparison of hematological parameters in patients with different MTRR polymorphic variants was reported.

Conclusions

In conclusion, among the Sudanese population, ALL is more frequent in children than adults and in males than females. The B-cell ALL subtype is more predominant than the T-cell type. MTRR (A66G) polymorphism is not associated with susceptibility to ALL.

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